Development of Tree Carbohydrate Budget Based Methods for Sustainable Management of Almonds under Changing Central Valley Climatic Conditions

Project No.: **17-PREC8-Zwieniecki**

Project Leader: Maciej Zwieniecki
Department of Plant Sciences
UC Davis
One Shields Ave.
Davis, CS 95616
(530) 752-9880
mzwienie@ucdavis.edu

Project Cooperators and Personnel:
Aude Tixier, UC Davis
Anna Davidson, UC Davis

Objectives:

- Using existing and novel tools to determine the seasonal pattern of carbohydrate management by the almond trees in relation to phenology
- Establish guiding principles on diurnal dynamics of carbohydrate content in almond.
- Establish experimental tools for studying dormant shoot physiology under variable environmental conditions in the lab and in the field including detailed analysis of starch and soluble sugar concentration dynamics in relation to gene expression patterns.
- Determine cumulative impact of environmental stresses on almond trees carbohydrate management.

Interpretive Summary:

Vegetative life of any plant can be described as a continuous struggle to acquire, transfer, and store energy that is necessary to grow, reproduce, and protect from environmental abiotic and biotic stresses. Carbohydrates (sugars) are responsible for the majority of long-distance energy transfers and long-term storage of energy in plants [1-3]. They are the ultimate currency that the plant produces during summer to 'pay' for all its physiological activity even during dormancy and this currency is preserved to allow for a healthy spring bloom. Thus, the understanding of carbohydrate management of plants responses to environmental stresses while accomplishing their reproductive functions (yield) is of key importance to yield predictions, determination of plant stress level, their ability to mediate salinity, drought, or winter survival [4]. Understanding of carbohydrate management is especially important in long-lived perennial crops like almond that must balance between diurnal, seasonal, and multi-year benefits that include tree survival and capacity to reproduce (nuts). Interestingly, species-specific sugar management is a trait that evolved that allows a species to thrive within its ecological niche. Almond evolved to survive in a Mediterranean climate therefore any slow changes in climatic conditions of Central Valley that significantly diverted from the average might result in loss of yield or challenge tree survivals.

Recently California's Central Valley experienced the most severe drought in more than 1200 years. Simultaneously the drought's effects are magnified by the slow climatic shift that is reducing the valley's fog cover [5]. The net result is an increased incidence of extreme thermal condition during winter including higher average temperatures, increased potential of frost nights followed by hot sunny days, and reduced winter precipitation. These factors combined with the increasing use of saline ground water supplies necessitated by decreasing surface water supplies have produced an unprecedented set of new abiotic stresses that affect horticultural production.

Orchard performance is dependent on a complex set of interactions involving the plant genotype, the physiological and developmental processes that occur within the crop plant, and the interaction of these processes within the plant's environment. Successful mediation of abiotic stresses by horticultural manipulation of the plant by the crop manager depends on precise knowledge of plant's physiological responses to applied treatments. Reported research focus is on the development of new approaches to measure trees' physiological status via temporal analysis of carbohydrate levels in trees that complement the currently used methods (water status and nutrient analysis).

We present a holistic approach to link basic research to orchard management by studying carbohydrate tree biology across temporal (hours to seasons), spatial scales (tissue to geographical regions), and biological (gene expression to phenology) scales.

To accomplish these ambitious tasks, we undertook a series of approaches:

- We have developed the Carbohydrate Observatory – a new analytical high throughput platform for collecting and analyzing wood and bark tissue from twigs for soluble carbohydrates and starch (non-structural carbohydrates – NSC), using samples supplied by individual growers from across Central Valley, CA.
- We analyzed diurnal and seasonal variation of NSC in mature almond trees including all tree parts (twigs, branches limbs, stem and root system).
- We conducted a chill exclusion study that included exclusion of chill from buds, stems or both to determine the impact of chill on flowering, the origin of signal for bud break, the role of the temperature on NSC management, and tree phenology in relation to gene expression.
- We designed and implemented research on the role of leaf surface hydration in recovery of almond trees from severe drought using artificial rain chambers.

The major accomplishments include:

1. Use of the Carbohydrate Observatory data to determine healthy levels of NSC during different parts of the season in relation to phenology and growth **(Project 1).**
2. Use of the Carbohydrate Observatory and tree carbohydrate analysis to develop a mechanistic model for dormancy chill accumulation **(Project 2).**
3. Use of seasonal analysis of NSC content in all tree's parts to show links between NSC content, growth and phenology **(Project 3).**
4. Use of diurnal NSC reserves analysis to show for the first time, for any tree species the highly dynamic aspect of NSC reserves formation and redistribution across tree parts and that xylem sap is a significant part of the NSC vertical bidirectional redistribution across the tree crown **(Project 4).**

5. Use of chill removal experiment to determine shifts in phenology, flower health, flowering synchrony and gene expression involved in signaling path-triggering bloom **(Project 5).**

We aim to use outcomes of our research to develop a set of recommendations, practices, and tools that utilize information on NSC in day-to-day orchard management. Specifically, we aim to:

- Develop a forecasting model that uses fall NSC content to predict orchard yield potential using location, variety, and orchard age.
- Develop a mechanistic model for analysis of chill and heat accumulation during dormancy for forecasting bloom time and intensity using NSC content as measurable proxy for gauging dormancy progression. First generation model is being tested.
- Develop postharvest NSC analysis schedule to guide orchard management that allows the orchard to accumulate adequate amounts of NSC consistent with geography, variety and orchard age.
- Develop an RNA test that can help to determine bloom readiness and forecasting.
- In collaboration with other researchers, develop irrigation practices consistent with NSC tree management especially for post-harvest and dormancy period.

Project 1. *Use of the Carbohydrate Observatory data to determine healthy levels of NSC during different parts of the season in relation to phenology and growth*

Materials and Methods:

The Carbohydrate Observatory is based on a "citizen science" research model for which, participants collect samples and send them to us for analysis. In the case of the Carbohydrate Observatory, farmers collect samples of one-year old branches (three branches per orchard) and ship them to UC Davis Z-Lab where samples are analyzed for starch, soluble sugars and total non-structural carbohydrates (in best scenario every 2-4 weeks from each location).

Our method for sugar analysis was previously described in Tixier et.al. (2017)[(6)], with cost reducing methods described in Earles et. al (2018)[(7)]. Briefly, we measured soluble carbohydrates in woody stem tissue following removal of the bark and presumably phloem using a fresh razor blade. 1 ml of deionized water was added to 50mg of dried tissue, vortexed, heated to 72°C for 15 min, and spun at 21 000 g for 10min. A 50 µl aliquot of the supernatant was diluted (925) and mixed with 150 µl of sulfuric acid (98%) and anthrone (0.1%, w/v) solution in a 96-well microplate. The precipitated pellet was reserved for later starch analysis. The plate was cooled on ice at c. 4°C for 10 min, then heated to 100°C for 20 min, and finally left to adjust to room temperature for 20 min (22°C). We determined the sugar concentrations as glucose equivalents from the colorimetric reading (Thermo Scientific Multiskan, Waltham, MA, USA) of absorbance at 620 nm (A620) using a predetermined standard curve (0, 0.01, 0.03, 0.1 and 0.3 mg l^{-1} glucose), and multiplied the result by a measured average wood density of 0.63 g cm^{-3}. After extracting the soluble carbohydrates as described earlier, the remaining pellet was processed further to determine tissue concentrations of starch. After two washes with 80% ethanol, the pellet was exposed to 100°C for 10 min and submitted to enzymatic digestion for 4 h in an acetate buffer (pH = 5.5) with 0.7 U of amylase and 7 U of amyloglucosidase at 37°C. Once the digestion was finished, samples

were centrifuged for 5 min at 21 000 g, and the supernatant was diluted 1:20 and quantified using the method described earlier.

