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

Project No.: PREC8.Zwieniecki

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A. Summary

California's Central Valley is affected by a slow climatic shift that is reducing the Valley's fog cover. The net result is an increased incidence of variable thermal conditions during winter including higher average and maximum temperatures and more severe frost nights that can affect tree phenology. These factors combined with the increasing use of saline groundwater supplies have produced an unprecedented set of new abiotic stresses that affect horticultural production. The vegetative lifespan of any perennial plant can be described as a continuous struggle to acquire, transfer, and store energy that is necessary to grow, reproduce, and protect from abiotic and biotic stresses and allow survival of dormancy.

Non-structural carbohydrates (NSC, including sugars and starch) are responsible for the majority of long-distance energy transfers and long-term storage of energy in plants. They are the ultimate currency that the plant uses to interact with the environment. The understanding of NSC physiology as plants respond to stress while accomplishing their reproductive functions is of key importance for predicting yields and a plant's ability to mediate salinity, drought, or winter survival. Understanding NSC management is especially important in long-lived perennial crops like almond that must balance short-term (seasonal) versus multi-year benefits with effects being carried out over multiple years.

We are in the third year of a project that allows for the analysis of carbohydrates in almond orchards across California at an unprecedented scale (the only such database in the world). High seasonal variation of sugars and starch reserves was found pointing at mid-summer as the most important period for a reversal in NSC trend from supply to storage. This suggests that management practices during post-harvest might influence future orchard performance and, ultimately, the following year's yield. Specifically, using direct analysis of correlations, we found that NSC content in the January-February period was positively correlated with the following year's yield, while that in August-September was negatively correlated. We are currently developing a yield prediction model using available data from the Carbohydrate Observatory and spatial climate information.

B. Objectives

1. 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:

- a. 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.
 - b. Provide easy and informative access to NSC information for almond growers.
 - c. Describe and publish seasonal patterns of NSC dynamics within the almond trees that link NSC management with tree phenology.
 - d. Develop a crop predictive model that utilizes carbohydrate data and determines the impact of NSC content on yield.
 - e. Theoretically and experimentally test the role of NSC reserve levels and their redistribution on bud development and bloom.
2. Outputs and milestones:
- a. Despite the impact of COVID we had maintained ~50% capacity of sample analysis and received ~80-90% of orchard samples.
 - b. We maintained online access to NSC data <http://zlab-carb-observatory.herokuapp.com/>. In addition, we communicated and discussed farm-specific data with growers upon request.
 - c. Manuscripts (see F).
 - d. Using available data, we developed an online platform for the estimation of the NSC content during winter months on yield <http://zlab-yield-model.herokuapp.com/>.
 - e. We experimentally tested the importance of fall NSC content on time of bloom and further improved the model linking winter thermal conditions and sugars to phenology (see F).

C. Annual Results and Discussion (*This is the core function of this report*)

- a. Throughout 2016-20 we have received or collected twig samples from over 170 orchards resulting in more than 15,000 NSC content analyses. Collection sites are located across the entire Central Valley spanning latitudes from 34.99 to 39.74 and longitudes from -118.81 to -121.84 providing highly diverse climatic (temperature and precipitation) and soil conditions. In addition, samples reflect the range of almond varieties, orchard ages, management practices, etc. The generated data set is the only one in the world with such large geographic and temporal scales. The technical analysis of these three-year data is under consideration for publication in *Scientific Reports* (see F).
- b. The data is presented on the website of the Carbohydrate Observatory for orchard owners and managers to review the performance of their sites (Figure 1). Currently, our interface allows for the comparative analysis of NSC content of a single orchard across time, across multiple orchards, 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 representing the average NSC content of individual orchards that have been analyzed so far. Features include the capacity to zoom into any portion of the graph. In addition, it is possible to look at the specific type of NSC and the tissue where they are located, i.e. soluble sugars and starch in wood and bark separately. This level of insight allows individual growers participating in the study to explore their own data and evaluate how their management decisions are reflected in the NSC status of their orchard.

