Dormancy and Chilling research notes

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Epigenetic silencing of callose synthase by VIL1 promotes bud-growth transition in lily bulbs
Authors

Methylation / Callose / Myosin / FT / FLC / VIL1 / PRC2 / H3K27me3 / H3K9ac / CALS3 / NFYA7 / HDA13 / HDA14 /

Study system: *Lilium* 'Siberia'

CHILLING (LTCT): 4 degC
WARMING: 12 degC
DAYLENGTH: not mentioned
CHILL LENGTH OPTIMUM/MINIMUM: 8 weeks

- **The overall model**
    - NFYA7 → VIL1-PRC2 –(H3K27me3)–| CALS3 –| growth transition
    - VIL1-PRC2 –| FLC –| FT
    - VIL1-PRC2 → H3K27me3
    - HDA13/14 –| H3K9ac
    - HDA13/14 |—| NFYA7 → H3K27me3/H3K9ac
- **Bud development**
    - Long term cold treatment (LTCT) for at least 8 weeks led to central bud development and emergence; treatment of 12 degC afterward led to floral meristem differentiation
        - Plasmodesmata closed in dormant buds but open in buds of bulbs exposed to LTCT for 8 weeks
        - Callose accumulation significantly higher in dormant buds
    - Transcriptome revealed upregulation of genes involved in histone lysine methylation and glucan metabolism
    - Treatment with myosin inhibitor BDM and callose biosynthesis inhibitor DDG led to acceleration of bud growth
        - Symplastic transport in the SAM was enhanced
    - Treatment with myosin ATPase blocker NEM led to delayed bud growth
        - Symplastic transport in the SAM was decreased
    - FT1 expression increased with extending LTCT
- **LoVIL1**
    - VERNALIZATION INSENSITIVE-LIKE 1 (VIL1): in Arabidopsis, involved in photoperiod detection and vernalization through master regulation of the FT repressors FLC and FLM, flowering loci
    - Dormant buds hardly express VIL1, but LTCT induces expression specifically within SAM and leaf-scale primordia
    - VIL1 silencing leads to delayed central bud growth and shorter buds in general
        - Also increased callose deposition at plasmodesmata
        - Decreased FT1 mRNA in the SAM
        - Sprouting and flowering time significantly delayed
    - WT VIL1 therefore induces floral meristem development and increases FT1 expression

    - Overexpression of VIL1 led to evergreen phenotypes that did not enter dormancy with very low plasmodesmata closure
- **LoVIL1 and LoCALS3**
    - CALS3: Callose synthase 3
    - High expression in the dormant SAM, decreasing expression when SAM entered a stage of active growth
    - Expression pattern is opposite of VIL1
        - VIL1 putatively represses CALS3 activity
        - VIL1 silencing led to upregulation of CALS3 expression
    - CALS3 silenced plants displayed significantly enhanced central bud elongation even after just 6 weeks of LTCT
        - Also led to a decrease in callose biosynthesis by 50% compared to WT
        - Accompanied by an upregulation in FT1 accumulation
- **LoNFYA7**
    - NFYA7: Nuclear Factor Y family subunit A7
    - MULTICOPY SUPPRESSOR OF IRA1 (MSI1): needed for transition into flowering in Arabidopsis; the LOF mutant leads to proliferation of unfertilized ovules and endosperm tissue
    - POLYCOMB REPRESSIVE COMPLEX 2 (PRC2): epigenetic repressor of gene expression; heavily involved in the accumulation of H3K27me3
        - VIL1-PRC2 also suppresses FLC expression, indirectly upregulating FT expression
    - Overexpression of VIL1 led to upregulation of trimethylation at H3K27 (H3K27me3)
        - VIL1 is a subunit of PRC2
        - Yeast-2-hybrid assay revealed NFYA7 as a candidate interactor with the VIL1-PRC2 complex
    - NFYA7 recruits PRC2 via recognizing and binding VIL1
    - High expression in dormant central buds, particularly SAM, and decreased after LTCT, the same as CALS3
    - NFYA7 silencing led to faster central bud, along with 33% decrease in callose accumulation at the plasmodesmata and also an increase in FT1 expression
        - NFYA7 therefore negatively regulates growth transition by increasing callose deposition
    - NFYA7 overexpression led to upregulation in CALS3 mRNA accumulation, and vice versa
        - NFYA7 directly binds to the promoter of CALS3 via CCAAT *cis*-elements (Cces)
    - If both NFYA7 and VIL1 are present, activity of the CALS3 promoter is significantly decreased
- **H3K27me3**
    - H3K27me3 was significantly enriched at the CALS3 locus under the presence of an NFYA binding site
        - VIL1 promoted H3K27me3 enrichment on the CALS3 locus in VIL1 overexpressed plants
        - NFYA7 alone can enrich H3K27me3, but co-overexpression of NFYA7 and VIL1 leads to a much larger enrichment
        - NFYA7 silencing, even in VIL1 overexpressing plants, significantly reduced H3K27me3 enrichment at the CALS3 locus, indicating the importance of VIL-PRC2 recruitment via NFYA7