Results and Discussion:

Data are included in our database and made available to the growers and the public in real time via a map-based interface on the Z-lab website: http://www.plantsciences.ucdavis.edu/plantsciences_faculty/zwieniecki/CR/cr.html

The annual pattern of carbohydrate reserves in almond trees is presented on **Figure 1.** Accumulation of NSC reserves in twigs starts around July and continues until the end of October (trees enter 'dormancy'). Interestingly, there is a continuous loss of reserves over the winter until bud break. The average level of NSC reserves for almond is ~225 mg/g DW (prior to dormancy), while the summer level is at 90 mg/g DW.

We predict that significant deviation from observed levels of winter maximum reserves may negatively affect the yield potential especially if adequate reserves are not acquired. Furthermore, we also predict that deviation from summer levels of NSC may reflect on the trees' health as an increase of reserves would suggest reduction in growth (stress) or problems with export (phloem disease) while excessive loss of reserves from twigs might reflect severe stress or reduction in photosynthesis.

Multiyear observations with an increased number of sites and different types of management would provide more robust assentation to our predictions.

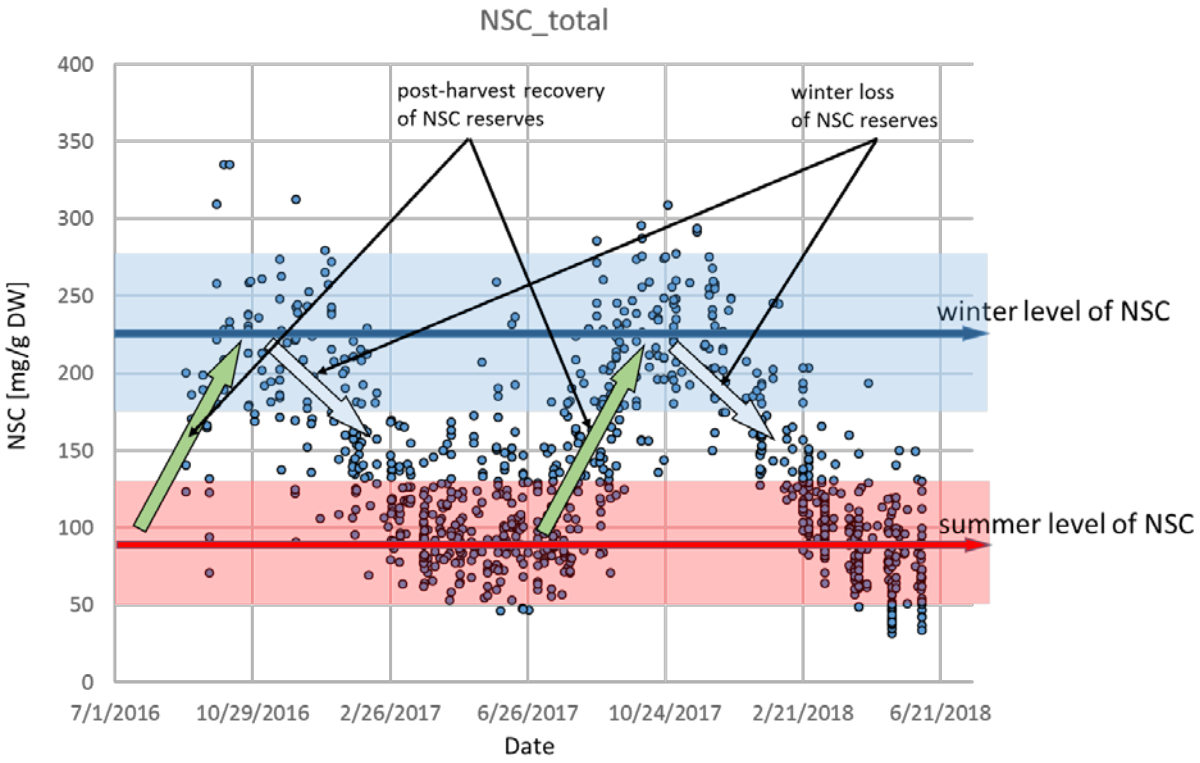

Figure 1. 2016-2018 dynamics of carbohydrate reserves in Almond twigs.

An initial analysis of NSC content at a geographical scale shows a significant difference between southern and northern orchards (**Figure 2**). Southern orchards accumulated higher levels of carbohydrates prior to dormancy (October-November) then northern located orchards. They lost a similar amount of NSC over winter (see parallel lines Oct-Nov and Dec-Jan). However, post bloom level (Feb-Mar) represents no south-north trend, thus suggesting that southern orchards used higher amount of NSC to bloom and initiate vegetative growth. Interestingly this high level of NSC in the winter correlates well with higher yields observed for southern located orchards (**Figure 2 – right**).

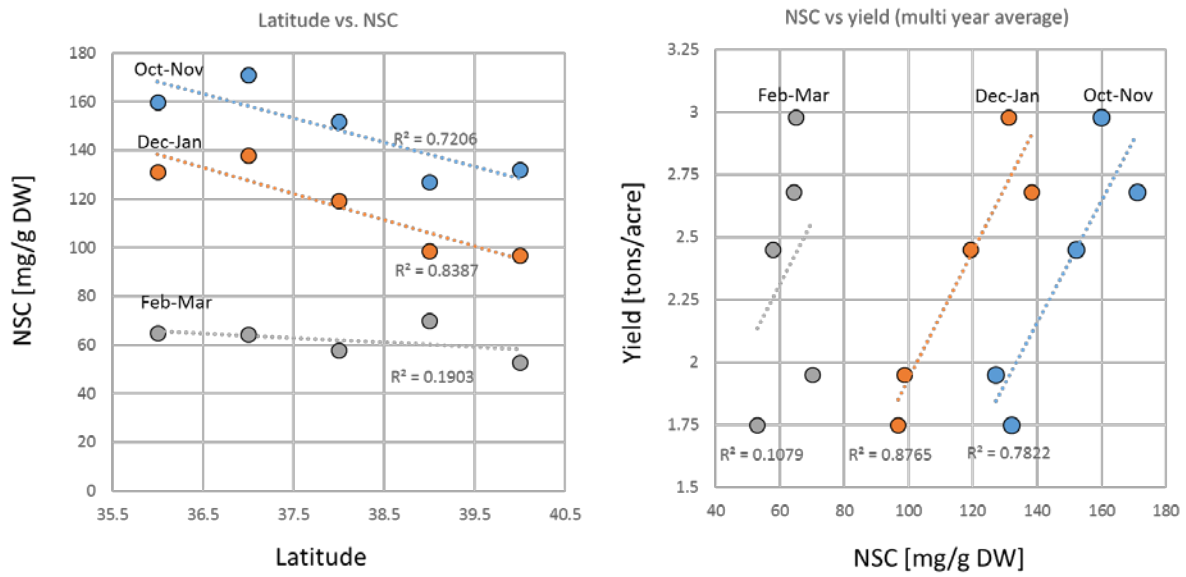

Figure 2. (left) NSC levels in twigs of almond trees along a latitudinal gradient. (right) Yield for a given latitude vs NSC recorded for that latitude.