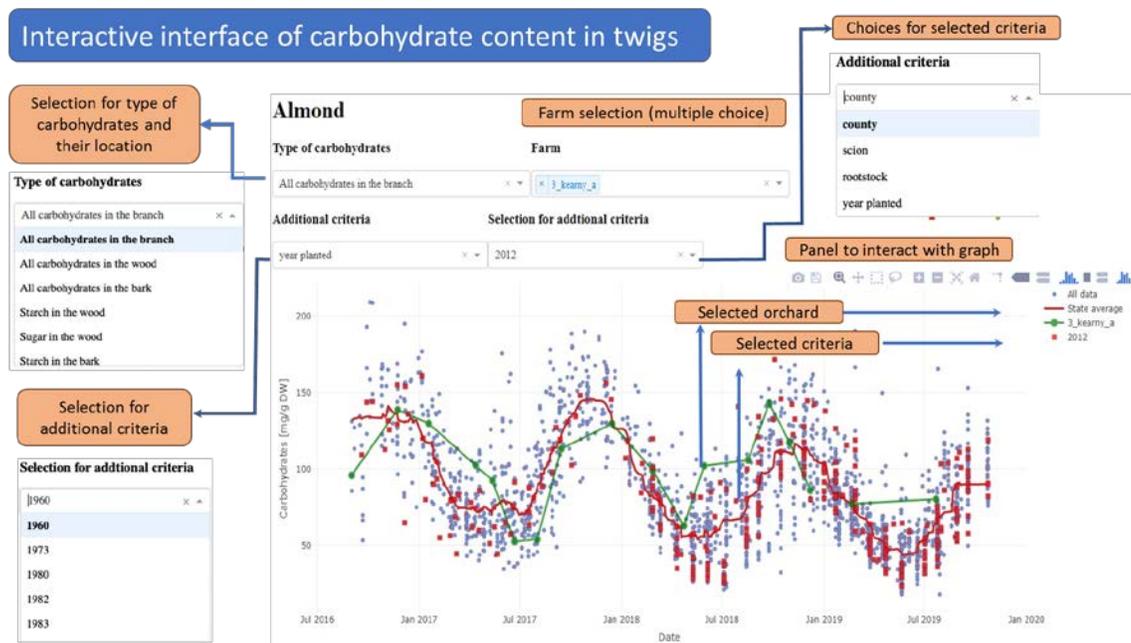


Figure 1. Interface for accessing NSC data: <http://zlab-carb-observatory.herokuapp.com/>

- c. We are publishing 3 manuscripts associated with this project (currently under review):
Scientific Reports: No time to rest – seasonal dynamics of non-structural carbohydrates in twigs of three Mediterranean tree species suggest year-round activity. Anna M. Davidson, Sylvia T. Le, Katelyn B. Cooper, Eden Lange, M.A. Zwieniecki

Perennial plants in temperate climates evolved short and long-term strategies to store and manage reserves in the form of non-structural carbohydrates (NSC; soluble sugars (SC) and starch (St)). NSC storage allows plants to survive seasonal periods of photosynthetic inactivity (dormancy). To study year-to-year seasonal patterns of trees' NSC dynamics that control phenology and yields, we established a large scale, multi-year study called the "Carbohydrate Observatory" using a citizen science approach with ~590 sites throughout the Central Valley of California. Monthly sampling tracked seasonal trends of starch and sugar levels in both xylem and phloem of twigs in *Prunus dulcis*, *Pistacia vera* and *Juglans regia*. Presented is the initial technical analysis of the first three years. With no exception, levels of reserves changed continuously throughout the year suggesting that even during dormancy, the average concentration of NSC, starch and sugars varies seasonally. In general, carbohydrate reserves are highest entering dormancy. During winter, NSCs slowly decrease to depletion during bloom time and remain low during summer until recovery near harvest. Starch is the major reserve compound in the wood of *P. dulcis* and *P. vera* while soluble sugars are the major reserves in *J. regia*. NSC content fluctuates throughout a season and significantly varies between years suggesting intrinsic and climatic effects on trees' energy reserves.

