Project/Proposal Title: Measuring storage reserves to assess severity of biennial bearing

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Cooperators:

Project Duration: 1 Year

Total Project Request for Year 1 Funding: $ 58,927
Other related/associated funding sources: None

Budget 1
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Item	**2023**
Salaries[1]	$29,315
Benefits[2]	$13,062
Wages[3]	$6,500
Benefits[4]	$650

Equipment	$0
Supplies[5]	$8,900
Travel[6]	$500
Miscellaneous[7]	$0
Plot Fees	$0
Total	$58,927

Footnotes:
[1] Salary is requested for 16.7% of a post-doc and 50% of a research intern (technician).
[2] Benefits are calculated at 39.9% for the post-doc and 46.5% for the research intern (technician).
[3] Wages are for covering summer salary for the graduate assistant
[4] Benefits are calculated at 10% for summer graduate students
[5] Supplies are for field and lab consumables to conduct applied experiments for objective 1 and 2.
[6] Travel funds are requested for frequent travel to the Sunrise research orchard for PIs and personnel.

OBJECTIVES

1. To establish baseline levels of carbohydrate concentrations in storage organs of apple trees that are in different biennial bearing cycles.

2. To establish cost-effective and industry-adoptable methods for measuring non-structural carbohydrates in apple trees.

SIGNIFICANT FINDINGS:

Based on data from 2022 and 2023 dormant sampling, carbohydrate levels were higher in high cropping trees across different plant tissues for 'Honeycrisp', while 'Gala' showed little to no differences at low and high crop loads. Nitrogen levels had a more inconsistent response, showing greater variation from year to year.

- 2022 had better relationships between crop load and carbohydrate and nitrogen content than 2023, likely because of leaves freezing on the trees and poor translocation of reserves to stored tissues.
- Crop load affects end-of-year carbohydrate stored in woody tissues but with an unexpected positive relationship in some cases. We are continuing to explore these relationships further.
- Nitrogen can be related to crop load but appeared to be dependent on year (2022 vs. 2023)
- There are simple methods of assessing soluble sugars in woody tissues but not starch. We will continue to evaluate simpler methods of assessment during the winter of 2025 like visual iodine staining of different tree parts with low and high crop loads from the previous year.
- We are testing agronomic practices that improve the stability of return bloom in Honeycrisp apple that are based on this data

RESULTS AND DISCUSSION:

In 2022, carbohydrate levels of 'Honeycrisp' apple trees were significantly higher in high-cropped trees for all tissue types that were sampled. Stored carbohydrates accounted for 9-12% of total dry weight of different woody tissues. These values align with those reported for other perennial horticultural species (Fernandez et al. 2018). There were no differences in stored carbohydrates in tissues in 2023 (Figure 2) compared to 2022 (Figure 1). 'Honeycrisp' apple trees in 2023 showed no significant difference in carbohydrate levels among different bud types, although slightly higher carbohydrate levels in high-cropping trees were observed (Figure 2). Carbohydrate levels of 'Gala' apple trees in 2023 did not show significant differences at different crop loads (Figure 3). However, spurs and terminal shoots had lower carbohydrate levels in high cropping trees.

Nitrogen levels of 'Honeycrisp' apple trees in 2022 displayed an inverse relationship to carbohydrate levels. Nitrogen levels were significantly lower for different bud types in high cropping trees (Figure 4). Nitrogen content ranged from 0.8 to 1.2% of total dry weight for dormant above-ground woody tissues. There were no differences in stored nitrogen content in 2023 compared to 2022. Gala had slightly lower nitrogen content in stored tissues compared to Honeycrisp (Figures 5 and 6). Nitrogen levels of 'Honeycrisp' apple trees in 2023 were not significantly higher for different bud types, although non-significant differences were observed (Figures 4-6). Higher nitrogen levels were observed among different bud types of high cropping 'Honeycrisp' trees. Nitrogen levels of 'Gala' apple trees with high crops were lower in spurs, apical buds, and bourse shoots (Figure 6). Conversely, terminal shoots had higher nitrogen levels in high-cropping trees.