Horticultural perspective
Restitution of NSC levels prior to winter is crucial for the winter survival and heathy bloom. NSC reserves are reconstituted during early fall especially during the post-harvest period. Winter concentration of non-structural carbohydrates in almond trees is decreeing along an increasing latitude (south to north). Regional average concentration of sugars in xylem of twigs in December-January period is highly correlated with an average regional yield.

Project 2. *Use of the Carbohydrate Observatory and tree carbohydrate analysis to develop a mechanistic model for dormancy chill accumulation*

Material and Methods:

Wood sampling
During the years 2016 and 2017 we received dry twigs (1st year of growth) by mail from Californian almond farmers through the 'Carbohydrate Observatory' project (a 'Citizen Scientist' initiative) and analyzed their NSC contents. We complimented the farmers' work by intensively sampling almond, pistachio, and peach trees at research sites in California (UC Davis) and Israel (Gilat, ARO). Sugar analysis was performed using methods described in Result 1 section.

Meteorological and phenology data
We used hourly air temperature data from the Californian Irrigation Management Information System (CIMIS, www.cimis.water.ca.gov) for four almond farming sites in California – Davis, Shafter, Durham, and Manteca. We included data for the years 1982-2017 (if available), excluded outliers, and ran statistical computations in an R GNU environment (R Studio Version 1.0.136). We included Julian days above 274 (October 1st, start of physiological senescence)

in the year and referred to winters, and days past senescence (DpS), for all later computations and discussions. We computed the chill portions (CP) status in each year for each site by hourly temperature data for 200 days of winter (October through April) according to the Dynamic Chill Model [DCM, (Erez et al., 1990)]. We also computed the growing degree hours (GDH) by the asymmetric curvilinear model (Anderson et al., 1986). We tracked almond phenology at UC Davis during 2016-2018, combined with temperature records, to asses and parameterize the winter models. Finally, we used a 26-year long phenology data set (1982-2008) concerning the annual time of 10% bloom in three key almond cultivation sites in California: Durham, Manteca, and Shafter to calibrate and test our novel approach of projecting almond bloom by temperature changes.

Model

The proposed model aims at a mechanistic understanding of chill and heat hours that impact dormant trees. We use a variant of a closed-loop control system that aims to maintain carbohydrate homeostasis in dormant plants. The biological assumptions for the model are:

- In temperate climates, stem parenchyma cells in trees remain biologically active during winter dormancy under permissive temperatures and parenchyma cells aim to maintain homeostasis of soluble carbohydrate concertation (SC_{hom})
- Homeostasis results from simultaneous transformation of SC to starch, and starch to SC, through parallel starch synthesis (s) and starch degradation (d) pathways and loss due to respiration (R).
- Synthesis and degradation pathways have unique and different temperature coefficients such as $Q_{10\text{-}s} > Q_{10\text{-}d}$ resulting in the existence of equilibrium temperature (T_{eq}) that amount of starch synthesis is equal to that of starch degradation.
- Continuous variation in diurnal temperature results in perturbation to SC_{hom} such that high temperature ($T > T_{eq}$) force SC transformation to starch and results in a drop of the SC while low temperature ($T < T_{eq}$) results in accumulation of SC.
- Capacity of synthesis and degradation pathway (A_{sld}) depends on the amount of available proteins that are controlled by de-novo expression of involved proteins and natural decay processes. Specifically, A_s capacity increases when $SC > SC_{hom}$ while A_d is allowed to decay and A_d capacity increases when $SC < SC_{hom}$ while A_s is allowed to decay. We assume no substrate limitations.

We have formulated the model into iterative computer program schematics of the model and the formulations are presented in **Figures 3 and 4**.

Results and Discussion

The presented model is a first attempt to explain how chill requirements work from a physiological perspective. This proposed mechanistic model has several unique features including the fact that all parameters are based on true physical quantities and that there are no artificially formulated temperature thresholds. In addition, all hours of the winter are accounted for either as heat or chill.

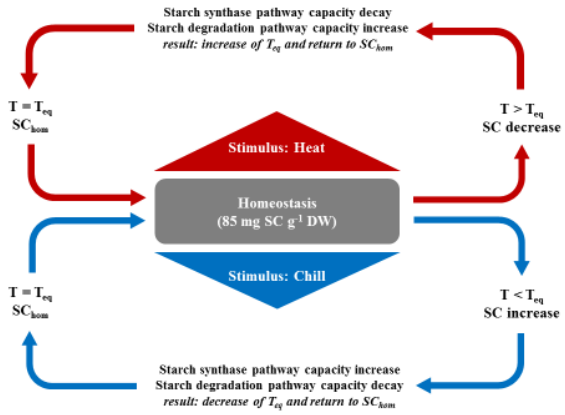

Figure 3. Schematic representation of the model

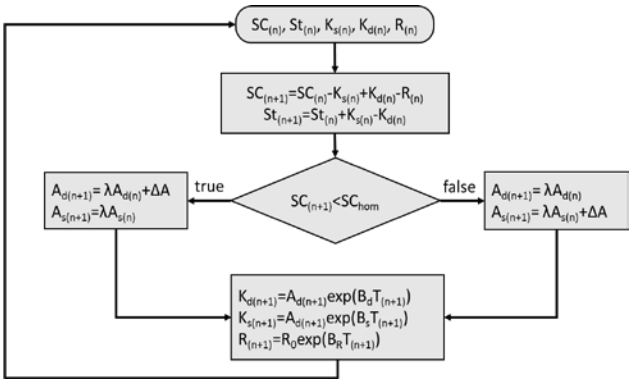

Figure 4. Formulation of feedback iterative model

Model predictions in relation to NSC content suggests that during spring warmup prior to bud break, we can expect a sudden excessive loss of soluble carbohydrates in wood tissue and concurrent increases in starch content. Interestingly, an increase of starch prior to bloom in twigs of almond, peach, pistachio in California and Israel was observed (**Figure 5 A**) at the level of single orchards. Additional support for this model comes from an analysis of average starch levels in twigs of almond in the Central Valley, Ca (data from carbohydrate observatory; **Figure 5 B**) where a significant increase of starch was observed prior to bloom. The model was tested to predict bloom date against the phenological data on almond bloom time for three locations: Durham, Manteca and Shafter (CA). Despite the low quality of historical data, our new model shows a very good statistically significant fit (**Figure 5 C**), suggesting that its further development might provide a bloom forecasting tool for almond growers that include an analysis of starch and winter temperature.