Tree Physiology: Spring phenology is affected by fall non-structural carbohydrates concentration and winter sugar redistribution in three Mediterranean nut tree species. Adele Amico Roxas, Jessica Orozco, Paula Guzmán-Delgado and Maciej A. Zwieniecki

Deciduous trees mostly rely on non-structural carbohydrates (NSC – soluble carbohydrates and starch) stored prior to dormancy to sustain both spring bloom and the initial phase of spring growth prior to the transition of leaves from sink to source. Winter management of NSC, their loss due to respiration, reallocation patterns and remobilization during spring, seems to be key to a timely and synchronous bloom. To assess tree dependence on NSC during dormancy, we tested whether the interruption of local branch NSC accumulation prior to dormancy by defoliation and the interruption of NSC translocation by phloem girdling influence spring phenology in three major deciduous Mediterranean nut crop species: *Prunus dulcis* (Mill.) D.A Webb, a hybrid between *Pistacia integerrima* (J. L. Stewart ex Brandis) and *P. atlantica* Desf. (referred to as *P. integerrima*), and *Juglans regia* L. Defoliation treatments had different effects on NSC concentration in different species depending on the time of application. However, despite the significant initial impact (increase or decrease of NSC concentration), with time this impact diminished resulting in overall similar concentrations between control and defoliated branches suggesting the presence of NSC reallocation during dormancy. Phloem girdling in *P. dulcis* and *P. integerrima* resulted in reduced export activity and greater NSC concentrations, while in *J. regia* girdling resulted in lower NSC concentrations, indicating that this species requires a net import of NSC during dormancy. Bud break was distinctly delayed by both defoliation and phloem girdling in all the three species, providing evidence of the significant roles that fall NSC accumulation and winter NSC management play in priming trees for spring growth resumption.

Tree Physiology: Winding up the bloom clock - do sugar levels at senescence determine how trees respond to winter temperature? Or Sperling and Maciej A. Zwieniecki

Variable winter temperatures cause a year-to-year discrepancy in the phenology of deciduous trees. It implies that an intrinsic 'winter clock' synchronizes bloom with the progression of winter to spring. The carbohydrate-temperature (CT) model established a mechanistic association between carbohydrate metabolism in dormant trees and hourly winter temperatures. By winter conditions across California and Washington state, and species-specific hereditary metabolic parameters, the model predicted the bloom times of *Prunus dulcis*, *Malus domestica*, *Pistachia vera*, and *Juglans regia* trees. There was a one-to-one correlation between actual and predicted bloom dates, yet with a deviation (RMSE) of 4-7 days. Incorporating variability in soluble carbohydrate concentrations at senescence (SC0), as observed in the field, significantly improved the CT model's predictions (RMSE 0.9 to 1.8 days). Interestingly, the CT model projected that high SC0 advances bloom and that low SC0 could delay bloom. After field trials support this finding, it could guide post-harvest field applications to manage fall SC0 and alter bloom time. Considering that genetics can alter plant metabolism (and not the weather), breeding programs could optimize the fit between crops and their environment, or their resilience to climatic shifts, by manipulating fall SC0.

Considering the current stage of the review process, we predict the three manuscripts will be published in the next few months.

d. Our large-scale approach allows for a first look into the relationship between seasonal NSC content in twigs and yield (Figure 2). Analyses reveal that there is a positive correlation between NSC content in winter (January and February) and yield. The total amount of sugars and starches in wood turns out to be the most important factor followed by sugar and starch in wood during January. There is also a very strong negative correlation for all types of sugars during summer and early fall, suggesting that high yield results in the exhaustion of reserves. The monthly-specific data can be used to predict yield, but we have to remember that NSC content is only a necessary component but not sufficient to warrant high yield as weather and management have a high post estimate influence on orchard performance. In Figure 3, I presented the results of yield prediction using the Extra Gradient Boost Regression. The features used in the model (variables) and their relative importance for yield prediction are listed in Table 1. The important finding is that NSC and starch explain ~30% of model variability. Finally, previous year's yield is one of the most important parameters for predicting yield, stressing the need to acquire robust yield data for the development of the model.

