# Chilling paper(s) notes

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## Contents



## 1 May 2025 outline for second part

The path forward should ...

1. Improve experiments
   (a) Redefine chilling for improved experiments
      i. Redefine chilling as an unknown physiological process—similar to 'floragen' period with vernalization, we need to be clear we have no marker
      ii. So treatments cannot be called chilling and forcing, just call them cool and warm treatments.
   (b) Begin a new generation of experiments that measure molecular stuff (this is critical)
   (c) Expect more experiments that do not measure molecular stuff ('old-generation'), measure more stuff, include stats that consider multiple hypotheses
      i. Measure cold hardiness
      ii. Make all chilling treatments include light or include light/dark as factor
      iii. Statistics should include alternative start date hypothesis if they don't measure the start date

2. Improve models to represent current understanding and lack thereof
    (a) We need to be less overly confident in what we know to make the best use of molecular insights. So ...
        i. Focus on simple models for now
        ii. Require uncertainty/non-identifiability to be better studied in models
    (b) Fit observational and experimental data together

## 2 November 2024 outline

**To do ...** See new file `_dothisChilling.md`

How to overcome the challenge of chilling in spring phenology models

1. Introduction
    (a) Why to care ...
        i. Critical to good forecasts (my current common baby) of forests, carbon storage ...
        ii. Lots of current work suggests it's changing in ways we cannot totally predict and it's even more critical in today's climate than we thought (decsens etc.)
        iii. But these new studies make a lot of assumptions about chilling
        iv. And we have a gazillion models of chilling, suggesting something is amiss
    (b) Chilling has long been a struggle for plant physiologists
        i. Debates about rest vs. quiescence in 50s-60s (maybe earlier?)
        ii. Conundrum of it in 60s-70s and peaches
        iii. Work in 2012 (Guy), 2026 (Chuine) and more have highlighted how little we know if we want to forecast with climate change
    (c) But this is a special moment for chilling
        i. More important than ever to understand it.
        ii. And new molecular insights are suggested we know parts of the story that could reshape how we measure and model it
    (d) Here we ...
2. What is chilling?
    (a) Fundamentally, it's the idea that cool temperatures of dormant buds/plants accelerate a phenological event that later occurs after warm temperatures
    (b) Considered to have evolved to protect plants from leafout during brief warm snaps in middle of otherwise cold winters.
    (c) Applied across a lot of fields and study systems ...
        i. Satellite measures of greenup
        ii. Small scale cutting studies of forest tree species
        iii. Also, lots of similar work on crops
        iv. Molecular studies

v. Lots of models of it for forest trees and crops
vi. Generally applies to leafing and flowering, with different fields focused on different events (much of molecular is flowers and vernalization, while forest trees and satellite is almost exclusively for leafout)

(d) Experimental context for it: things get chilled at 5°C (molecular and phenology people)

(e) Models of it define it as what is needed for endodormancy break or leafout ...
i. Most common model is often sequential (explain it, include optimal chill idea)
ii. But lots of variation, alternating, more complex versions of alternating (parallel etc.), whatever Luedeling is called.

(f) And then there are attempts to estimate chilling using observational data in crops (but often when planted outside range) and forest trees ... and basically always based on assumptions from existing models and/or experiments (peaches again).

3. The problem with chilling

(a) Refers to either:
i. a latent accumulation before an event
ii. an experimental treatment
iii. But the problem with this dichotomy is rarely if ever acknowledged

(b) Non-identifiability of chilling (unrecognized)

(c) Experimental and observational data often don't match

(d) Resulting models from this research are poor, and thus we build a million different models

4. New molecular insights could reshape the field and its models

(a) Molecular has always been a focus, but new results are particularly exciting and more studies are coming out across more species and the pathway is coming together (cite figure)

(b) We mention two major ones: callose and time-dependent stuff
i. Review callose
ii. If callose is correct, it may rule out long periods when chilling and forcing should act together (e.g., parts of alternating and parallel models) but also times when they may co-act and warm temperatures can negative 'chilling' because there is also ..
iii. Time-dependent growth (Zhu)

(c) New molecular insights hold promise to remove part of the non-identifiability, but the field needs to advance to take them on and evaluate them carefully given they will likely come from different species and possibly different events ....

5. Be more aware of assumptions and limitations (including non-identifiability) of current models and current data

(a) Model assumptions should be more explicit
i. How is chilling and endodormancy defined?

ii. What parameters are non-identified and how variable can these be in the model.

iii. What assumptions are made about some of the known potential complexities of chilling (see Box) such as photoperiod, induction temperature, species variability etc..

iv. Treat current models as bad guesses, and consider building ones with fewer parameters to test against complex ones (explain value of this, and push this idea! Refer to Box to suggest we may miss other factors if we press on with complex non-identified models only)

(b) Acknowledge limitations of current data

i. Remember that chilling treatments in experiments are not the same definition as conceptual concept of chilling (and models)

ii. Be careful using any observational data to estimate chill

(c) And find better data

i. Experiment should be explicit: How is chilling measured? What is photoperiod, induction temperature etc.

ii. Look for observational data outside the range of species with extremely different chill

6. Interdisciplinary efforts to bridge experimental-observational divide (people always say you should be more interdisciplinary, but we really should be here)

(a) Models to date at best use one experiments or observational data to inform the other, but do not build models designed to predict experimental and observational data together; but we could and we should.

(b) Building such models would benefit from far greater interdisciplinary efforts, bridging:

i. Phenology observations and experimental and modeler folks

ii. Molecular experimentalists and modelers

iii. Crop biologists and their modelers

iv. Hardiness folks

(c) Start building a metanalytic database (see table in Supp perhaps) that can bridge these fields by including all conditions and also what exactly is measured as induction and endodormancy break etc.

(d) Make testable predictions from these advances, and test them

Box: What we know matters to chilling ... and might matter.

1. What are we fairly sure matters (across many studies and species)

(a) Time matters ... is it only time, no also time at different temperatures: $< 0$, 0-10, $> 10$

(b) Temperature ...

2. What additional factors likely matter, but we're not sure which species or how big effects are...

(a) Stuff related to population/site differences

   i. Fall temperatures (don't forget we have OSPREE papers on induction temperatures)
   ii. Photoperiod
   iii. Population variation
(b) Stuff related to temperature variability
   i. Subzero temperatures (Fredi's paper, but see OSPREE lit where old temperature did not matter)
   ii. Intermittent warm periods
   iii. This is why hardiness has come up more

3. Species identity ... it matters! Consider focusing on one model species and race models against each other.

## 3 Next steps: from alt 2022 paper

**Not applicable to totally new version of paper written above, but saving here in case Fredi or someone does want to do any of this...**

**Make tables & figures?** ... Update old notes below to do that.

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 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

*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

## 4 Old 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 (Fredi'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 temperatues
      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

# 5 Meeting notes

## 5.1 8 December 2023: Lizzie meets with Loren Riesberg

Full notes in my gray and green notebook, but some major points to rememeber 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.

### 5.2 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

### 5.3 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`

### 5.4 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 temperatues
    (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 ...

### 5.5 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 is 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?