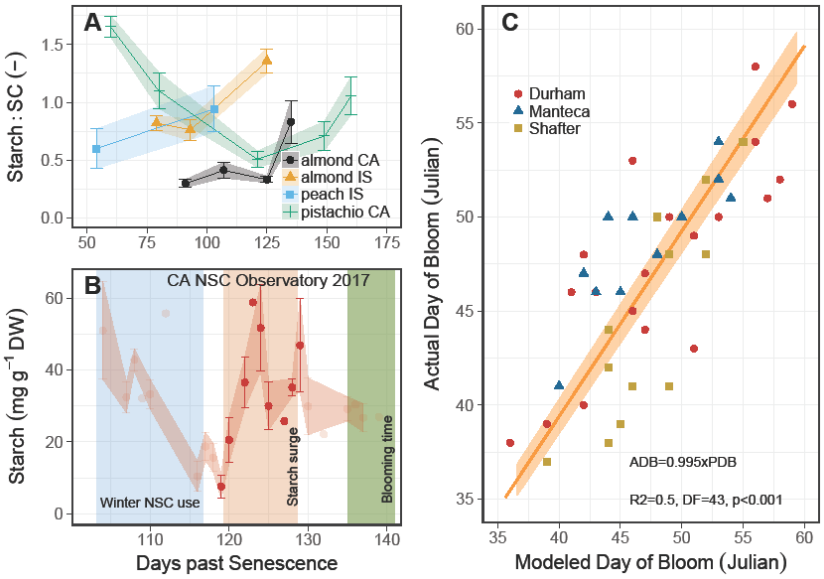

Figure 5. (A) Measured starch content in twigs of almond, pistachio and peach in Israel and CA. (B) Temporal starch content in CA orchards of almonds in 2017. (C) Correlation between modeled (predicted) bloom time of almond vs. real bloom time for three CA locations.

Horticultural perspective
This is the first fully mechanistic model that after multi-year tuning, will be capable of non-stochastic determination of winter chill progression in almond orchards and forecasting the bloom.
If the model will be accompanied by real time measurements of starch content in trees, its forecasting precision will be greatly improved.
Mechanistic bases of the model allow for forecasting precision even under highly variable conditions.
The ability to use measurable simple components of winter progression in the form of NSC and starch synthase/degradation pathways capacity can be used in the future to guide breeding programs aiming at specific winter chill requirements.

Project 3. *Use of seasonal analysis of NSC content in all tree's parts to show links between NSC content, growth and phenology*

Materials and Methods:

The understanding of non-structural carbohydrate (NSCs) allocation trade-off between storage, growth and reproduction is considered one of the key pieces of information to predict yield and tree productivity. Spatial and temporal variations of NSC storage in trees were measured and compared with radial growth and tree phenology.

Spatial and temporal variations of NSCs concentration in five, seven-year-old, well-watered, healthy *P. dulcis* trees grown in an orchard at the University of California, Davis in Central California (38.32318,- 121.47474**)**. Over a period of 16 months, we collected tissue samples every two weeks during the winter season and every month during the growing season to analyze seasonal NSCs dynamics. Extension shoot, spur-bearing branches, cores from the trunk and root samples were collected and phenology was assessed over the course of the sampling period. The L-almond model was used to estimate the above ground almond tree total biomass and then NSCs total storage. Radial growth was measured using precision dendrometers from Phytech company. NSCs storage, phenology and growth were compared to evaluate the carbohydrate allocation trade-off during the growing season.

Results and Discussion:

Aboveground tree total NSC reaches a maximum of 4 kg at the end of the growing season (**Figure 6**). Half of the total NSC are stored in the trunk of the tree. This storage is used during winter dormancy for maintenance metabolism and for the development of new organs (flower and leaves) during spring. Thus, the maintenance of photosynthetic healthy trees during the postharvest part of the growing season is crucial for the development of flowers and later nuts the following year. After bud-break, the total NSC storage reaches a minimum of 1 kg in April. This low level of NSC storage is maintained during the beginning of the growing season and it coincides with a phase of active growth (**Figure 7**). Once the radial growth slows in June, a massive accumulation of carbohydrate is observed. Similar trends are observed in response to drought stress when growth stops before photosynthesis, leading to the temporary accumulation of carbohydrate in stems. These results suggest the presence of a temporal trade-off between growth and the accumulation of carbohydrate reserves in almond trees. This

exciting result provides interesting perspectives for orchard management. Indeed, future experiments aiming at modifying this trade-off could offer the possibility to shorten the growth phase of the growing season in large full-grown orchards and promote the accumulation of storage carbohydrate to ensure higher yield the following year. Furthermore, this research project suggests that the fruit and stem growth represents a significant proportion of the diurnal carbon budget (i.e. reserves would not sustain growth) of the almond tree and that NSC storage is strongly influenced by the growth budget. Another interesting outcome of this research project is that the low level of NSC during the early phase of the growing season implies a higher susceptibility to biotic and abiotic stress. Indeed, the stored NSC buffers asynchrony between demand and supply of carbon typically during periods of stress. This higher susceptibility coincides with nut fill, and thus implies that extra caution is necessary during that phenological phase of the growing season.

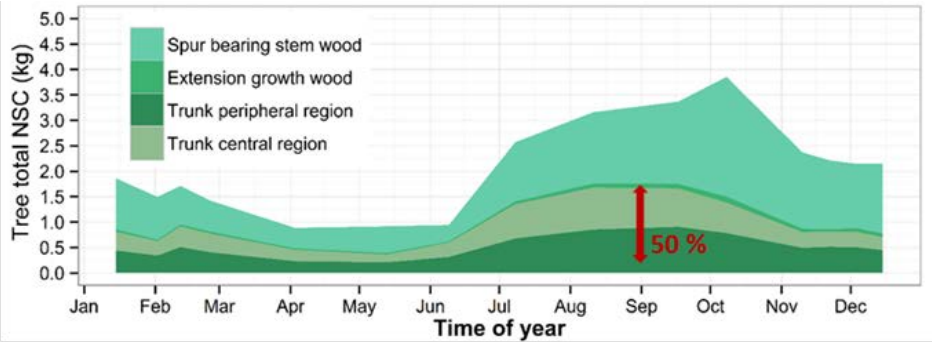

Figure 6. Seasonal variations of above ground almond total NSC storage. Tree total NSC reaches a maximum of 4 kg at the end of the growing season. This storage is later used during winter dormancy (winter respiration) and bud break. The plant tends to operate at minimum reserves of 1 kg NSCs from April to July. Data presented here were obtained from five 7-year-old almond trees grown at the UC Davis orchard. Biomass was estimated with the L-almond model and a total of 1400 samples were measured.

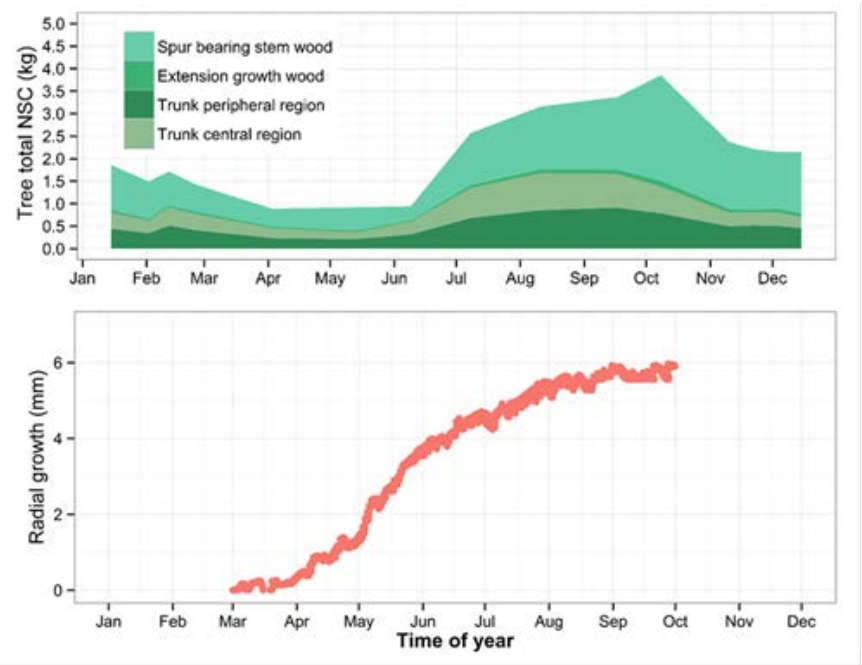

Figure 7. Comparison of seasonal patterns of carbohydrate accumulation and radial growth in almond trees. During the growing season, the cessation of growth coincides with the acceleration of carbohydrate accumulation

and highlights a temporal trade-off between growth and NSCs accumulation.

