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

Project No.: PREC8

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Project Cooperators and Personnel: N/A

Grantee(s) of the Almond Board are REQUIRED to address sections A through G. These should be **submitted in PDF**, using Arial font size 12 for the main text, and be five to seven pages in length.

A. Summary

Tree growth and yield are dependent on a complex set of interactions involving genotype, physiological and developmental processes, and the interaction of these processes with the environment (abiotic and biotic stress). Sugars (carbohydrates) are the products of photosynthetic activity providing both building blocks for the developing tree structure and temporary storage of energy in the form of non-structural carbohydrates (NSC; starch (ST) and soluble carbohydrates (SC) like sucrose, glucose or fructose). NSC are the key energy used to 'pay' for all biological services that includes growth, respiration, nutrient uptake, defense against pathogens, reproduction, and protection from abiotic stress. Evolved management of NSC reserves is the key process allowing the tree to survive adverse climatic conditions during the vegetative growth portion of the year (spring-summer-fall) and dormancy (winter). As the Central Valley climate becomes more erratic and abiotic stresses more severe (summer-like temperatures in the fall and prolonged fall drought, loss of winter fog, large daily swings of temperature, and increasing probability of winter frost), understanding the mechanisms responsible for tree energy reserves management is crucial. Reserves are fundamental for tree survival and reproductive capacity (yield). Knowledge on how orchard trees generate adequate NSC reserves and how climate and orchard management practices affect NSC reserves usage can provide a basis for the development of horticultural techniques that will improve orchard performance and mitigate the impact of current and future abiotic stresses while maintaining high productivity. This knowledge will also add additional 'tools' to management practices 'toolbox'.

To achieve the goal of better understanding trees' management of NSC, we developed a program called the Carbohydrate Observatory (CO) that allows for gaining a unique insight into the temporal dynamics of NSC in almond trees along multiple vectors of system diversity including geographical location, tree age, variety, rootstock, scion combination, management practices and thus shortening the discovery time. Carbohydrate status of almond orchards is presented at http://zlab-carb-observatory.herokuapp.com. Currently, we have determined that productive almond orchards present a risky strategy of NSC management resulting in large swings of NSC reserves between summer and dormancy. These large variations seem important for

yield capacity as indicated by a significant positive correlation between NSC reserves in the winter and yield the following year (high NSC content in twigs leads to high yield). However, we did observe a significant negative correlation of NSC content in the summer with yield the same year (low level of NSC in summer is associated with high yield). This suggests that trees ending summer with low NSC reserves have to rebuild their yield potential during the post-harvest vegetative phase to maintain high productivity.

In addition, analysis of the NSC seasonal pattern showed an interesting aspect of NSC's role in timing dormancy. The experimental approach to manipulate NSC availability in the fall by defoliation and their redistribution by girdling revealed a complicated pattern that suggests a significant impact of winter NSC storage on bloom success. In general, any disruption to natural senescence and redistribution resulted in delayed bloom and reduced synchrony of the bloom. However, the most pronounced effect was seen after early fall defoliation and girdling in the spring – a situation that reduces NSC storage and does not allow for late spring retrieval of distally located sugars. This insight provides prospect for the development of a model that uses climatic data and trees' NSC management to assess dormancy progression and tree readiness for synchronous bloom presented at http://zlab-chill-heat-model.herokuapp.com.

B. Objectives *(300 words max.)*

Our main objective is to develop a carbohydrate analysis method as a tool to determine almond's physiological status that would complement the currently used methods such as water potential and nutrient analysis. The goal is to use carbohydrate analysis as a new option for sustainable orchard management.

Specific Objectives:

(1) Continue to maintain and possibly expand a network of almond orchards that provide samples to a large-scale, state-wide study of seasonal dynamics of carbohydrate that minimizes time and costs for research.

(2) Provide easy and informative access to NSC information for almond growers by continuous improvements to our online platform: http://zlab-carb-observatory.herokuapp.com (Figure 1)

(3) Describe and publish seasonal patterns of NSC dynamics within the almond trees that links NSC management with tree phenology

(4) Use already data collected to determine impact of NSC on yield.

(5) Experimentally test the role of NSC reserve levels and their redistribution on bud development and bloom time. A new model of phenology that links winter temperature and NSC management was proposed and published. Model is available on the website: http://zlab-chill-heat-model.herokuapp.com/ (Figure 10)

C. Annual Results and Discussion

Determination of carbohydrate content in almond trees in relation to yield. Over the period of 2016-19 we received and collected samples from over 150 orchards resulting in more than 10000 NSC content analysis. The data is presented on the website (Figure 1) for orchard owners and managers to review the performance of their sites.