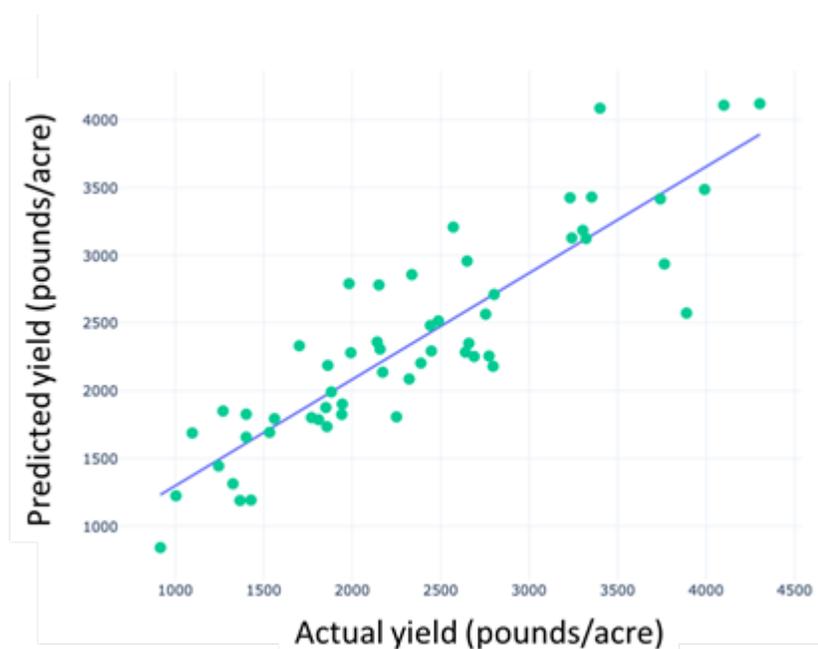


Figure 3. Regression between predicted and actual yield for test sing Extra Gradient Boost Regression model for data available in April. Features used in the model include previous year yield, orchard age, geographical location, monthly mean temperature, monthly total precipitation, total NSC, starch in wood and NSC use in the spring as the ratio between content in February and March or April. Mean Absolute Error: 296.99 pounds/acre (RMSE 393 pounds/acre) with accuracy of 86.3%.