Horticultural perspective
Analysis of seasonal trends in NSC content at the tree level in the context of phenology, physiology and growth provides an interesting perspective for orchard management and future experiments aiming at modifying existing NSS-stress and NSC-growth trade-offs to possibly shorten the growth phase in large full-grown orchards and promote the accumulation of stored carbohydrate to ensure higher yield the following year.

Project 4 - *Use of diurnal NSC reserve analysis to show for the first time, for any tree species, the highly dynamic aspect of NSC reserve formation and redistribution across tree parts and that xylem sap is a significant part of the NSC vertical bidirectional redistribution across the tree crown*

Materials and Methods:

Sample collection was done on five, 7-year-old, well-watered, healthy *P. dulcis* trees. Over a period of 48 hours we collected tissue samples every four hours totaling 12 collection times (photosynthetic mature leaves, wood and bark (phloem and cambium) from current year extension shoots, two-year-old shoots, 5-year-old limbs, the tree trunk, structural roots and small active roots). Following collection, samples were dried in the oven at 75ºC for 48 hours and then analyzed for soluble sugars and starch content. We also measured stem water potential (pressure bomb) and collected xylem sap for sugar content analysis. To track the redistribution of newly assimilated carbon, we performed a pulse labelling of ^{13}C enriched with $^{13}CO_2$. This experiment was conducted in the same orchard as described above. Three large, 4 m-long branches were placed in translucent plastic bags and pulses of ^{13}C enriched $^{13}CO_2$ were injected into the bag. Bags were placed on the branch at 14:00 for ~2 hours and then the bags were removed. Sampling for carbon analysis was done every 6 hours at 13:00 prior to exposure of the branch to enriched $^{13}CO_2$, then 17:00, 23:00, 5:00 and then again 13:00. Sunset occurred at approximately 21:00 and sunrise 6:00. Simultaneously, samples were collected from adjacent limbs of similar size located across the crown. Sampling entities included mature leaves, wood and bark of the extension shoot, two-year-old branches, limb, trunk and small roots. Samples were immediately placed in the oven and dried at 80ºC for 48 hours. Multiple leaves and stem segments from each sampling localization were the powdered using a ball grinder and similar weight from each subsample was mixed to increase the homogeneity of the samples prepared for the ^{13}C analysis. Analysis was performed at the Stable Isotope Facility at UC Davis.

Results and Discussion:

Leaf soluble sugar concentration remained relatively constant over the course of a day (**Figure 8**) with no significant changes in the range of 60 to 80 mg/g of leaf dry weight (DW). Leaf starch concentration changed significantly over the 24-hour period. Starch was accumulating in the morning hours reaching a maximum of ~ 7.5 mg/g of leaf DW around midday and remained constant during the afternoon. Overnight starch content in leaves dropped to near zero concentration and was recovered at 13:00 the next day (**Figure 8**). Bark of extension shoots showed limited diurnal variation in soluble sugar concentration and starch content. However,

soluble sugar content in wood was variable with a daytime level ~ 45 mg/g DW, and night SCs content gradually increasing to nearly ~ 80 mg/g DW. Starch concentration was also variable in a similar pattern to SCs. A minimum starch level was observed during the daytime (~40 mg/g DW) and a maximum was observed prior to sunrise (~ 68 mg/ g DW). While it is known that there are large seasonal changes in NSCs content, it is also being shown that starch stored in xylem parenchyma may reside in wood tissue over 50 years and never be used. This permanency of stem storage underlines the notion that this NSCs pool is relatively stable and does not undergo any short-term changes. We provide evidence that NSC storage is highly dynamic at the diurnal scale with remobilization patterns in the almond tree architecture. Parsimonious and efficient redistribution of NSC store is essential for the establishment of well-distributed nuts in the canopy of almond trees. The understanding of this redistribution process may be a useful decision tool for pruning practices.

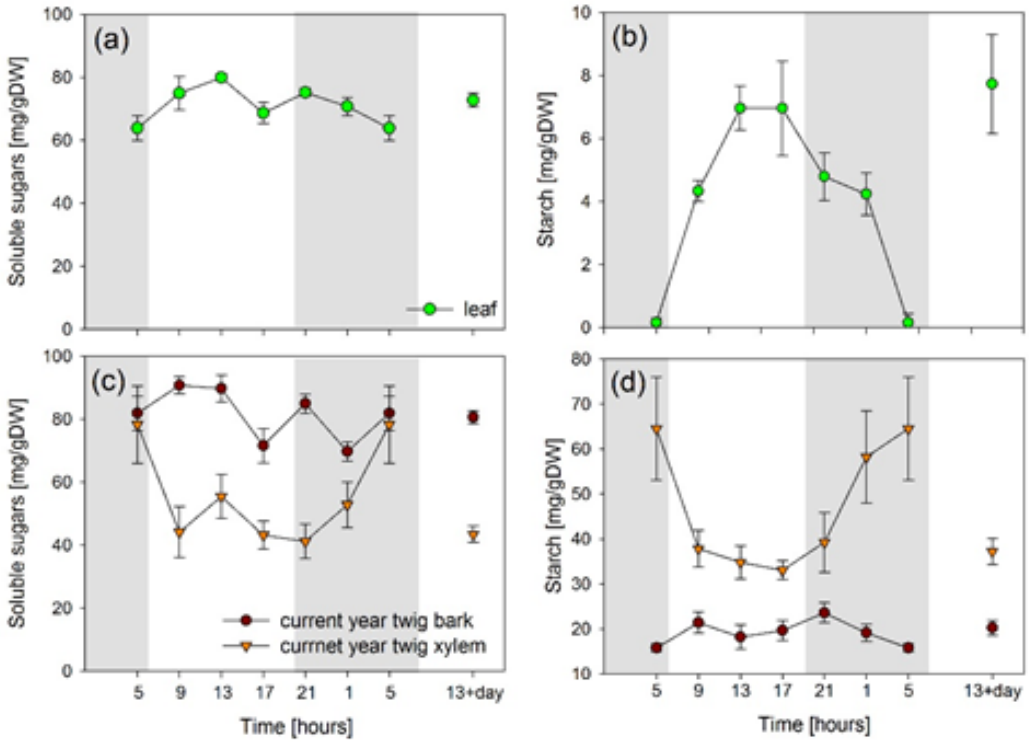

Figure. 8: Diurnal dynamics of soluble sugars (a-c) and starch (b,d) leaves (a-b) wood and bark of The extension shoot (c-d). Samples were collected every 4 hours. Data points represent mean values from five trees. Error bars represent SE. Shaded areas represent nighttime.