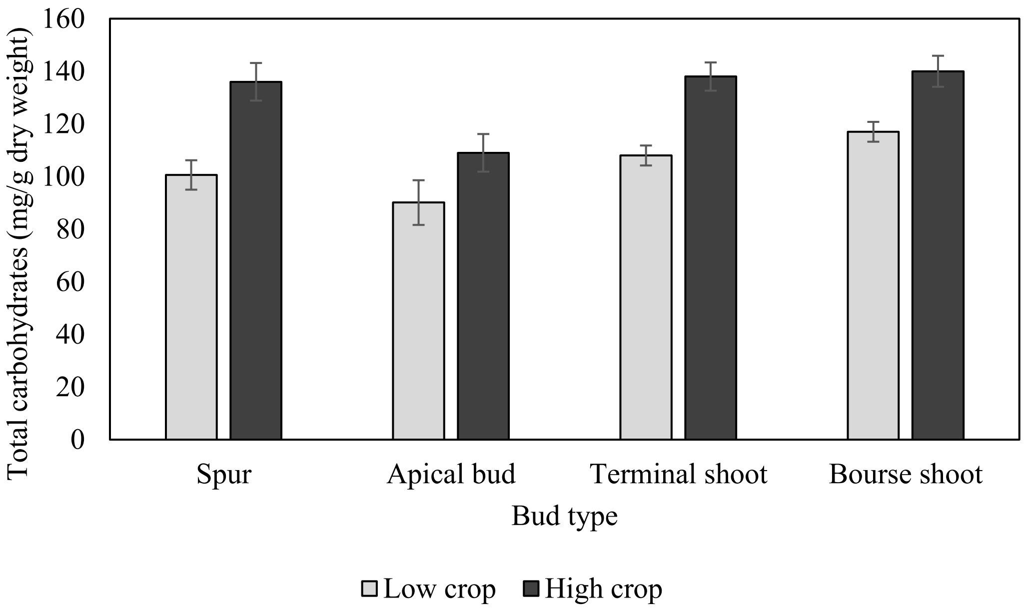

Figure 1. Total soluble carbohydrate (TSC) content (soluble sugars and starch combined) of spurs, apical buds, terminal shoots, and bourse shoots for 'Honeycrisp' apple trees in 2022.