Currently, our interface allows for the comparative analysis of multiple orchards, NSC content to each other and against additional parameters that include: rootstock, scion, age, and county locations. Permanently displayed values are: running average of the NSC content for the entire state and all data points collected/analyzed so far. Features include the capacity to zoom to any portion of the graph. In addition, it is possible to look at a specific type of NSC i.e. soluble sugars and starch in wood and bark separately.

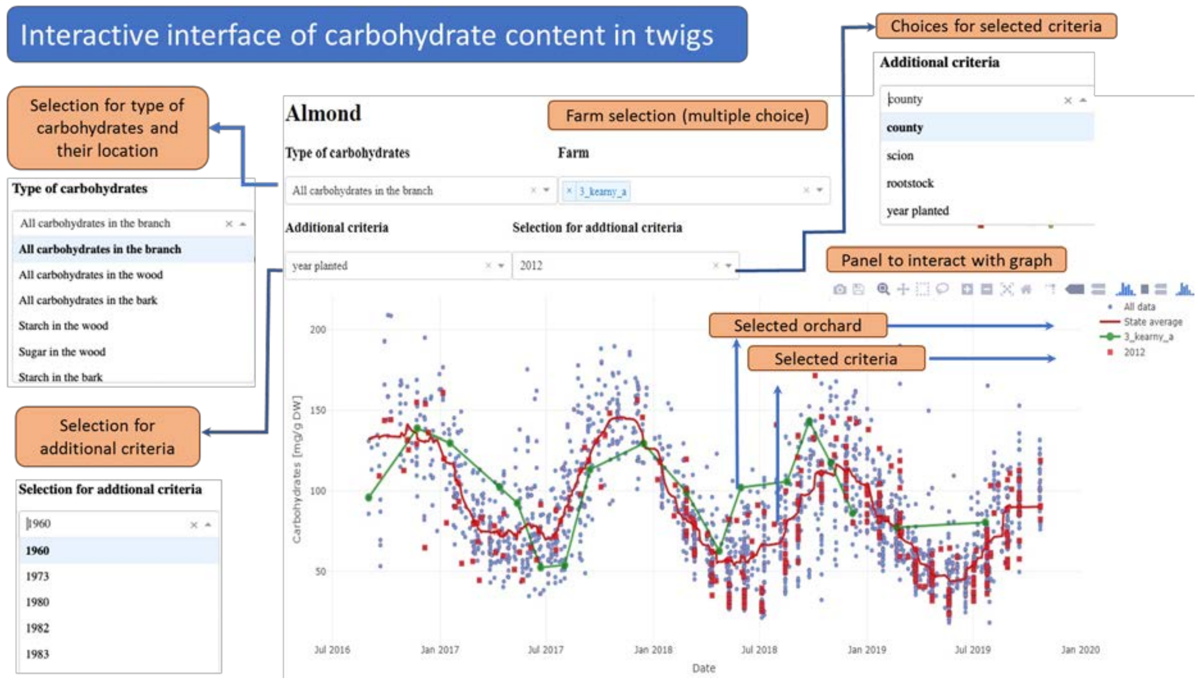

Figure 1. Snapshot of the website allowing for comparative analysis of NSC in almond trees.

This level of insight allows individual growers participating in the study to compare their orchards against all specific management practices they use or orchard properties that were not revealed to us and make decisions on how to explore and use their own data.

Analysis of carbohydrates in bark and wood reveal that major swings in the NSC content occur in wood (Figure 2). Specifically, there is a relatively slow but progressive use of reserves from maximum content in October until July, then content is at its minimum until harvest and the recovery is observed between harvest and leaf senescence. These findings in association with NSC content in the fall and yield (see below) underlines the necessity of post-harvest management aiming at recovery of NSC reserves prior to dormancy.