In order to gain knowledge on the allocation of NSC in tree architecture, we applied $^{13}CO_2$ to limbs of almond trees that resulted in a very strong increase of ^{13}C content in the leaves (**Figure 9**). Elevated levels of the ^{13}C in leaves in all three applications remained constant for a few hours and then steadily decreased. The export of the ^{13}C from leaves was matched by increase in the ^{13}C in the wood and bark of the current extension shoots. Following this initial and expected accumulation and release of the ^{13}C from leaves and wood of young branches, we observed an increase of the ^{13}C in mature leaves of the treated branches. These reoccurring spikes happened either in the morning or in the late afternoon suggesting a return of ^{13}C. The explanation for the return of ^{13}C to the leaves could be that sugars are flowing with the transpiration stream. We observed an overnight accumulation of SC in xylem sap followed by a sudden drop in the morning most likely reflecting the transport of accumulated sugars via

the transpirational stream. The proposed hypothesis that the xylem offers a secondary pathway for sugar redistribution suggests a novel mechanism to explain NSC supply and equalization of sugar content across the tree architecture.

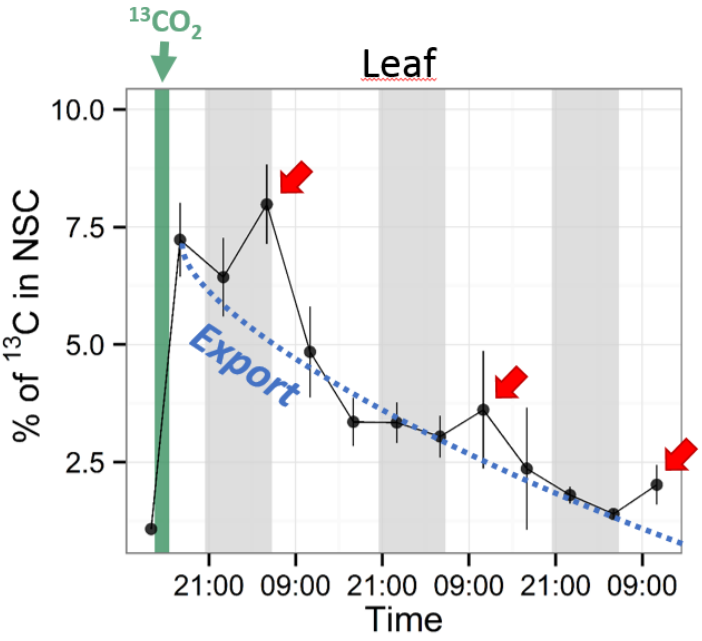

Figure 9: Diurnal dynamics of new assimilates allocation in the leaves of the almond tree. Pulse labelling of 13C was applied to a limb (limb 13C) for 2 hours (green vertical bar). Levels of 13C were quantified in NSC of leaves for 3 days every 6 hours. Data points represent mean values from three trees. Shaded areas represent nighttime.

Horticultural perspective
Observation of large (>50%) variation in starch and soluble sugar content in stems and branches of almond at a diurnal scale suggests that even a short perturbation to photosynthetic activity might lead to significant effects on growth and delays in fruit production.
Xylem participation in vertical redistribution and equalization of NSC across the tree crown of NSC suggest that well designed irrigation might be key to growth and formation of uniform trees.

Project 5. *Use chill removal experiment to determine shifts in phenology, flower health and flowering synchrony and gene expression involved in signaling path-triggering bloom*

Materials and Methods:

To develop a predictive mechanistic chilling model, we have designed a heating device that imposes different temperature/winter climatic scenarios to buds, stems, or both (**Figure 10**). These micromanipulations offer very versatile possibilities in regard to predicting warm winter effects on phenology and reproductive success by testing different winter climatic scenarios for almond plants having experienced the same growing season conditions. Effectiveness of the system is presented on **Figure 11**. Imposing different chilling accumulation to adjacent organs permits us to evaluate the effect of temperature gradients on NSC storage and bud demand to ensure successful bloom. Combined with RNAseq analysis, this approach allows for the

molecular dialogue between storage organs and buds. We want to develop a mechanistic link between NSC winter/spring metabolism and underlying gene expression patterns. Whole genome transcriptome analysis with spatial (stems, bud) and temporal (winter/spring progression) resolution is being performed using clustering gene approaches. A total of 256 buds and bud-twig combinations were used in the study. The different chilling models are applied to our experimental system to test them and we intend to parametrize these models on a physiological and molecular basis. Phenology, carbohydrate storage, growth and respiration are analyzed in combination with comparative analysis of gene expression using RNAseq.
Experiment 1: Identify physiological and transcriptational shift during winter and spring progression in control almond plants. This experiment allows us to identify the chronology of functions resumption in the wood and buds and the level of synchronism between both organs. Growth, respiration, NSC and gene expression are being studied. Samples were collected six times over the course of winter.

RNAseq experiment 2: Identify the effect of chilling deprivation on phenology, NSC content and local gene expression. Identify a potential molecular dialogue between the storage tissue wood and the bud during winter. Growth, NSC and gene expression are being studied. The treatment/winter scenario prevents buds, twigs or both to experience chilling hours (**Figure 8)**. We will use the abbreviation NO CHILL in the rest of the document.

RNAseq experiment3 : Identify the effect of warmer spring temperatures ("forcing" phase of phenological models) on phenology, NSC content and local gene expression. Identify potential molecular dialogue between storage wood tissue and bud. Growth, NSC and gene expression are being studied. The treatment increases experienced temperature by 5ºC in comparison with control samples once the trees accumulated their chilling requirement. Thermal treatment was applied to the bud, stem or both. We will use the abbreviation MORE HEAT in the rest of the document.

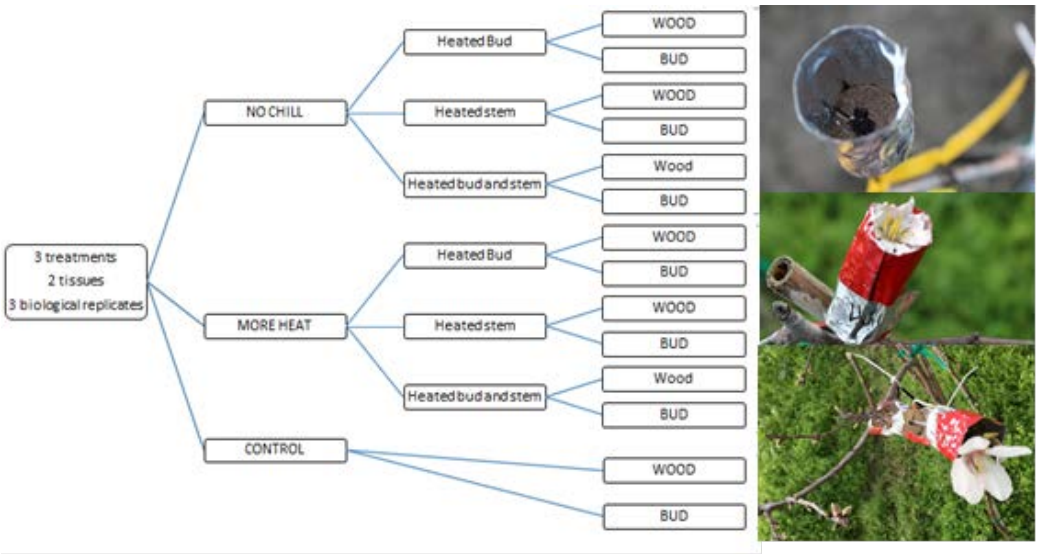

Figure 10: Experimental design. For each thermal treatment three combinations were performed: applying thermal treatment to bud, stem or both. Two tissues are studied for RNAseq: wood and buds. The thermal treatments were applied locally with heating devices controlled by thermocouples mesuring bud temperature (pictures).