- **H3K9ac**
    - NFYAs are often associated with acetylation of H3K9 (H3K9ac) and compete with HISTONE DEACETYLASE (HDA13) in soybean (GmHDA13)
    - NFYA7 silencing led to significant decrease in H3K9ac enrichment, while overexpression led to an increase in enrichment
        - Lily orthologue HDA14 competes against NFYA7 to regulate CALS3 expression through histone modification (methylation H3K27me3 or acetylation H3K9ac)

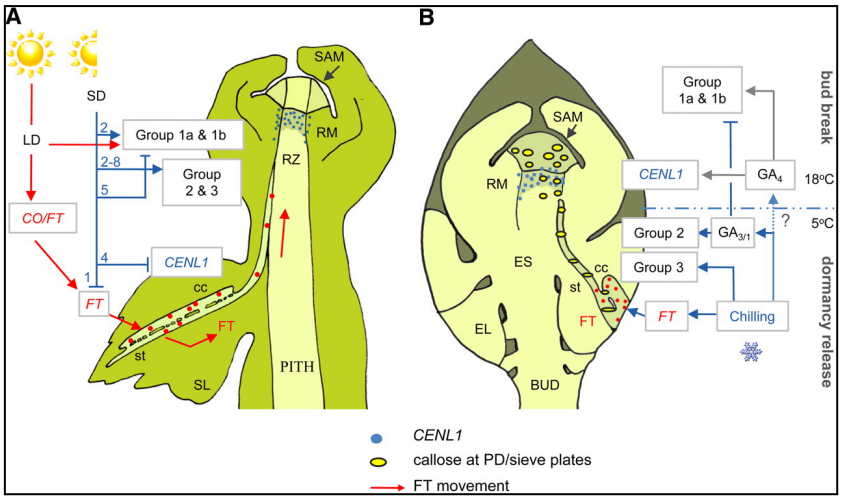
[Chilling of Dormant Buds Hyperinduces FLOWERING LOCUS T and Recruits GA-Inducible 1,3-β-Glucanases to Reopen Signal Conduits and Release Dormancy in Populus]
Authors

FT / Glucanase / GA / GA3ox / GA20ox / CO / CENL1 / DELLA / GID / GIP / Lipid bodies / GH17

Study system: *Populus tremula* × *tremuloides*

CHILLING: 5 degC
WARMING: 18 degC
DAYLENGTH: 10 hour SD / 18 hour LD
CHILL LENGTH OPTIMUM/MINIMUM: 6 weeks

- **The overall model**



- **Chilling on primordia**
    - Six weeks of chilling optimal for bud burst, elongation, and leaf growth
- **GA_3 and GA_4**
    - GA_4 induced canonical bud burst while GA_3 failed and instead led to callus-like growth at the leaf bases
    - GA_4 induced shoots had smaller and paler leaves than those that were chilled, suggesting only partial substitution
- **Transport in dormant buds**
    - Dormancy is enforced by blockages in the symplastic pathways in the phloem and shoot apex
    - Chilled plants had symplasm blockages released
    - GA_4 improved this transport capacity
    - GA_3 led to no change in transport capacity compared to dormant plants