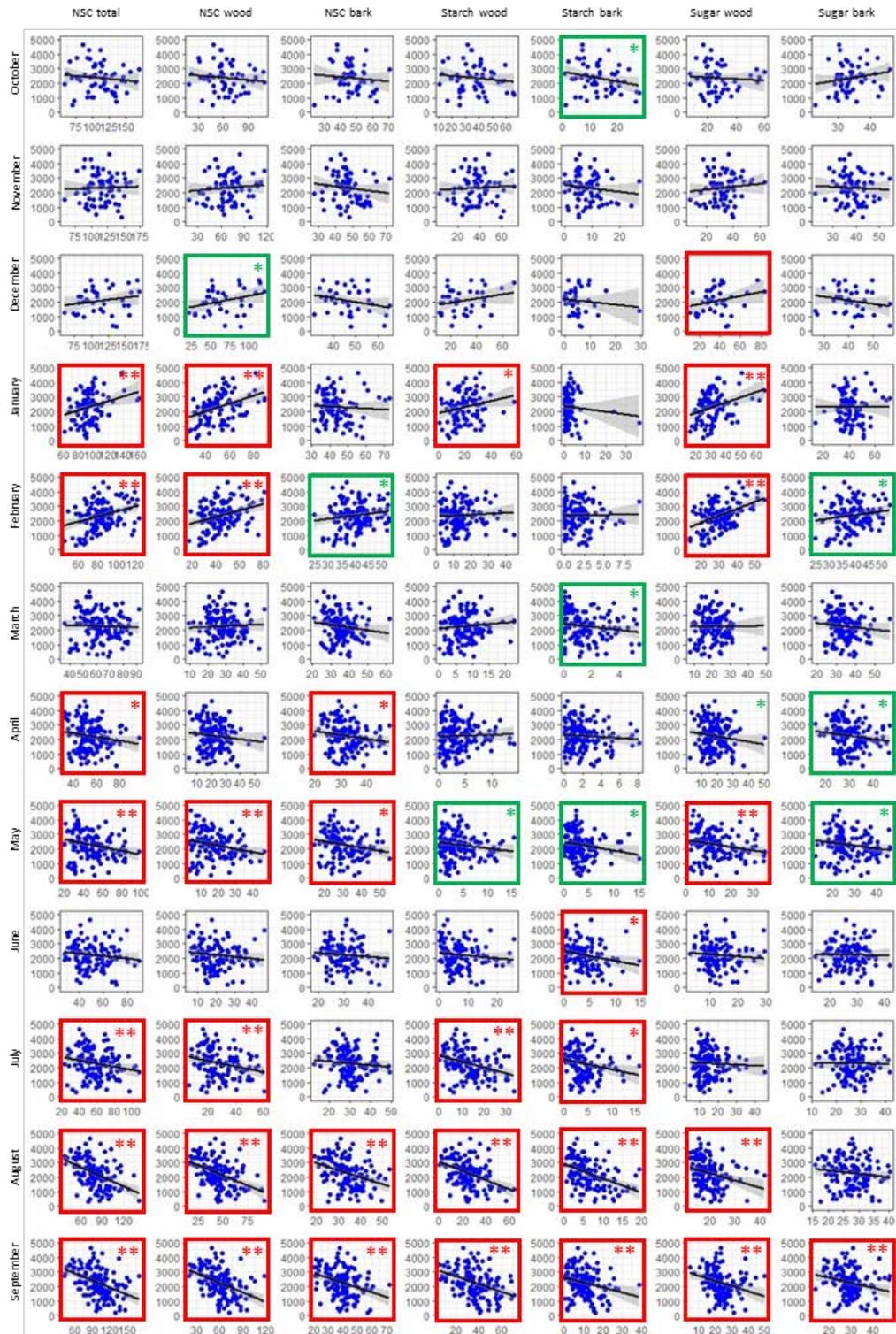


Figure 2. Visualization of relation between NSC content in twigs of almond and yield. Red boxes depict correlations with $p < 0.05$ and green with $p < 0.1$. The correlations in winter are generally positive and highly significant for January and February. Correlations in summer are mostly negative reflecting exhaustion of reserves if high yield was present.

Table 1. Relative importance of features in the yield prediction model. Feature include climatic and NSC variables. Colors underlines importance from high (blue) to low (red).

| Feature | Importance | Feature | Importance |
|-----------------------------|------------|------------------------------|------------|
| Mean temperature in March | 0.203 | NSC total in January | 0.013 |
| Starch in wood in October | 0.162 | Ratio of NSC February/April | 0.010 |
| Previous year yield | 0.076 | Starch in wood in April | 0.008 |
| Mean temperature in January | 0.054 | NSC total in March | 0.004 |
| Precipitation in January | 0.052 | Orchard age | 0.004 |
| Precipitation in March | 0.051 | Starch in wood in November | 0.003 |
| Precipitation in February | 0.050 | NSC total in December | 0.003 |
| Mean temperature in March | 0.046 | NSC total in October | 0.003 |
| Precipitation in October | 0.041 | Starch in wood in March | 0.003 |
| Precipitation in April | 0.036 | Ratio of NSC March/April | 0.002 |
| Precipitation in November | 0.032 | NSC total in February | 0.002 |
| Starch in wood in February | 0.030 | Mean temperature in October | 0.002 |
| Ratio of NSC February/March | 0.027 | NSC total in November | 0.002 |
| Mean temperature in March | 0.023 | Mean temperature in Dec | 0.001 |
| NSC total in April | 0.023 | Mean temperature in February | 0.000 |
| Starch in wood in January | 0.018 | Starch in wood in December | 0.000 |
| Precipitation in December | 0.018 | | |

The goal of the modeling effort is to provide growers with easy access to yield prediction specific to their orchard location and NSC content. A test site with one of the tested models is availed at zlab-yield-model.herokuapp.com. The interface allows users to enter site-specific information and if data are missing, the model will use average state values.

- e. A detailed analysis of the temporal dynamics of NSC content in almond trees suggests that late fall and early spring are the most significant periods affecting NSC content and their redistribution. During late fall, trees restore the levels of NSC required to survive winter. During spring, NSC are mobilized for bud break. Experimentally induced perturbation to these patterns significantly delayed or even halted flower and bud development (Figure 4). Early defoliation delayed bloom **over 2 weeks**. Fall girdling also delayed bloom highlighting the importance of NSC redistribution. The combination of defoliation and girdling significantly delayed bloom time for both apical and lateral buds.