Results and Discussion:

The increase in bud temperature after the chilling requirement (More Heat) advanced the bloom date suggesting that buds are main sensors for the forcing phase (**Figure 12**). However, preventing the accumulation of chilling hours advanced the bloom date significantly only when buds and stem were experiencing the temperature treatment. This result suggests that the progression of chilling hours requires molecular dialogue between NSC storage site (stem) and buds. A lack of chilling requirement induces earlier bud break but also delays the formation of fully developed flowers, effectively resulting in significant asynchrony of flowering. Interestingly, heating stems didn't affect the date of blom but affected the mortality rate of buds. These results show that twig temperature as well as bud temperature are important for meeting chilling requirements thus underlining the importance of a whole tree approach in studying dormancy break. This report of the influence of stem temperature on blooming date highlights the potential to use white paint to mitigate warm winters in almond orchards. The increase stem albedo from white paint, decreases stem winter temperatures that can potentially delay too early of a bloom and limit erratic blooms in orchards (see Tixier et al., 2017)

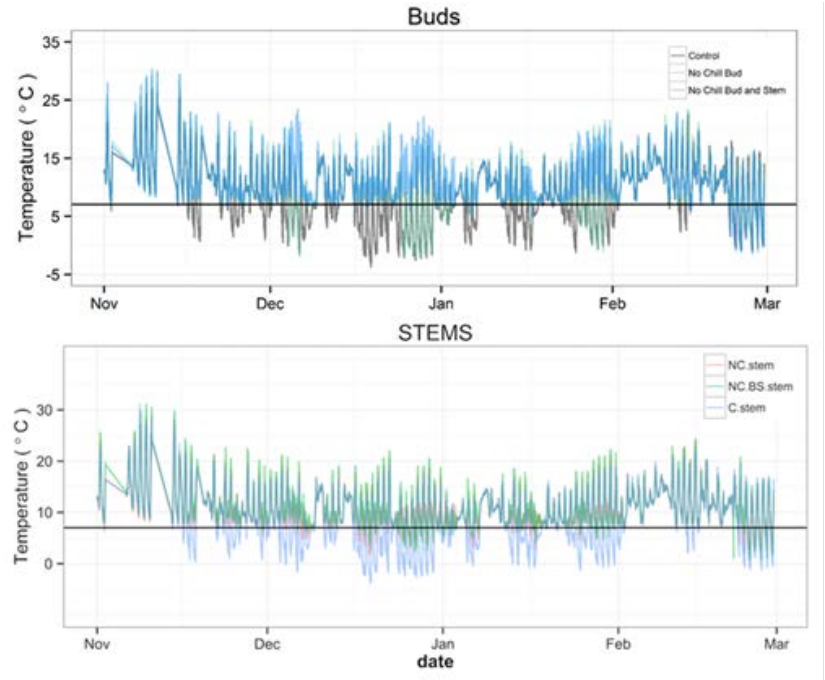

Figure 11: Record of bud and stem temperature of the No Chill treatment in comparison with organs from control almond organs.

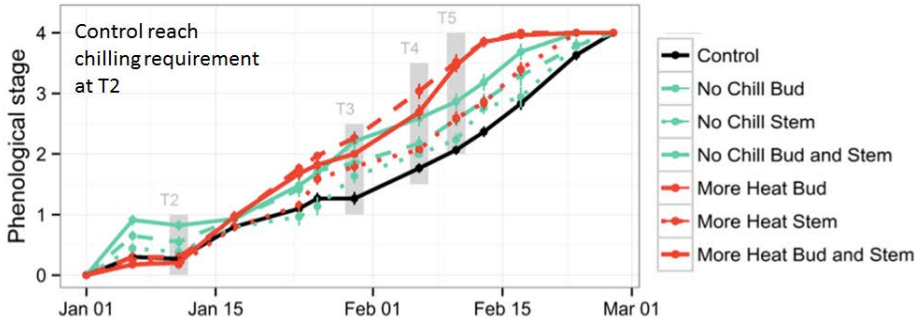

Figure 12: Influence of chilling hours and forcing hours on Almond bloom and phenology. The increase in bud temperature after chilling requirement (More Heat) advanced the bloom date. Preventing the accumulation of chilling hours advanced the bloom date only when the buds and stem were experiencing the temperature treatment.

We compared outputs from empirical chilling models classically used in horticulture (Chilling hours model, Utah Model, Dynamic model) with our phenological data from our experimental design. According to the Chilling hours, Utah model, and Dynamic model, control buds reached a chilling requirement before the No chill treatment and thus should have bloomed earlier (**Figure 13**). However, we observed opposite trends (9). The buds from the No Chill treatment bloomed significantly earlier that the control buds. These results highlight the necessity to parametrize almond bloom models with physiological/ molecular information from trees in the orchard as we have developed in Project 2.

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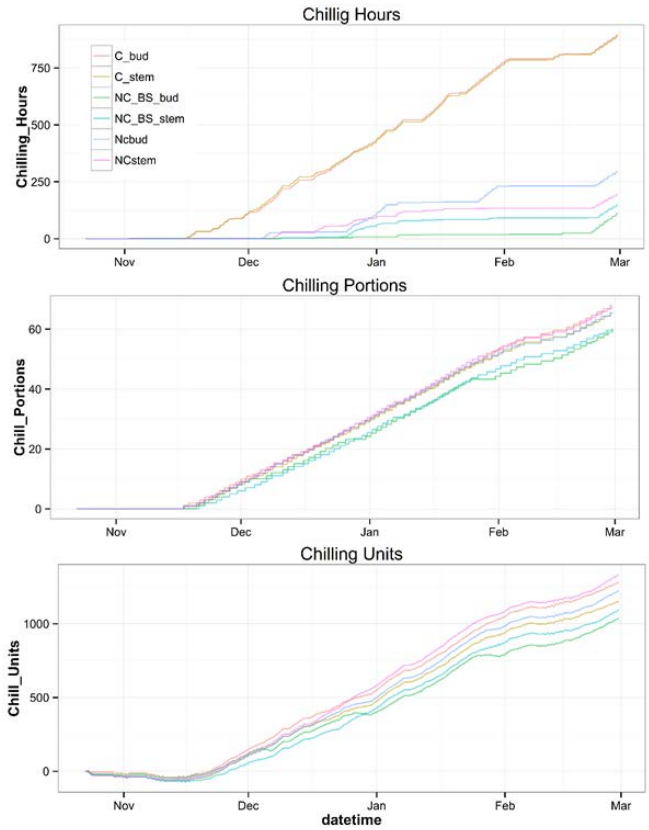

Figure 13. Comparison of Chilling hours model, Utah model and Dynamic model on control No Chill and More Heat treatments performed on almond organs (bud and stem). The 3 classical empirical models predicted earlier bloom for control buds, but phenological data showed the opposite. These results highlight the necessity to shift horticultural models to mechanistic models.

The comparison of NSC concentrations between no Chill and control wood showed that an increase in temperature of wood in NoChill stem lead to higher levels of NSC. Classical model usually describes NSC use during winter as maintenance respiration. As maintenance respiration has an exponential relationship with temperature, our results show that NSC mobilization during winter can't be explained solely by respiration (**Figure 14**). One of the hypotheses we are testing is that temperature also influences transport of NSC during winter and that locally increasing temperature to branch allows for transport of NSC from adjacent cold parts. Also, the model developed in the second section of this report will be applied to present data to have a holistic understanding of NSC management during winter.