- **GH17 genes**
    - GH17: 1,3-β-glucanases of the glucan hydrolase family 17
        - Glycosylphosphatidylinositol (GPI) anchor: a lipid anchor for cell-surface proteins that allows attachment of a protein to the lumen or extracellular surface
        - CBM43/X8 motif: a protein motif that enables callose binding
        - LB: lipid bodies
    - Callose is characterized by glucan bonds of this 1,3-β class
    - GH17 groups:
        - Group 1a: CBM43, sometimes GPI anchor, localized at the cell wall or plasmodesmata
        - Group 1b: GPI anchor, localized to plasmodesmata
        - Group 2: lipid-body associated proteins
        - Group 3: GPI anchor, localized at plasma membrane
    - GA induction
        - GA_4 upregulated expression of 1a and 1b GH17s
        - GA_3 upregulated expression of Group 2 GH17s
        - Group 3 was not significantly affected by either GA application
    - Chilling induction
        - Group 1a peaked at 0 weeks of chilling and decreased with chilling
        - Group 1b peaked at 2 weeks of chilling and decreased after
        - Group 2 peaked at 6 weeks of chilling, but declined right after, aligning with the finding that 6 weeks was optimal for inducing all 3 elements of dormancy release (budburst, elongation, leafing)
        - Group 3 peaked at 2 weeks of chilling
    - Short days induction
        - Groups 1a and 1b peaked early on during short day treatment but declined with increasing short day condition length
        - Group 2 peaked later in SD (5 weeks) except for 1 (GH17_101)
        - Group 3 increased with longer short day conditions
- **GA biosynthesis and signalling**
    - GA3ox1 and GA20ox8 are upregulated during chilling
    - GA biosynthesis gene families have non-redundant functions in this dormancy mechanism
    - GA2ox1, involved in GA deactivation, were upregulated near the end of chilling
    - GID1 (GIBBERELLIN INSENSITIVE DWARF 1), a nuclear GA receptor, was upregulated during short day conditions
    - GIP-like1 (GIBBERELLIN INDUCIBLE PROTEIN-like1) was downregulated during short days, but expression increased following warming if chilling was sufficient
    - DELLA-like1 was downregulated during short days
        - DELLAs are GA signalling repressors
        - Longer than 6 weeks of chilling led to the upregulation of DELLA-like1
- **Flowering locus T (FT), CONSTANS, and CENL1**
    - CONSTANS (CO): a protein that induces floral meristem differentiation and photoperiod sensing, involved in short-day and long-day detection mechanisms
    - CENTRORADIALIS-LIKE 1 (CENL1): an orthologue of Arabidopsis TFL1, a regulator or floral development both temporally and physiologically; involved in transition into dormancy in *Populus*

    - FT protein is expressed in leaf vasculature and localizes to the shoot apex to facilitate elongation
        - Short days cause FT downregulation
        - Chilling induces rapid FT upregulation
        - Budburst leads to rapid FT downregulation
    - Dormant buds have elevated CONSTANS (CO) expression after 2 weeks of chilling, after which it plateaus
        - CO expression further increased when plants are exposed to warm LD conditions after sufficient chilling (8 weeks here, not 6)
    - CENL1: a *P. trichocarpa* orthologue of AtTFL1, encoding a signalling peptide that is FT-like
        - Localized at the rib meristem (RM) underneath the shoot apical meristem (SAM)
        - Chilling alone did not induce CENL1 expression; needed a period of warmth after sufficient chilling
        - Similar expression pattern to long day operating genes like GA biosynthetic GA20ox and GA3ox, GA inactivity GA2ox1, CO, DELLA-like1, and GIP-like1
        - CENL1 expression peaks just before bud burst, which occurs first in the lower lateral buds which also burst first
            - CENL1 therefore positively correlates with the event of budburst itself
    - GA_4 also induced CENL1 expression before budburst
- **GA_4 and lipid bodies**
    - After 5 days of GA_4 feeding, lipid bodies no longer aligned with the plasma membrane and plasmodesmata
    - Lipid bodies congregate at the SAM and RM during dormancy
        - All disappear after GA_4 exposure
    - Coincided with bud swelling
    - Typically, GH17-decorated lipid bodies localize to the cell wall/plasmodesmata; these GH17-LB aggregates are formed during short day conditions and chilling causes them to move to their targets
    - Consequently, reopening of the plasmodesmata via GA_4 application is likely not due to lipid bodies

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Temperature-dependent growth contributes to long-term cold sensing
Authors

VIN3 / NTL8 / NTL14

Study system: *Arabidopsis thaliana*

CHILLING: 4 degC
WARMING: 20 degC
DAYLENGTH: 16/8 photoperiod for flowering
CHILL LENGTH OPTIMUM/MINIMUM: not mentioned, but VIN3 RNA increased linearly with cold duration (experiment stopped at 40 days ~6 weeks)

- **The overall model**
    - NTL8/14 → VIN3

    - VIN3
- **NTL8/14**
    - VERNALIZATION INSENSITIVE 3 (VIN3): PHD protein involved in the PRC2 complex to silence FLC expression epigenetically
    - NAC TRANSMEMBRANE MOTIF1-LIKE 8 (NTL8): a NAC TF involved in various biological processes like salt signalling, trichome development, etc.
    - VIN3 overexpression mutants (VIN3 expression even during warmth) displayed premature termination of translation of NTL8 and dwarfism and leaf deformation
        - NTL8 lacking in the C-terminus putatively led to their constitutive activation
    - In the LOF *ntl8* mutant, VIN3 expression was not affected
    - The closest genetic relative NTL14 can induce VIN3 expression and the NTL8 overexpressing mutant also displays VIN3 constitutive expression
        - The *ntl8 ntl14* double mutant displayed significantly reduced VIN3 expression, even in the cold
    - *ntl8* LOF also displayed reduced silencing of FLC
    - NTL8 and NTL14 therefore represent two redundant members of the NAC TF family that are involved in cold induction of VIN3 and floral development
    - NTL8 overexpressing mutants did not display uniform upregulation of cold-stress response genes, indicating that specificity of NTL8-induced VIN3 upregulation for vernalization
- **Slow degradation of NTL8**
    - NTL8 isoforms have relatively short half-lives (2 days)
    - In warm conditions with rapid growth; dilution of NTL8
    - In cold conditions of slow growth or dormancy; accumulation of NTL8
- **Mathematical model**
    - NTL8 is stable over many weeks during the cold
    - Visualization of localization of NTL8 with GFP revealed accumulation in reduced growth and vice versa
    - Warm-induced new growth had low GFP signal; GFP was only strongly observed in the root tip and cells that were no longer actively growing