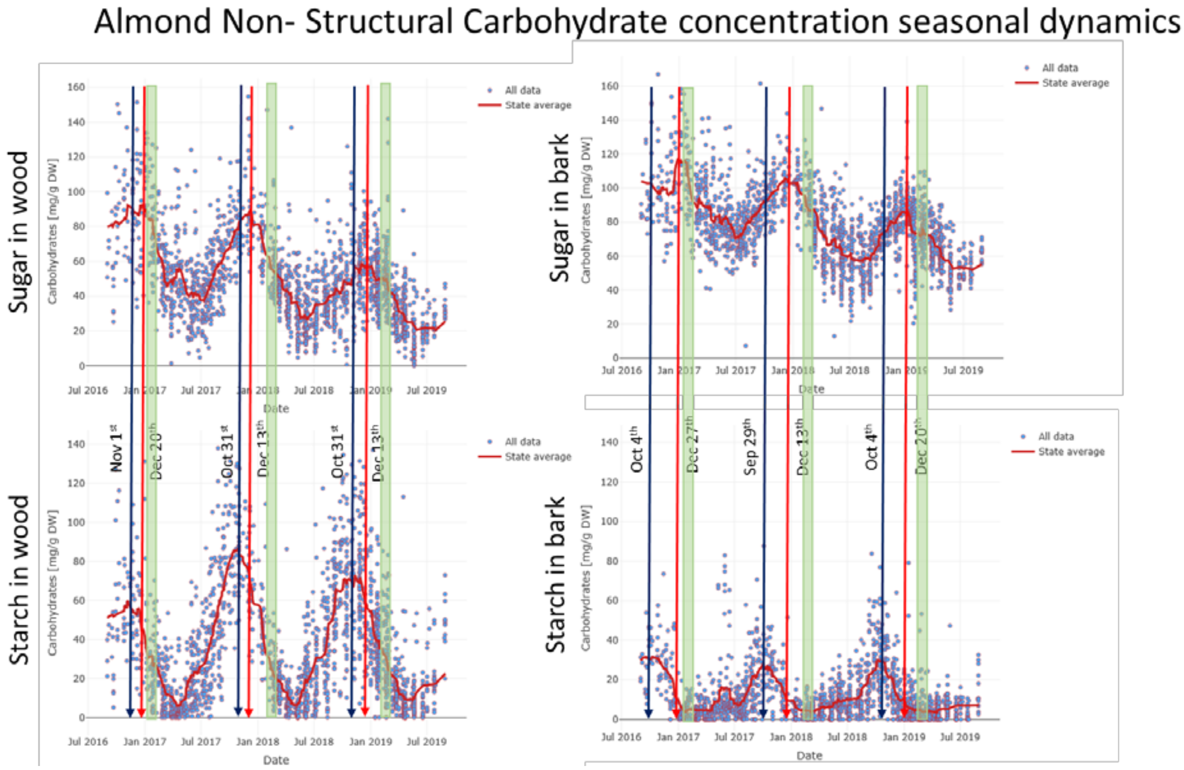

Figure 2. Seasonal pattern of soluble sugars and starch concertation in wood and bark of almond trees with maximum of soluble sugars marked by red arrow starch blue arrow and bloom time by green bars

A small number of farms that allowed use of their yields provided an opportunity to gain the first insight to determine the role of branch NSC in crop performance (Figure 3). Higher number of yield reports would improve this portion of research exploration and possibly provide further support to presented results. However, even with limited information, we can show a positive significant correlation between NSC content in late fall (October) and in the spring (February) preceding bloom with the following summer yield. This is an important finding that suggest potential use of the fall information as a tool to either predict future yield or apply fall management aiming at restoration of high NSC content in trees.

How high accumulations of NSC prior to dormancy translate to high yields is not yet known. This might happen via multiple pathways. (1) Survival of dormancy by providing energy to maintain high vitality of meristems (both apical in buds and secondary in cambium); (2) Recovery of xylem transport capacity by providing energy for restoration of hydraulic continuity in xylem vessels – i.e. generation of positive pressure in xylem during spring; (3) assuring a high level of reserves at bloom time thus, providing energy to develop healthy flowers and sustain fast growth of new leaves before they achieve photosynthetic independency. An experiment to determine if indeed manipulation of NSC content prior to dormancy and their redistribution, suggests that there is a significant impact on bloom time and synchrony of bloom. Specifically, we have found several interesting aspects of both branch defoliation (aiming at the reduction of NSC content in branches) and girdling aiming at reducing the redistribution of NSC potential that shows unique aspects of walnut biology.