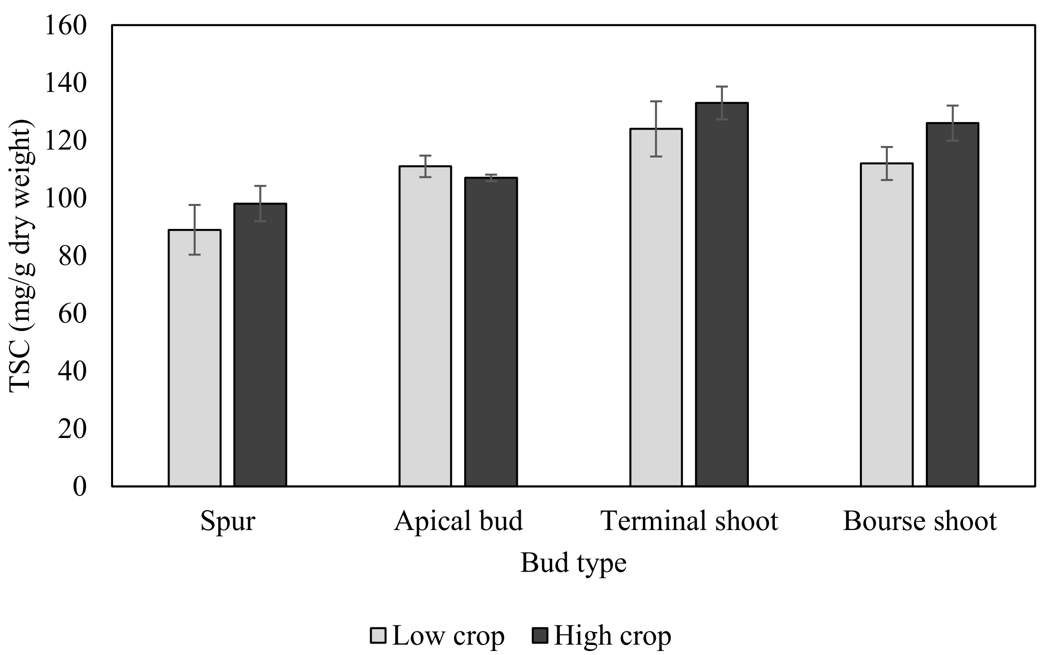

Figure 2. Total soluble carbohydrate (TSC) content (soluble sugars and starch combined) of spurs, apical buds, terminal shoots, and bourse shoots for ‘Honeycrisp’ apple trees in 2023.

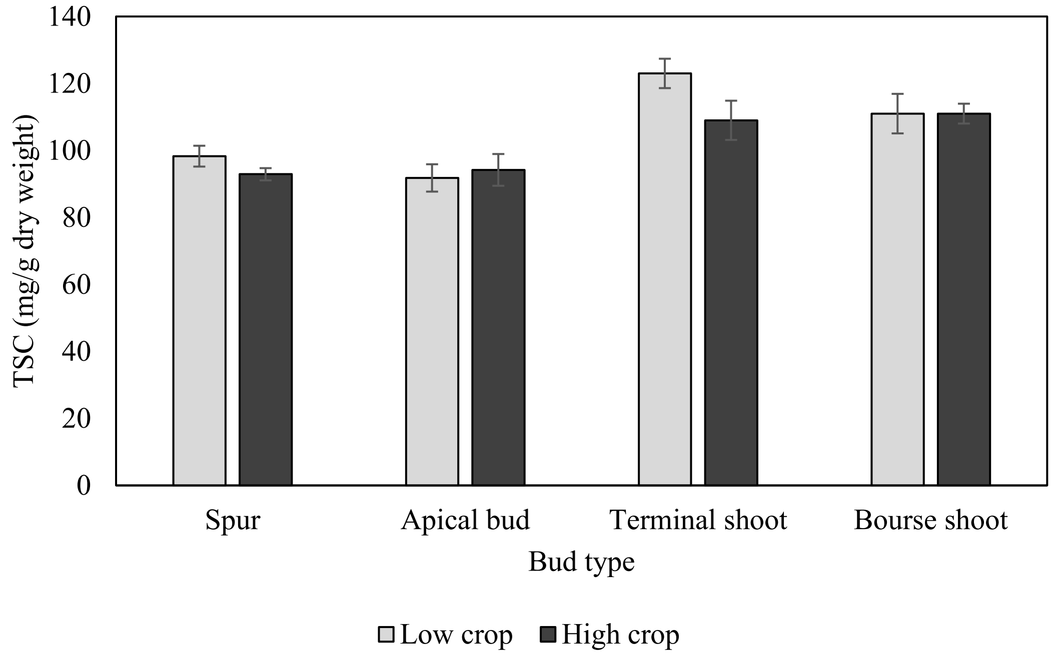

Figure 3. Total soluble carbohydrate (TSC) content (soluble sugars and starch combined) of spurs, apical buds, terminal shoots, and bourse shoots for ‘Gala’ apple trees in 2023.