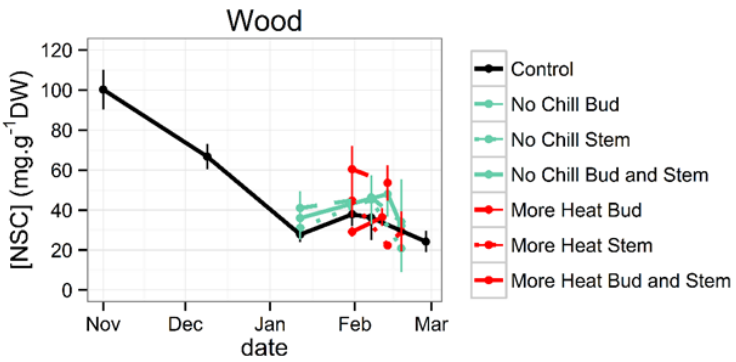

Figure 14. Comparison of NSC concentration in almond wood of control, No Chill and More Heat treatment performed on almond organs (bud and stem). As NSCs are being mobilized during winter, the heating treatment leads to higher levels of NSC in the wood located in the vicinity of flower buds.

The RNAseq experiment succeeded in the obtaining of high-quality mRNA without RNAr contamination as well as clean reads from RNA sequencing (**Figure 15)**. These reads have been mapped against the peach genome and showed 80% coverage. This percent coverage is satisfactory to analyze the transcriptional shifts during dormancy and bud break. Genes are currently being annotated with function and expression levels done on the basis on reads count. These encouraging preliminary results of RNAseq ensures that the bioinformatics analysis can be performed with a clustering approach that will be developed during winter 2018 to identify molecular dialogue between buds and stems and identify key genes of dormancy breaking.

Horticultural perspective
The presented analysis suggests that indeed, temperature is a key aspect of dormancy progression and bloom synchrony, timing, and health. It underlines the fact that buds are not the only receptors of temperature but branches also participate in response/signaling to winter thermal conditions.
This project offers a look into links between physiology and expression level signaling association. Adding a molecular component to project 2 provides an additional pathway for precise forecasting of bloom and can improve decision making in regard to winter orchard management.

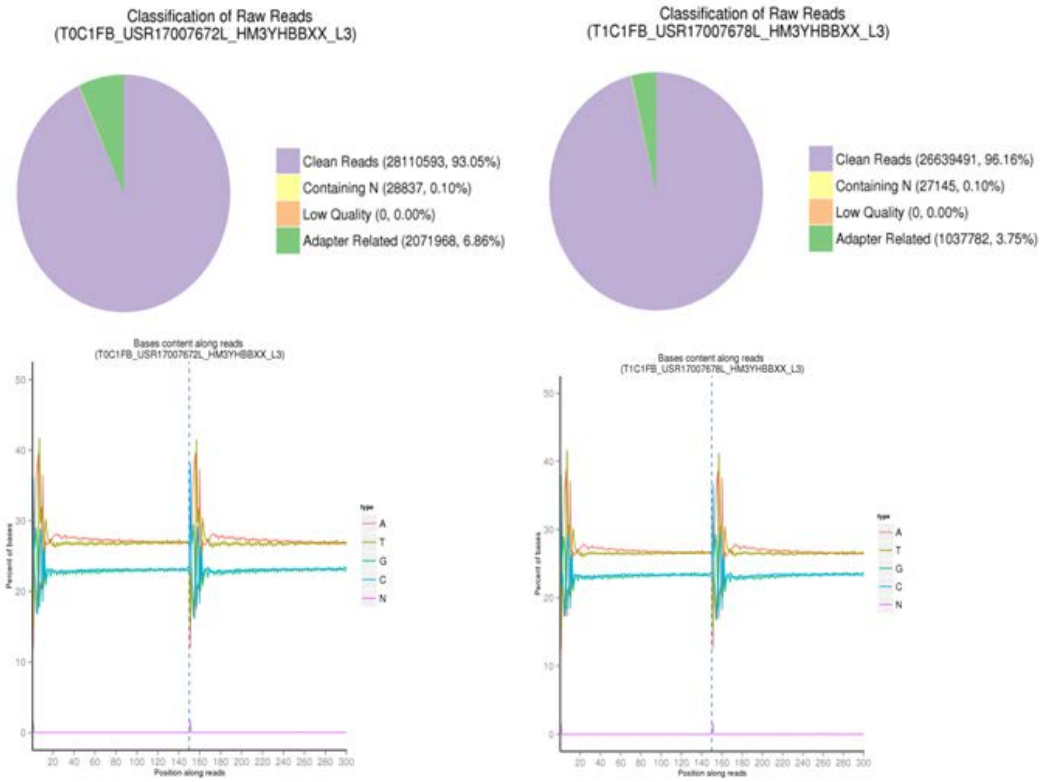

Figure 15. Quality check of RNAseq library performed on control buds. The RNA seq experiment lead to the abstention of high-quality mRNA without RNAr contamination as well as clean reads from RNA sequencing. These reads have been mapped against the peach genome and we observed a satisfactory 80% coverage.

Research Effort Recent Publications:

In Review/Preparation

Or Sperling, Tamir Kamai, Aude Tixier, Anna Davidson, Katherine Jarvis-Shean, Eran Raveh, and Maciej A. Zwieniecki (2018) To bloom or not to bloom? Exploring dormancy using novel carbohydrate-temperature metabolism model (in preparation)

Aude Tixier, Jessica Orozco, Adele Amico Roxas, J. Mason Earles, Maciej A. Zwieniecki (2018) Diurnal variation in non-structural carbohydrate content in trees and vertical mixing - Is xylem a secondary redistribution system for sugars? Plant Physiology (in review)

Published

Guzman-Delgado, P., Earles, J.M., Zwieniecki, M.A. 2018. Insight into the physiological role of water absorption via the leaf surface from a rehydration kinetics perspective. Plant Cell and Environment (in press) https://doi.org/10.1111/pce.13327

Sperling O, Silva L, Tixier A, Théroux-Rancourt G, Zwieniecki MA (2017) Temperature gradients assist carbohydrate allocation within trees. Scientific Reports 7, Article number: 3265 (2017) doi:10.1038/s41598-017-03608-w, IF: 5.8

Tixier A, Sperling O, Orozco J, Lampinen B, Amico Roxas A, Saa S, Earles JM, Zwieniecki MA (2017) Spring bud growth depends on sugar delivery by xylem and water recirculation by phloem Münch flow in Juglans regia. Planta. Doi: 10.1007/s00425-017-2707-7. IF : 3.2

Secchi, F., Zwieniecki, M.A. 2016. Accumulation of sugars in the xylem apoplast observed under water stress conditions is controlled by xylem pH. Plant Cell and Environment. 39:2350-2360 DOI: 10.1111/pce.12767

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Earles, J., Knipfer, T., Tixier, A., Orozco, J., Reyes, C., Zwieniecki, M.A., Brodersen, C., McElrone, A. 2018. In-vivo quantification of plant starch reserves at micrometer resolution using X-ray microCT imaging and machine learning. New Phytologist 218:1260-1269 DOI: 10.1111/nph.15068