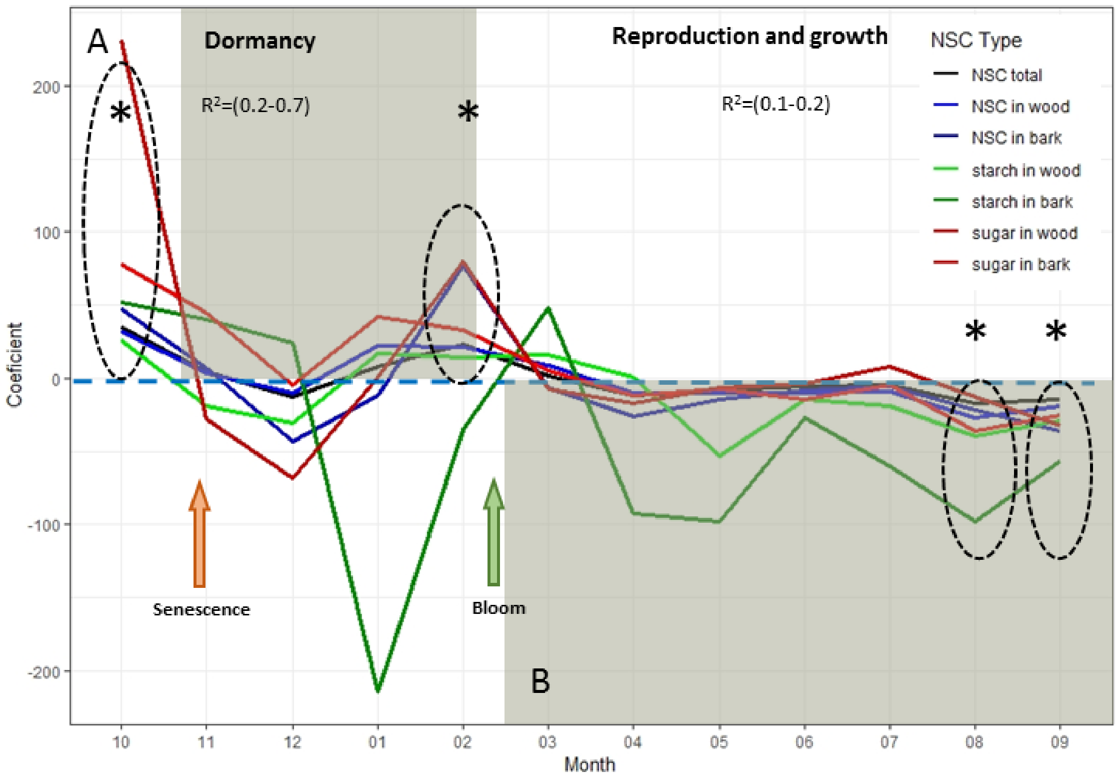

Figure 3. Shown are respective slopes of correlation between carbohydrate content and yield (coefficient of linear function formulated as: yield= coefficient*NSC Type + offset). Region 'A' shows a generally positive correlation (coefficient>0) of winter NSC levels with the following summer yield. Region 'B' shows coefficients in summer as generally not correlated with the current year's yield. '*' denote significance with $p<0.05$ for the correlation for at least one NSC type.

October defoliation, resulted in a lower content of NSC in wood and bark in November, but late defoliation had the opposite effect with a higher initial accumulation in December. However, these initial effects were not detectable later in the winter, suggesting that winter redistribution of NSC is very active in almond trees, equalizing NSC access across a tree (Figure 4). This is especially evident if we compare the content of defoliated branches with branches where redistribution was affected by girdling of the phloem. In general, fall girdling resulted in a slightly higher content of NSC prior to bloom in January (Figure 5), that translated to a higher availability of NSC during bloom time in branches with reduced reallocation of NSC. Impact of both defoliation and girdling was similar to the impact of girdling only, thus again, suggesting that redistribution of NSC in the winter is very active and can control levels of NSC availability during spring.