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EARLY BUD-BREAK 1 and EARLY BUD-BREAK 3 control resumption of poplar growth after winter dormancy

Authors

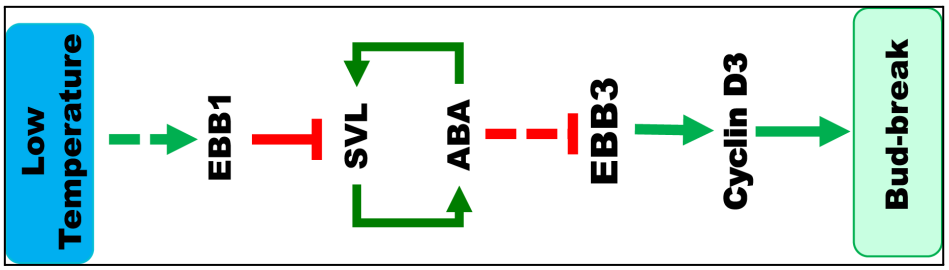
EBB1 / EBB3 / SVL / FT / CYCD3.1 / AP2/ERF / H3K27me3 / ABA / GA / GA2ox1 / GA3ox2

Study system: *Populus tremula* × *P. alba*

CHILLING: 4 degC
WARMING: 20 degC light, 18 degC dark
DAYLENGTH: 8 hour SD / 16 hour LD
CHILL LENGTH OPTIMUM/MINIMUM:

- Dormancy induction: short days for 10 weeks
- Chilling: 5 weeks

- **The overall model**
    - EBB1 –| SVL --| EBB3 → CYCLIND3.1 → bud cell proliferation
        - EBB1 binds to GCC-box on SVL and represses
        - SVL putatively downregulates EBB3 through ??? mechanism
        - EBB3 binds to GCC-box on CYCD3.1



- **The *ebb3D* mutant displays early bud break phenotype**
    - In this mutant, L34e-like and AP2/ERF-containing TF like PtERF113 are upregulated
        - L34e is a ribosomal protein
        - AP2 refers to APETALA2 and ERF is ethylene-response element (ERE) binding factor; the AP2/ERF domain is a common component in proteins that bind to the GCC-box
            - The GCC-box plays a role in ethylene and jasmonate response
- **EBB3 is PtERF113**
    - Overexpression → early budbreak; Suppression → delay
        - WT function is therefore to initiate budbreak
    - Strong expression in active apical meristem
        - Expression is downregulated during entry into dormancy
        - Highly upregulated during the winter and spring, suggesting positive signalling through low temperatures
        - Expression peaks just before budbreak in spring but downregulated shortly after
    - Trimethylations are sensitive to low temps in peach and pear
        - Particularly at H3K27 (H3K27me3)
        - Low temperature induced demethylation at H3K27
            - Temporal correlation between release from trimethylation and EBB3 upregulation indicates that methylation is responsible for suppression of its expression in warmth
- **EBB1 and SVL**
    - EBB1: AP2/ERF TF
    - SVL: SHORT VEGETATIVE PHASE-LIKE protein; a repressor of FLOWERING LOCUS T (FT)
        - SVL is upregulated during short day conditions and binds to FT2 promoter as a transcription repressor
            - FT2 induces shoot elongation during long days
                - FT2 downregulation induces growth cessation and bud set
                - FT2 also upregulates 13-hydroxylation pathway in GA biosynthesis and also regulates the expression of some GA biosynthesis genes like GA2ox1 and GA3ox2
        - SVL Decreases bioactive GA concentration
    - Consequently SVL accelerates growth cessation and bud set
    - EBB1 upregulated during low temperatures but downregulated during short days