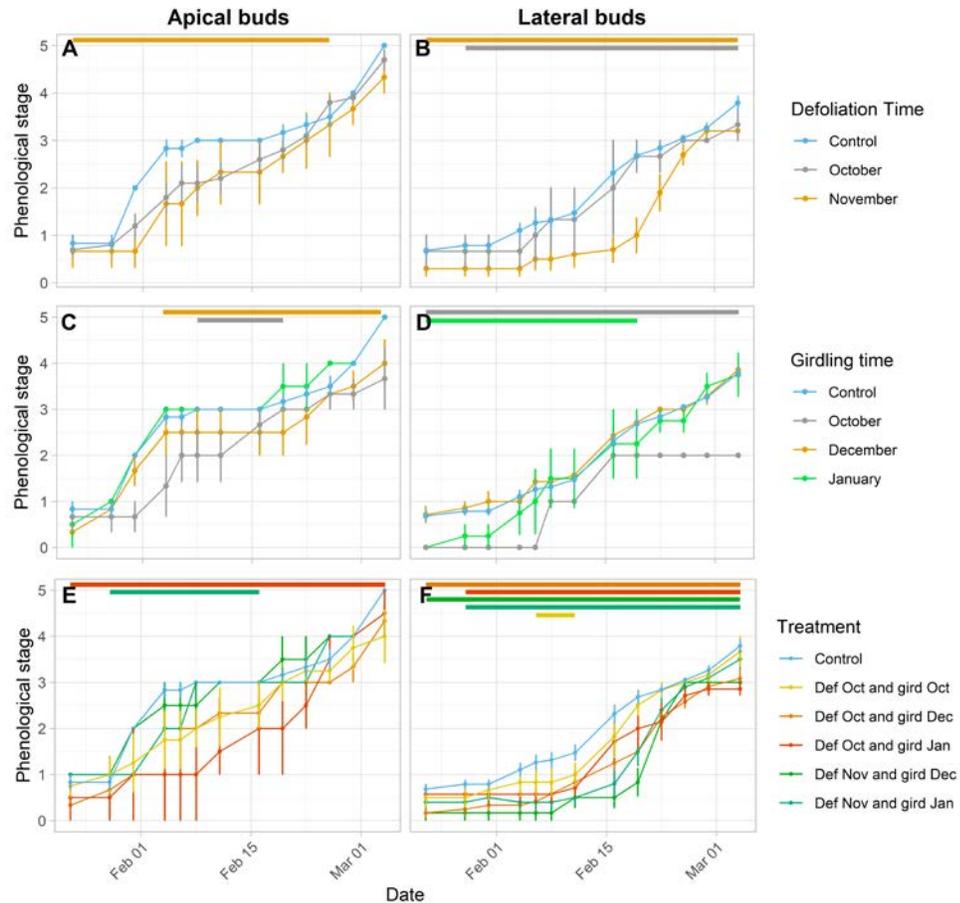


Figure 4. Impact of fall defoliation and girdling treatments on almond phenology. Horizontal bars at the top of each graph represent periods when there is a significant difference in bud phenological stage between control and treated twigs. (A and B) effect of defoliation, (C and D) effect of girdling, and (E and F) effect of the combination of girdling and defoliation. The defoliation, girdling and combination of the two resulted in a significant delay of apical and lateral buds development. (Pictures show the phenological stages used in the study)

Using 25 years of bloom data, we developed and parametrized a phenology model that utilizes fall NSC and winter hourly temperature as inputs. The analyses (Figure 5) revealed that soluble sugar content impacts phenology with earlier bloom being associated with higher levels of sugars in the fall.