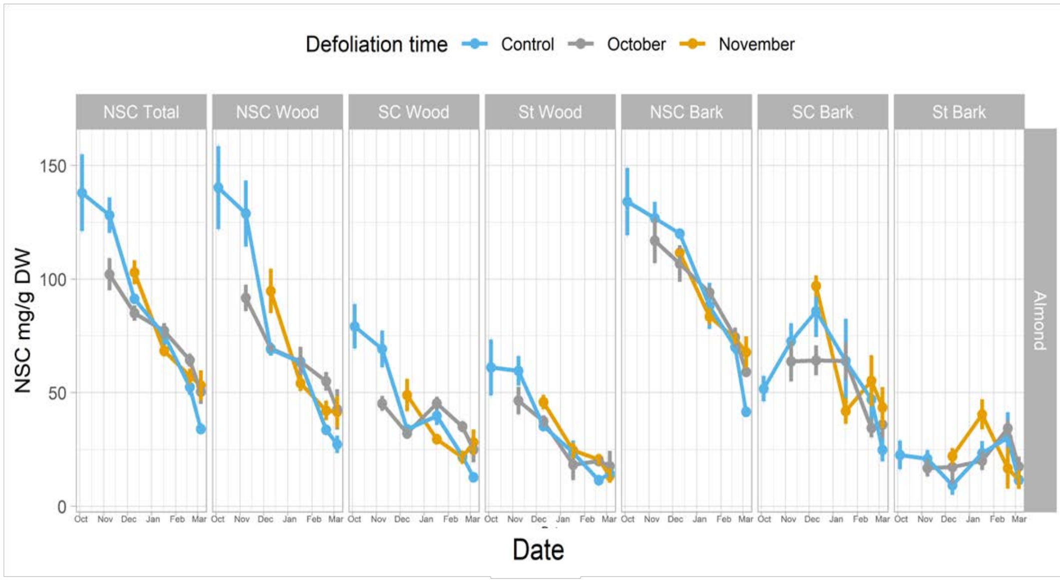

Figure 4. Impact of defoliation time on NSC content in almond twigs. NSC – non-structural carbohydrates, SC – soluble sugars, St - starch

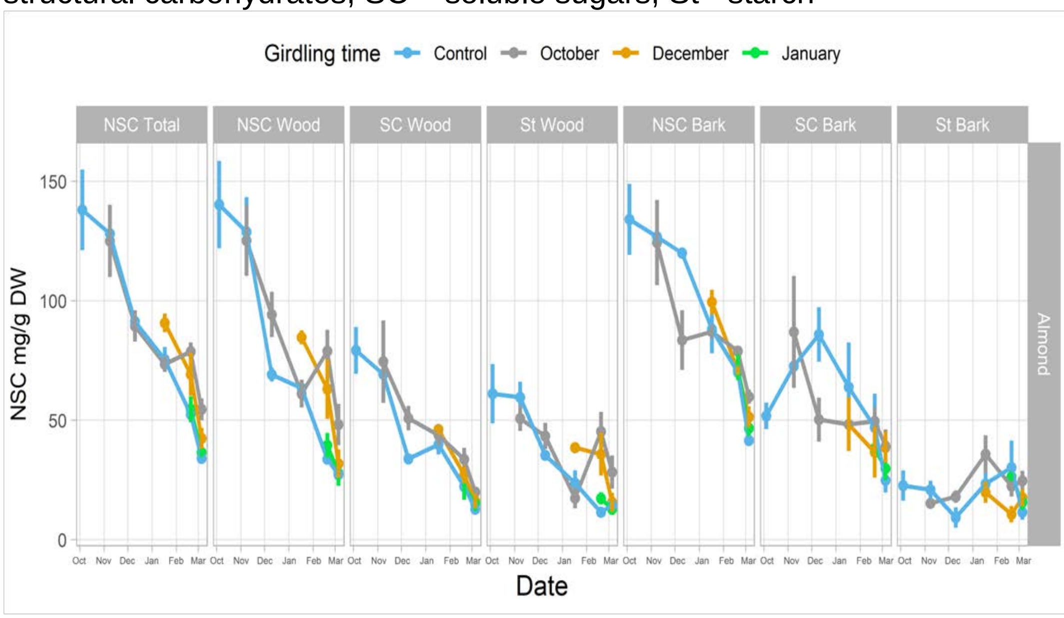

Figure 5. Impact of girdling time on NSC content in almond twigs. NSC – non-structural carbohydrates, SC – soluble sugars, St - starch

Early and late defoliation despite relatively insignificant impact on NSC content in branches led to significant delay and asynchrony of bud break (Figure 6 and 7). The impact of fall girdling had similar effect and resulted in delayed, asynchronous bloom (Figure 6 and 8), although pre-bloom girdling had no effect on bloom timing. This suggests that the impact of NSC winter redistribution activity is important while end of January level had no further impact on bloom time in almond. In addition, any combination of defoliation with girdling leads to delayed bud break and significant asynchrony of bloom.