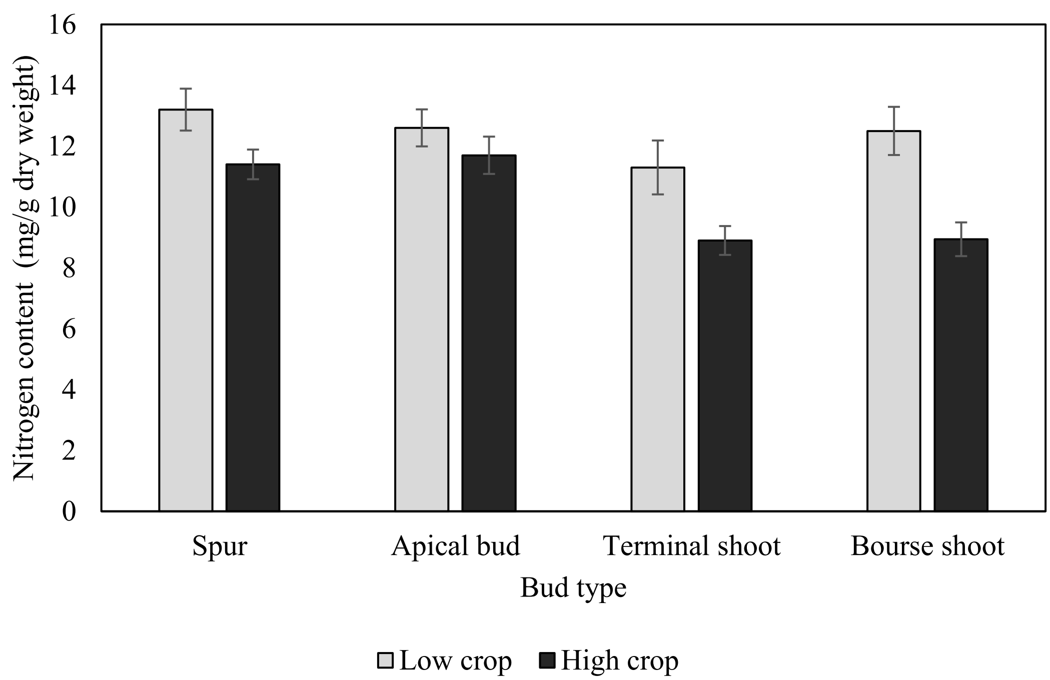

Figure 4. Nitrogen levels of ‘Honeycrisp’ apple trees in 2022 by bud type (spur, apical bud, terminal shoot, bourse shoot).

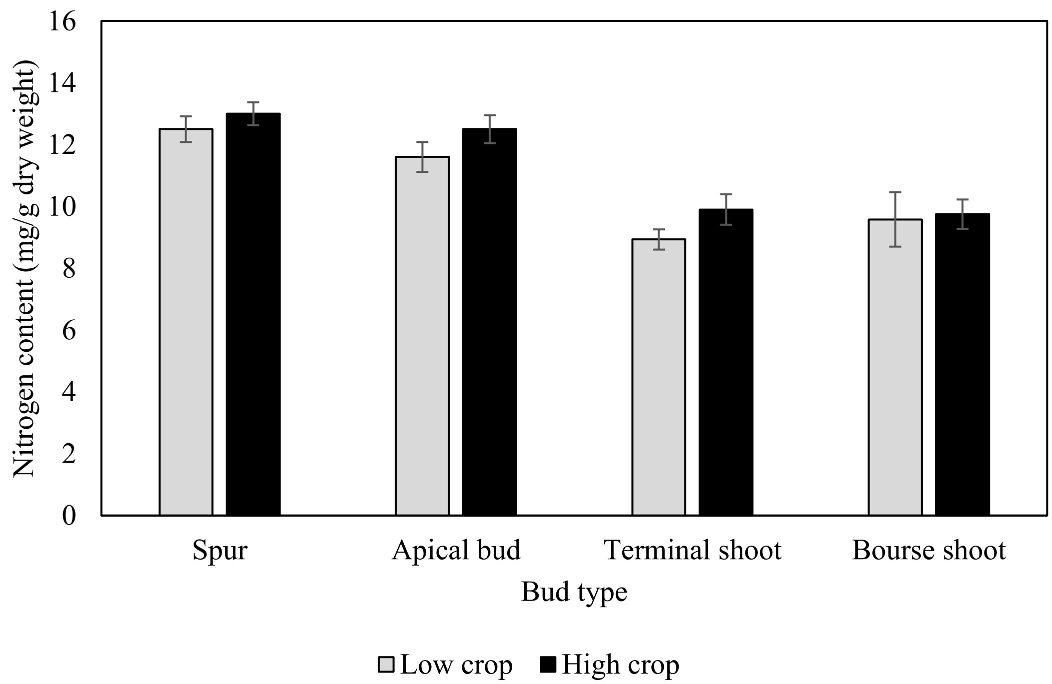

Figure 5. Nitrogen content of ‘Honeycrisp’ apple trees in 2023 by bud type (spur, apical bud, terminal shoot, bourse shoot).