        - After low temp, warmth and long days further upregulate EBB1 expression, i.e. budbreak conditions
        - SVL expression is the exact opposite of EBB1
    - EBB1 likely acts upstream of SVL as a negative regulator
    - SVL contains a GCC-box that the AP2/ERF domain in EBB1 can bind to
        - EBB1 directly binds to the SVL promoter at the GCC-box
- **EBB3 is downregulated in the SVL overexpression mutant**
    - Suggesting its placement downstream of SVL
    - EBB1 is therefore upstream of SVL and EBB3
- **Role of ABA**
    - ABA upregulates SVL and SVL reciprocally upregulates ABA biosynthesis genes
    - ABA downregulates EBB3 expression
        - EBB3 is not responsive to increasing GA; that is, the upregulation of EBB3 in the ABA biosynthetic LOF mutant is not through increased GA but specifically reduced ABA
- **CYCLIND3.1**
    - the only statistically significantly downregulated gene in EBB3 knockdown plants
    - CYCD3.1 is the key driver of progression past the G1/S checkpoint in mitosis and its expression correlates strongly with bud reactivation during budbreak
    - CYCD3.1 is upregulated in EBB3 overexpression mutants; CYCD3.1 is downstream of EBB3
    - CYCD3.1 bears a GCC-box with which EBB3 can bind to as a direct target

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Cold-induced Arabidopsis FRIGIDA nuclear condensates for FLC repression
Authors

FLC / FRIGIDA / COOLAIR / H3K27me3 / H3K36me3

Study system: *Arabidopsis thaliana*

CHILLING: 5 degC, 8/16 light/dark photoperiod
WARMING: 20 degC, 16/8 light/dark photoperiod
DAYLENGTH: 8 hour SD / 16 hour LD
CHILL LENGTH OPTIMUM/MINIMUM: 2 weeks (4 weeks was also used for supplementary figures, but 2 weeks just showed even further repression of FLC)

- **The overall model**
    - Cold → COOLAIR → FRIGIDA → FLC
    - Cold –| FRIGIDA activity → FLC
- **FRIGIDA**
    - A protein needed for flowering in Arabidopsis, associated with vernalization
    - Forms nuclear condensates under cold conditions
    - Nuclear condensates accumulate both in size and number, but are delocalized away from the FLC locus
    - Warmth leads to rapid dissociation of FRIGIDA nuclear condensates which then localize around FLC locus
    - FLC transcription is thus enabled under warm conditions, initiating the reproductive phase

- **COOLAIR**
    - Heterochromatin region at near the terminus of the FLC locus
    - Produces long non-coding RNA in the antisense form of the FLC open reading frame
    - Inducible by small RNA
    - Increasing trimethylation at H3K27 (H3K27me3) leads to further repression of FLC expression, opposite occurs when H3K36me3 is decreased (i.e. stronger trimethylation at H3K36 leads to higher FLC expression)
    - H3K36me3 is deposited during cold-induced, FRIGIDA-mediated transcription of FLC

The effect of climatic conditions on dormancy development of peach buds. I. Temperature
Erez, A. & Lavee, S.
Study system: *Prunus persica* cultivars 'Redhaven', 'Elberta', 'Early-Red-Fre'

CHILLING: 3-10 degC, in darkness
WARMING: 23 degC, 16/8 light/dark photoperiod
CHILL LENGTH OPTIMUM/MINIMUM: LOCAL optimum at 90 days according to Figure 2, don't know if it extends beyond that

Key findings:

- This paper explicitly showcases that different temperatures have different efficacies for bud burst.
- **Figure 2**: bud opening percentage depending on how long the plants were chilled for
    - The paper also states that bud burst increases steeply after a minimum of 67 days with more than 1600 hours of chilling time
- **Figure 3:** the effect of chilling temperature (constant duration) on the bud burst of lateral and terminal buds
    - "**Maximum rest-breaking efficiency**" occurred at a higher 8 degC for terminal buds compared to the 6 degC found for lateral buds
- **Table 1:** Measuring bud burst when plants were chilled at different temperatures
    - Bud opening relative to 6 degC showed that temperatures above and below 6 degC are not as optimal
    - The degree of bud opening relative to opening at 6 degC was 0.9 for 3 and 8 degC, and 0.5 for 10 degC
- **Table 2**: placing plants under intermittent chilling periods with long or daily cycles
    - Looked at the effect of continuous or intermittent chilling
    - When maximum temperatures did not exceed 18 degC, bud burst was highest
    - After 21 degC there is a "disappearance of the chilling effect"
        - That is, when plants are placed under an intermittent chilling regime, entering the 18-21 degC range is dangerous because it is warm enough to reverse the chilling required

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The effect of limitation in light during the rest period on leaf bud break of the peach (*Prunus persica*)
Erez, A., Lavee, S., Samish, R. M.
Study system: *Prunus persica* cultivars 'Redhaven', 'Elberta'

CHILLING: 6 degC, 8/16 light/dark photoperiod
WARMING: 23 degC, 16/8 light/dark photoperiod
CHILL LENGTH: 50 days of cold and 19 days of warmth

- For darkness, plants were covered in a nearly-opaque shade cloth

Key findings:

- **Table 2**: plants chilled and warmed in the dark and light with bud burst percentage measured
    - **Terminal buds:** 99.0% in the dark and 95.2% in the light, non-significant
    - **Lateral buds:** 57.5% in the dark and 42.4% in the light, significant
    - "Chilling in the dark considerably increased the leaf bud opening as compared to that in the light"
- **Table 3**: plants were placed outdoors over a three-year period and preconditioned with natural daylight or darkness, budburst measured
    - **P. persica 'Elberta':**
        - 1964 experiment
            - Terminal: 2% in daylight and 36.1% in darkness, significant
            - Lateral: 14% in daylight and 52.8% in darkness, significant
        - 1965 experiment
            - Terminal: 10.7% in daylight and 41.9% in darkness, significant
            - Lateral: 12% in daylight and 21.4% in darkness, significant
    - **P. persica 'Redhaven' (1966):**
        - Terminal: 65% in daylight and 87% in darkness, significant
        - Lateral: 6.3% in daylight and 19.3% in darkness, significant
- **Table 4:** varying degrees of light exposure on the effect of bud burst after chilling, measuring initial budburst on 10 April and later budburst on 16 April
    - Heavy shade, but not pure darkness, was best for initiating bud burst
        - **Terminal:**
            - Darkness: 78.5% / 97.7%
            - Heavy shade: 79.3% / 95.8%
            - 1 hour light daily: 65.8% / 94.3%
            - 4 hours light daily: 42.8% / 61.0%
            - Natural daylight: 0% / 46.0%
        - **Lateral:**
            - Darkness: 36.4% / 54.5%
            - Heavy shade: 45.3% / 65.7%
            - 1 hour light daily: 26.9% / 58.8%
            - 4 hours light daily: 31.5% / 56.4%
            - Natural daylight: 14.7% / 43.2%
- **Discussion:**
    - They briefly mention that photo-oxidation of auxin and the higher concentration of GA in etiolated plants might be responsible for why dark-preconditioned plants burst at higher percentages
        - Further things to look at are how light affects the biosynthesis and signalling function of auxin and GA?

Phenor package models

Ecodormancy only

- **Thermal Time (TT)**
  - Cannell & Smith, 1983
  - Chuine, Cour, & Rousseau, 1999
  - De Reaumur, 1735
  - Hänninen, 1990
  - Hunter & Lechowicz, 1992
  - Kramer, 1994
  - Leinonen, Repo, & Hänninen, 1997
  - Wang, 1960
- **Chilling Degree Day (CDD)**
  - (Jeong & Medvigy, 2014)
- **Photothermal-time (PTT)**
  - Črepinšek, Kajfež-Bogataj, & Bergant, 2006
  - Masle, Doussinault, Farquhar, & Sun, 1989
- **M1**
  - Blümel & Chmielewski, 2012

Both eco- and endodormancy

- **Alternating (AT)**
  - Cannell & Smith, 1983
  - Murray, Cannell, & Smith, 1989
- **Sequential (SQ)**
  - Hänninen, 1990
  - Kramer, 1994
- **Parallel (PA)**
  - Hänninen, 1990
  - Kramer, 1994
  - Landsberg, 1974
- **Unified (UN)**
  - Chuine, 2000
- **Growing Season Index (SGSI, AGSI)**
  - Xin et al., 2015
- **Grassland Pollen model (GRP)**
  - García-Mozo et al., 2009
- **Sequential M1 (SM1)**
- **Parallel M1 (PM1)**
- **Unified M1 (UM1)**

ChillR Package Models

- **Chilling Hours**
  - Weinberger JH (1950) Chilling requirements of peach varieties. Proc Am Soc Hortic Sci 56, 122128
    - **Couldn't access the paper**
  - Bennett JP (1949) Temperature and bud rest period. Calif Agric 3 (11), 9+12

        - **Study system: Hardy pear**
        - **Summary:** Cold temperatures of 37 F (2.8 degC) for between 56 to 81 days permitted full bud break, temperatures higher than 45 F and/or days shorter than 56 were unconducive and/or had a delaying effect
            - "The requirements are a temperature between freezing and about 45 F and a period of continuous exposure of two to three months."
            - "In the intermediate temperature range - 45" F to 60" F - the results of continuous exposure vary with the kind of plant, but in general, an incomplete ending of the rest occurs."
            - "A relatively short exposure to excessively high temperature-a few hours or days at 110" F to 130" F-will often start resting buds into growth"
            - "The picture shows that trees which.had been stored at 37" F continuously for 56 to 81 days opened most of their buds, as they do after a normal winter outdoors"
            - "With decreasing length of cold treatment below 56 days, an increasing proportion of buds remained inactive until at no exposure and at 10 days exposure only two injured buds grew feebly"
- **Utah Model**
    - Richardson EA, Seeley SD, Walker DR (1974) A model for estimating the completion of rest for Redhaven and Elberta peach trees. HortScience 9(4), 331-332
        - **Study system: *Prunus persica* 'Elberta'** and ***P. persica* 'Redhaven'**
        - **Summary:** temperatures between 37 and 48 F were most conducive to acquiring chill units where 1 chill unit = 1 hour of exposure to temperatures below 6 degC, like a quantification of Erez & Lavee's findings
        -

Table 1. Conversion of selected temperatures to Chill Units.