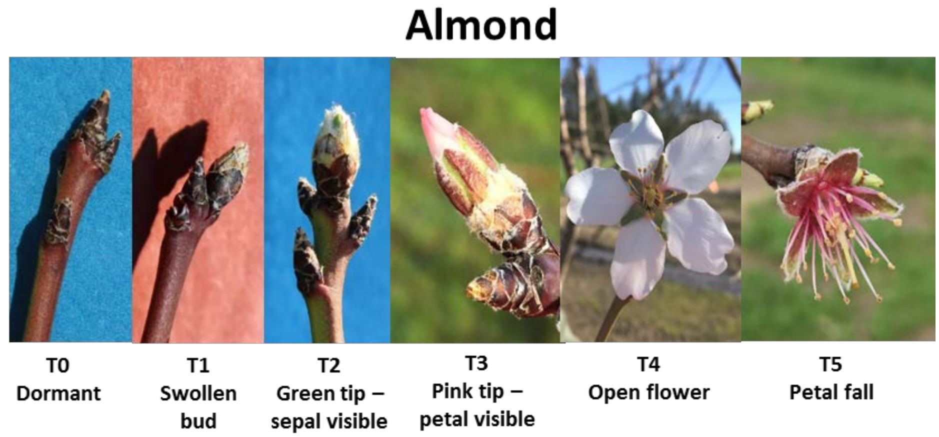

Figure 6. Flower stages used in the study

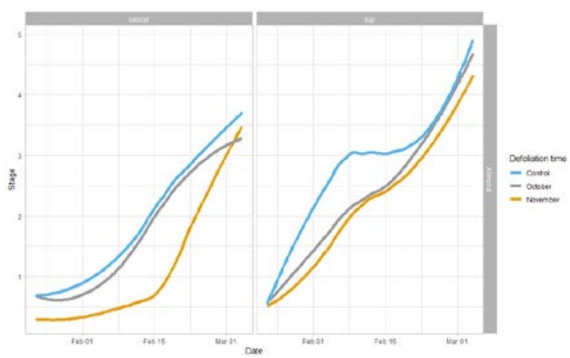

Figure 7. Impact of defoliation on bloom time. Early (October) defoliation significantly delayed the bud break and bloom asynchrony.

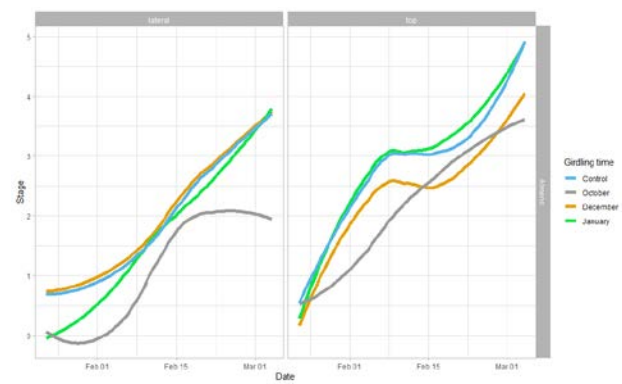

Figure 8. Impact of girdling on bloom time. Late (March) girdling lead to a significant delay in bud break and bloom asynchrony.

Trees in seasonal climates gauge winter progression to assure vital and productive blooming. However, how dormant plants assess environmental conditions remains obscure. We postulated that it involves the energetic reserves required for bloom, and therefore studied winter carbohydrate metabolism in deciduous trees. We quantified non-structural carbohydrates throughout winter in almond, peach, and pistachio trees in California and Israel and characterized winter metabolism. We constructed a carbohydrate-temperature (C–T) model that projects changes in starch and soluble carbohydrate concentrations by temperature mediated kinetics (Figure 9). Then, we tested the C–T model projections of bloom times by 20 years of temperature and phenology records from California. The C–T model attributes winter carbohydrate regulation in dormant trees to continuous updates of metabolic pathways. The model projects a surge in starch synthesis at the end of winter, and critically low concentrations of soluble carbohydrates, that trigger bloom. This is supported by field measurements of starch accumulation at the end of winter (˜50 mg g−1 DW in almonds) that preceded bloom by ˜10 days. The C–T model provides a physiological framework for bloom forecasts in deciduous orchards. It integrates contrasting notions of chill and heat and elucidates why abnormal winter temperatures may compromise bloom in deciduous orchards. The access to model and its prediction is available at http://zlab-chill-heat-model.herokuapp.com/, with model scientific background published in *Predicting bloom dates by temperature mediated kinetics of carbohydrate metabolism in deciduous trees. 2019. Or, S., Kamai, T., Tixier, A., Davidson, A., Jarvis-Shean, K., Raveh, E., DeJong, T., Zwieniecki, M.A. Agricultural and Forest Meteorology:* ***276-277*** *https://doi.org/10.1016/j.agrformet.2019.107643*