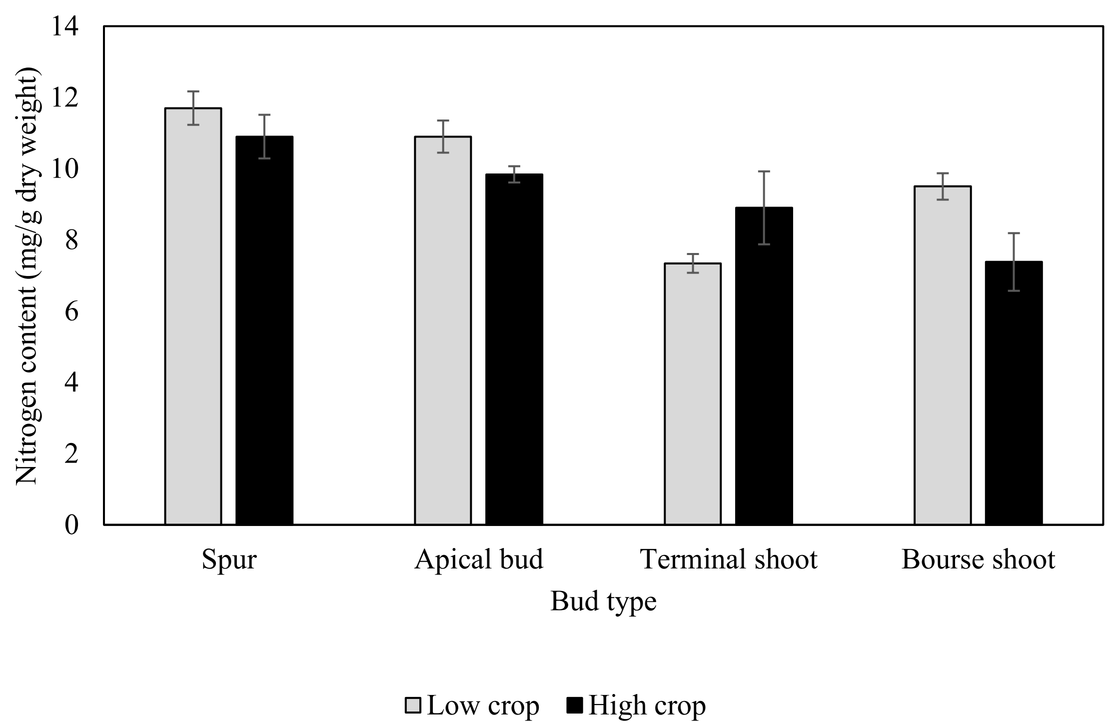

Figure 6. Nitrogen levels of 'Gala' apple trees in 2023 by bud type (spur, apical bud, terminal shoot, bourse shoot).

Crop load was positively related to stored carbohydrate levels in plant tissues at the beginning of dormancy. In 2022, effects were significant across different plant tissues. This relationship was less clear in 2023, although trees with higher crop loads still had higher stored carbohydrate levels than lower crop loads. Effects were inconsistent from year-to-year, highlighting the complex factors affecting stored carbohydrate content in apple trees. This is contrary to previous publications, where low-cropped trees were found to have higher carbohydrate levels in plant tissues. Higher starch concentrations have also been reported in flower buds when crop loads were low, supporting a negative correlation with tree crop load (Goldschmidt and Golomb, 1982).

Nitrogen was also measured from the dormant plant tissues that were collected in 2022 and 2023. Trees with low crop loads had significantly higher nitrogen concentrations in 2022. In 2023, the effect was not significant for any type of plant tissue. In general, nitrogen levels varied less with different crop loads when compared to carbohydrate levels. Year-to-year variation, however, was apparent in nitrogen content.