Temperature °C	Temperature °F	Chill units contributed
< 1.4	< 34	0
1.5 – 2.4	35 – 26	0.5
2.5 – 9.1	37 – 48	1
9.2 – 12.4	49 – 54	0.5
12.5 – 15.9	55 – 60	0
16 – 18	61 – 65	−0.5
> 18	> 65	−1

- **Dynamic Model**
    - Erez A, Fishman S, Linsley-Noakes GC, Allan P (1990) The dynamic model for rest completion in peach buds. Acta Hortic 276, 165-174
    - Fishman S, Erez A, Couvillon GA (1987a) The temperature dependence of dormancy breaking in plants- computer simulation of processes studied under controlled temperatures. J Theor Biol 126(3), 309-321
    - Fishman S, Erez A, Couvillon GA (1987b) The temperature dependence of dormancy breaking in plants- mathematical analysis of a two-step model involving a cooperative transition. J Theor Biol 124(4), 473-483

- **From Zhang & Taylor (2011)**
    - "The Dynamic Model (Fishman et al., 1987a, 1987b) was developed in the 1980s and defined a new concept for the negation process. Winter chill is assumed to accumulate in a two-step process. Cold temperatures initially result in the formation of an intermediate chilling product; high temperatures can destroy this product. Once a critical amount of this chilling product has accumulated, it converts to a chill portion, which cannot be destroyed. A certain chill portion accumulation indicates fulfillment of chilling requirement."
- Arose when it was observed that cyclic and intermittent exposures to cold and warm displayed different effects where longer cycles of cold and warm (that is, the exposure to cold lasted longer) displayed weaker warmth-induced negative of chill accumulation than plants that had frequent intervals of thermoperiodicity
    - This was built upon data from Erez *et al.* (1979)
- Zhang & Taylor (2011) also **summarize the other models that came before the Dynamic Model's conception;**
    - "Bennett (1949) quantified winter chill as the number of hours 0 to 7.2 C(32 to 45 F), whereas Weinberger (1950) suggested using the number of hours 7.2 C or less(45 F) during the winter season. Luedeling et al. (2009c) proposed that ''freezing temperatures did not contribute to winter chill accumulation'' and used 0 to 7.2 C and denoted it the Chilling Hour Model. However, it has been found that high temperatures have a negative chill contribution (Richardson et al., 1974). ErezandLavee(1971)reported that 10 was approximately half as efficient in breaking dormancy as 6C.The Utah Model, a weighted Chilling Hour Model with high temperatures having a negative effect on chilling accumulation, was developed in the 1970s (Richardson et al., 1974). This model has been adapted to adjust to varying climatic conditions. Norvell and Moore (1982) extended effective temperature ranges compared with the Utah Model. Shaltout and Unrath (1983) adjusted the relationship between chill units and temperatures by assigning greater chill contribution to lower temperatures and more negative effect to temperatures greater than 21 C(NorthCarolina Model). Disregarding chill units accumulated on days when there is a negative total has been found to be more suitable in marginal areas in South Africa (Allan et al., 1995). This model is called the Positive Utah Model (Linsley-Noakes et al., 1995)"
- **Growing Degree Hours**
    - Anderson JL, Richardson EA, Kesner CD (1986) Validation of chill unit and flower bud phenology models for 'Montmorency' sour cherry. Acta Hortic 184, 71-78
        - **Study System: *Prunus cerasus* 'Montmorency'**
        - **Summary:** Built upon its predecessors, using the 6 degC optimum as part of its model
            - "One growing degree hour was defined as 1 hour at a temperature 1 C above the base temperature. All temperatures above 25 °C were assumed equal to 25°C; thus the greatest accumulation for any 1 hour was 20.5 GDHs"
            - "Temperature data was entered into our computer system. Chill unit accumulation was determined by our chill unit model.