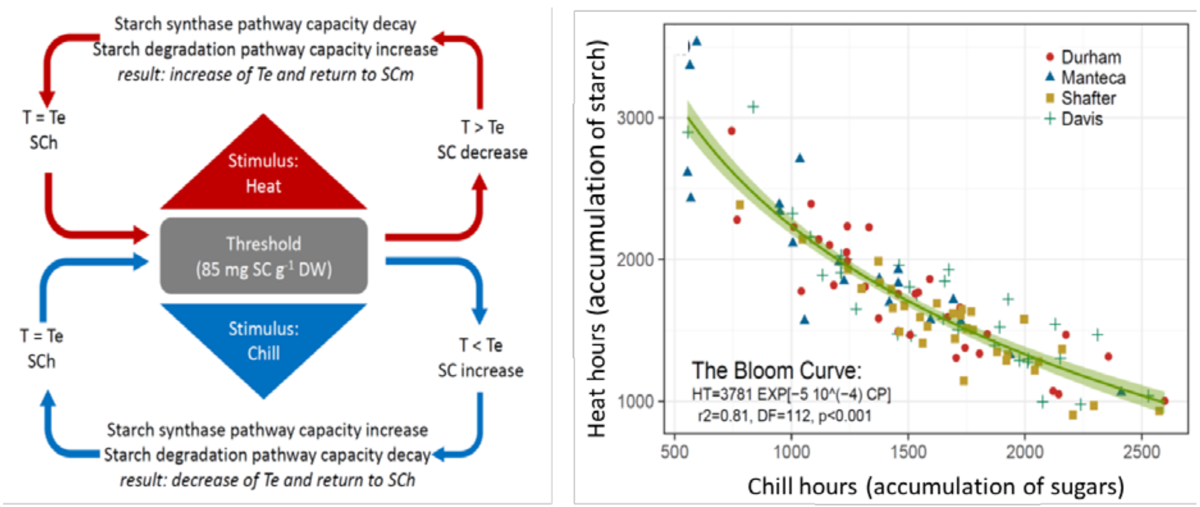

Figure 9. (left) A diagram to illustrate how chill (blue frames) and heat (red frames) stimuli activate metabolic adjustments to sustain permissive soluble carbohydrates (SC) concentrations through temperature changes. (right) The C–T model projections, processing historical temperature data (1983–2018) in Durham (red circles), Manteca (blue triangles), Shafter (orange squares), and Davis (blue crosses), for the time almond trees spent inducing their starch synthase frequency factor (As, 500–2,500 h) and its inverse exponential proportions ($r2=0.81$, $DF=112$, $p < 0.001$) to the time they spent inducing starch degradation (Ad, 1,000 and 3,000 h)