Carbohydrate and nitrogen levels changed significantly from 2022 to 2023, possibly due to environmental factors. In 2022, a quick sudden freeze in fall caused leaves to remain on trees throughout winter without undergoing regular senescence. Although it is well understood that leaf remobilization of carbohydrates and mineral nutrients contributes to stored reserves in woody tissue for the following year, the impact of this early freeze in this specific scenario is unknown. Without undergoing senescence, the leaves may have not been able to transport carbohydrates and nitrogen into the reserve pools of spurs and branches, increasing variation detected in 2022. However, this is a theory, and testing it is quite complicated. More long-term results are needed to identify trends and controlled experiments are needed. Most of the stored carbohydrates in woody tissue is already present prior to leaf senescence and it is possible that the final remobilization in healthy trees contributes a smaller portion of total stored carbohydrates than previously estimated.

In-season leaf/spur sampling:

Leaves were also collected throughout the 2023 growing season to study carbohydrate level fluctuations throughout the season. This was an addition to the project to better understand seasonal fluctuations in non-structural carbohydrate content prior to harvest. Leaves were collected starting on May 26 and continued weekly until November 6. Soluble sugars and starch content were measured on leaves collected throughout the season.

Soluble sugar content for low cropping trees was higher for the first four weeks of the season (Figure 7). This is the period where cell division is occurring, which is a significant resource demand, especially when crop load is high. Since the fruit are likely undergoing the peak rate of cell expansion in June and July, high cropping trees may be more affected by greater carbohydrate demands by fruit followed by an increase in leaf soluble sugar content later in the summer. A dramatic drop in sugar concentration occurred on August 18, which could be attributed to soluble sugar remobilization into fruit during the final stages of fruit development. This could be related to fruit ripening and maturation (harvest occurred on September 5 and 6). Soluble sugar content increased following harvest for trees with either low or high crop loads. Once fruits are harvested, carbohydrate demand would drastically decrease. Overall, sugar concentrations increase under both crop loads following harvest due to the lack of sink demand posed by fruits that were once on the trees.

Sugar concentration of 'Gala' leaves showed little differentiation throughout the season between high cropping and low cropping trees (Figure 8). Higher sugar levels in the beginning of the season are concurrent with photosynthetic activity and are subsequently used to fuel shoot growth, root growth, and fruit growth. A notable decrease in sugars from June 2 to June 9 could be attributed to the period where cell division is speeding up and fruits are placing greater demands on the overall soluble sugar supply in the tree. Another notable drop in sugars occurs on August 18 immediately following harvest. After harvest, sugars accumulate in leaves and increase, regardless of crop load. This lack of differentiation is explained by the lack of fruit on the trees after harvest, reducing the influence of crop load on sugar accumulation in leaves.