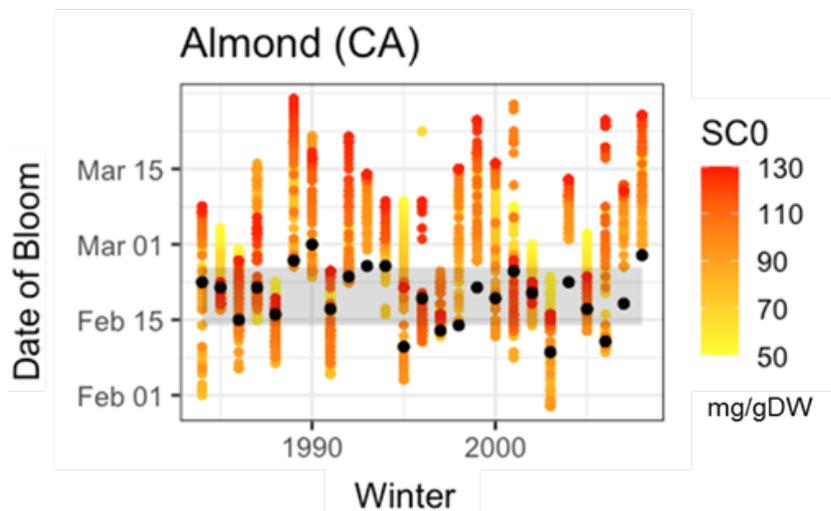


Figure 5. Impact of soluble sugar content in twigs of almonds in the fall on bloom time. Black dots represent true bloom time, and red to yellow dots represent the possible range of bloom time in a given year. Red color denotes high levels of soluble sugars (SC0) while yellow color denotes low levels. With only a couple of exceptions, high levels of soluble sugars in twigs during October resulted in earlier bloom.

Implications for orchard management:

- Twigs' NSC content in the winter is positively correlated with following summer's yield, while August-September NSC content is negatively correlated. This implies the need to develop robust post-harvest management practices that aim at the reconstitution of NSC content before natural defoliation.
- Any fall practices interrupting NSC accumulation, climatic impacts on NSC accumulation and disruption to phloem activity during dormancy can lead to a significant delay in bud-break time and asynchronous bloom.
- Carbohydrates play a significant role in models aiming at an early prediction of future yield. Our data suggest the potential to estimate yield in April with a relatively small mean error of ~300 pounds/acre. The yield model can be improved with an increased number of observations (e.g. NSC or previous years' yield) and including additional features (like bee hours). Additional data are also extremely important to avoid overfitting.
- NSC dynamics and its relationship with winter temperatures can be used to assess the progression of dormancy and help to provide insights for the use of bud-breaking chemicals.

D. Outreach Activities

1. Carbohydrate webinar (April 2020 – zoom meeting, ~40 attendees – growers, FAs and CEs)
2. Extension Article: Just Like People, Almond Trees Need Carbs To Thrive. <https://www.almonds.com/almond-industry/industry-news/just-people-almond-trees-need-carbs-thrive>.
3. Almond Work Group Zoom May 13th presentation about carbohydrate role in leafing and bloom.
4. Quarterly News Letters to participating growers.

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

The Carbohydrate Observatory is a research initiative providing analytical service to growers interested in a better understanding of NSC management of their orchards. Growers provide three twig samples (with wood and bark separated) per orchard that

are subsequently analyzed for soluble sugars and starch content in wood and bark. Analytical results are being published online at:
<http://zlab-carb-observatory.herokuapp.com>.

The 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 acidic buffer to hydrolyze all soluble carbohydrates.
- Using a colorimetric method (a spectrophotometer), the concentration of sugars is measured (Anthrone method) in a sample of buffer (50 μ L) and recalculated to express soluble sugar 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 via email (mzwienie@ucdavis.edu).

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

Additional information about methods used in specific studies and modeling are available in publications emerging from this work.

F. Publications that emerged from this work

1. List peer review publications in preparation, accepted or published
 - Anna M. Davidson, Sylvia T. Le, Katelyn B. Cooper, Eden Lange, M.A. Zwieniecki. 2021. No time to rest – seasonal dynamics of non-structural carbohydrates in twigs of three Mediterranean tree species suggest year-round activity. *Scientific Reports* (accepted)
 - Or Sperling and Maciej A. Zwieniecki 2021. Winding up the bloom clock - do sugar levels at senescence determine how trees respond to winter temperature? *Tree Physiology* (in review)
 - Adele Amico Roxas, Jessica Orozco, Paula Guzmán-Delgado and Maciej A. Zwieniecki. 2021. Spring phenology is affected by fall non-structural carbohydrates concentration and winter sugar redistribution in three Mediterranean nut tree species. *Tree Physiology* (in review)
2. Other publications (e.g. outreach materials)
Extension Article: Just Like People, Almond Trees Need Carbs To Thrive.
<https://www.almonds.com/almond-industry/industry-news/just-people-almond-trees-need-carbs-thrive>.
3. Please provide copies of publications