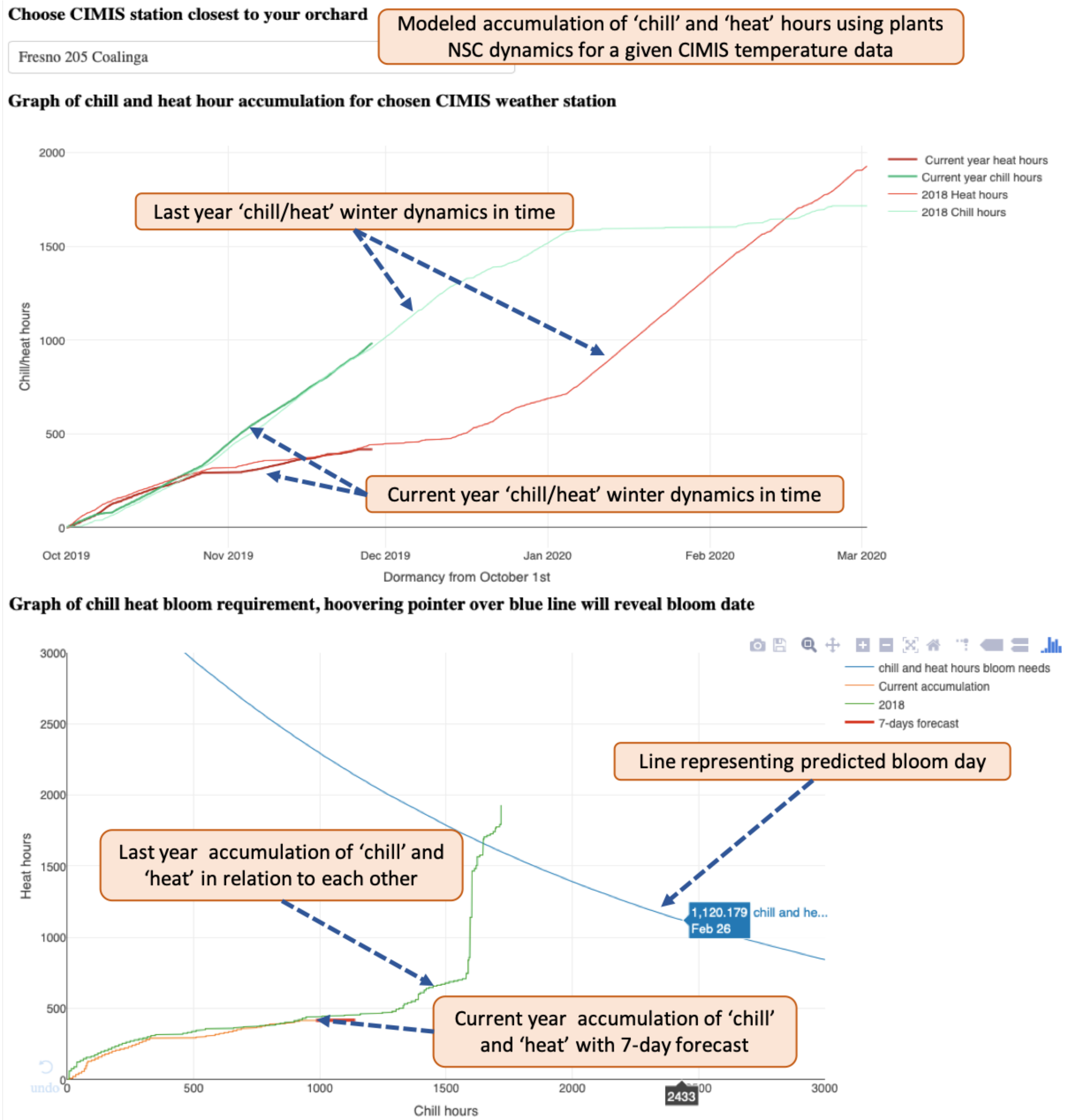

Figure 10. Interface of the proposed NSC based model for tracing the dormancy period (model developed for almond). Currently, the interface allows for comparative analysis of current year data with last year dormancy thermal dynamics.

D. Outreach Activities

1. Presentation at the almond short course, visits to growers, phone-calls

E. Materials and Methods *(500 word max.)*:

The Carbohydrate Observatory is the research initiative providing analytical service to growers interested in a better understanding of NSC management of their orchards. Growers provide three twig samples (with xylem and phloem separated) per orchard that are subsequently analyzed for soluble sugars and starch content in wood xylem –

water conducting tissue) and bark (phloem – sugar conducting tissue). Analytical results are being published on line at http://zlab-carb-observatory.herokuapp.com.
Received samples are processed in the lab following the procedure described below:

- Each sample is ground into powder. A small amount (25 mg) is then washed in 1 mL of pH buffer to dissolve all soluble carbohydrates.
- Using a colorimetric method (a spectrophotometer), the concentration of sugars is measured (Anthrone method) in sample of buffer (50 uL) and recalculated to express SC concentration per g of dry matter.
- The remaining material in the buffer is treated with two different enzymes that digest starch to form the soluble sugars. These are again measured in a spectrophotometer using the Anthrone method.
- For further details of the procedure please refer to published articles by the Zwieniecki lab, on our website or request the procedure vial email (mzwienie@ucdavis.edu).

Additional information related to each orchard is provided by growers on a voluntary basis that includes specific management practices, age, scion/rootstock combination and yield. This part needs further improvement due to a low participation from almond growers. The quality of analysis requires a high number of samples. Our lab is currently communicating with many growers to explain the reasoning for the need of additional data. In addition, CIMIS and NOAA weather data is used in the analysis. The additional site related information is crossed referenced with the NSC analysis database and used in subsequent analyses of the role of NSCs in annual orchard performance.

F. Publications that emerged from this work

Predicting bloom dates by temperature mediated kinetics of carbohydrate metabolism in deciduous trees. 2019. Or, S., Kamai, T., Tixier, A., Davidson, A., Jarvis-Shean, K., Raveh, E., DeJong, T., Zwieniecki, M.A. *Agricultural and Forest Meteorology* ***276-277***: https://doi.org/10.1016/j.agrformet.2019.107643

Comparison of phenological traits, growth patterns, and seasonal dynamics of non-structural carbohydrate in Mediterranean tree crop species. 2019. Aude Tixier, Paula Guzman Delgado, Or Sperling, Adele Amico Roxas, Emilio Laca, Maciej Zwieniecki. *Scientific Reports*