When 954 chill units were accumulated, rest was considered to be completed and GDH accumulation was begun as defined by our ASYMCUR fruit-tree model."
- "The ASYMCUR model consists of two cosine equations. Equation 1 (below) determines GDH accumulation at temperatures between the base and optimum temperatures. GDH = FA/2 (1 + cos(n+n(TH-TB)/(TU-TB)))"

Main questions:

- How to apply a model for trees across the world? These models were predominantly built on information from temperate orchards, with a lot of them taking that 6 degC finding from Erez & Lavee (Utah, Dynamic, and to an extent even the GDH model)
    - The paper on geophytes already tells us that different geophytes have different thermal optima for dormancy induction and dormancy in general, presumably due to ecological niches, and so for trees it is likely the same
- These models were done on observational data, but I can't think of way in which the Dynamic model can be explained given the premise of an intermediate chill unit or molecule that can degrade in warmth or be built up in cold, only to remain permanent after a sufficient cold duration is met
    - Can callose be this molecule? We know from the papers that callose is deposited during dormancy entry and that callose synthase expression declines as the cold temperatures progress until, by budbreak, callose is no longer actively deposited
        - If the dynamic model were to be supported AND our molecule was callose, we would need to find evidence that callose biosynthesis and/or signalling are impeded by oscillations in temperature and that a set amount of cold exposure is needed to 'concretize' callose deposition for dormancy entry
- Why don't temperatures below 0 degC do anything? This finding was observed by Bennett (1949) in the paper about the Chilling Hours model and then rehashed by Luedeling et al. (2009) in their summary of the chilling models
    - All the papers I've read about genetic pathways for chilling also use temperatures above 0 degC
    - Still can't find anything on thermal optimal for callose synthase…is this field just too new??
- Where to go forward?

Phenor package models

Alternating model

- **Cannell & Smith (1983)**
- study system: _Picea sitchensis_
- "presents an empirical model for the timing of budburst...based solely on thermal time and winter chilling."
- Predictive model on 2 to 10 year old trees for most of the provenances grown in Britain
- thermal time: T_x = sum(t_m - x) where T_x = day degrees with a base temperature of x degC and t_m = mean daily temperature on those days, i.e. basically GDD
- Chill days = n days between 1 Nov to budburst date where t_m <= x
- observed relationship was that increased chilling during april-may decreased the thermal time required for budburst

- least squares method for finding the optimal balance between thermal time and chill day/unit requirements led to decreasing exponential models fitting the best
- as such the model is thermal time = 67.4 + 4401.8e(-0.042 x chill days)

Sequential model
- **Kramer (1994)**
- study system: _Fagus sylvatica_
- leaf unfolding data collected over the course of 57 years in the Netherlands was used as the data to fit 6 pre-existing models, which were developed to only require temperature as an input
- This species responds to photoperiod cues, however, and so this was incorporated into the models that were tested by inserting an additive argument (just appended to R_chl, the rate of chilling)
- The model that apparently fit the data best was the Sequential model posited by Sarvas (1974) which "considered rest and quiescence as two strictly separate phases." with no gradation between these two stages unless the chilling threshold is met, and "no transition from quiescence to the active phase" unless the forcing threshold is met
- "This model was called the sequential model, because the state of chilling and the state of forcing increase sequentially in time (Model I of Hanninen 1990)."

However, Hanninen (1990) argues that not even this sequential model posited by Sarvas (1974) is sufficient;
- **Hanninen (1990)**
- Study system: _Pinus sylvestris_ and _Picea abies_
- From section 5, Synthesis model; based on previous models and experimental results
- C(t) = ((1 - C_min - deltaC(t))/(CU_crit - deltaCU(t) - CU_abs))*(S_chl(t)-CU_abs) + C_min + deltaC(t)
- The premise of this model is that it takes the data from many studies and also compares the accuracy of prior models and finds the model type that has the most empirical support
- they argue that "with increased chilling, the rate of ontogenetic development becomes less dependent on the forcing temperature."
- This paper argues that the prior models of all types from I to IV (outlined in Table 2 of this paper) are too extreme and/or insufficiently integrate environment cues in their predictions of effects of chilling

Parallel model
- **Landsberg (1974)** + Hanninen and Kramer
- study system: _Malus domestica_
- posits that apple bud development can be split into 3 phases, which are morphogenesis and dormancy induction, true dormancy, and burst to bloom
- from Kramer (1994): "even when the critical state of chilling has not yet been attained, response to forcing temperature must be possible."
- "This model was called the parallel model, because the state of chilling and the state of forcing increase together in time (Model II of Hanninen 1990)."
- this paper explicitly talks about endogenous factors that promote and inhibit growth, like GA, cytokinin, etc and cite a bunch of papers where these hormones are discussed
- Here they mention that base temperature is 5 degC because apparently below that threshold, "lower temperatures are not advantageous to the physiological processed leading to the condition in which growth can proceed at the maximum rate allowed by prevailing

conditions."; no citation for this though because "the 5 degC limit is arbitrarily chosen as a convenient value at about the level below which growth is usually assumed to cease"
- they do later mention that 5 degC might not be correct, but they still chose to follow through with it regardless
- the model is laid out as G = A/(I + be^(-k(I),P)) where G is the number of growth units
- also note that Hanninen and Kramer both critique this model in their respective papers