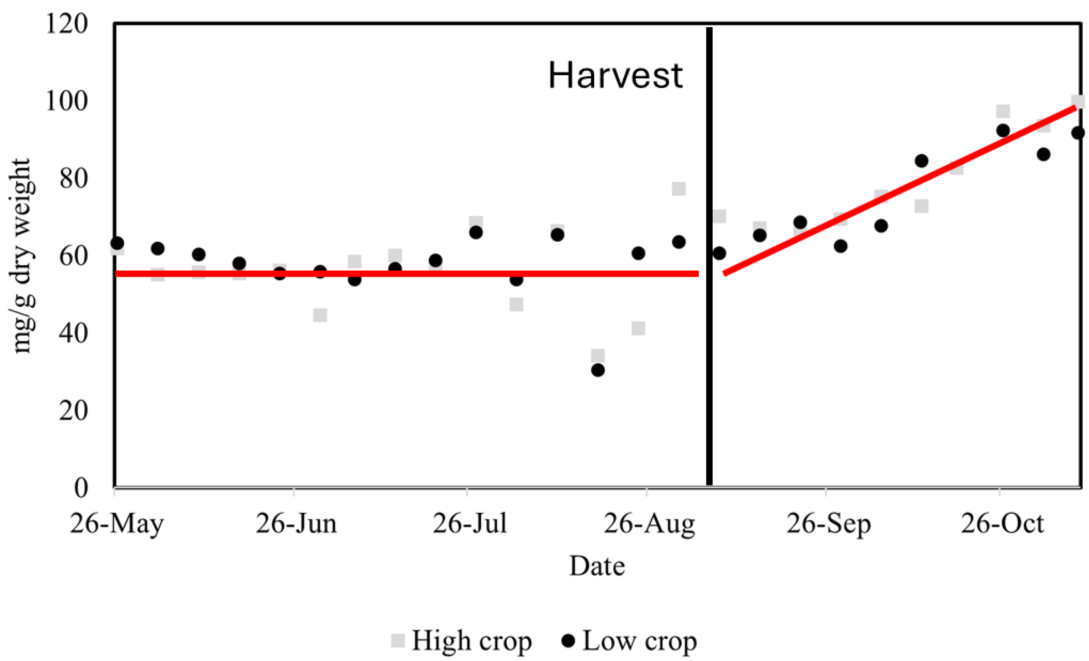

Figure 7. Weekly soluble sugar content of 'Honeycrisp' leaves in 2023 at low (black circles) and high crop loads (grey squares). Red lines are not statistical and are simply for illustrative purposes.

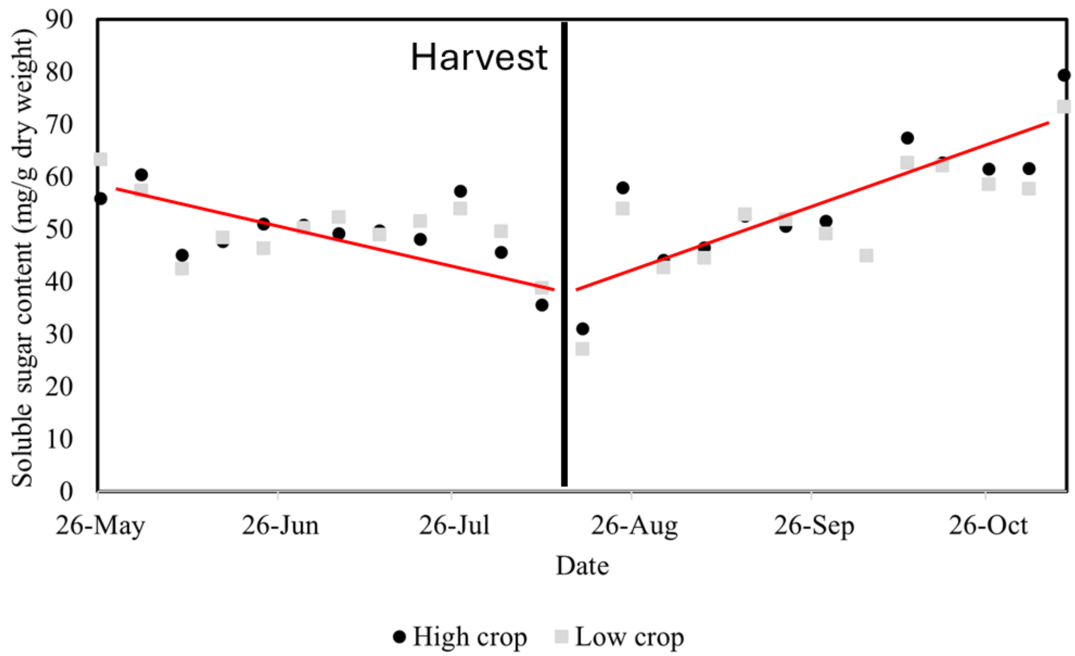

Figure 8. Weekly soluble sugar content of 'Gala' leaves in 2023 at low (black circles) and high crop loads (grey squares). Red lines are not statistical and are simply for illustrative purposes.

In 'Honeycrisp', starch concentrations were generally higher (although similar at certain points) for trees with low crop loads (Figure 9). These follow observations by Snyder-Leiby and Wang (2008) where starch grains accumulated in Honeycrisp leaves when crop loads were low. Trees with low crop loads had lower carbohydrate demand, which may contribute to higher starch content, as observed on June 30 and July 6. There was variability in starch content among sampling dates that could be caused by variability in environmental conditions (warm nights, cloudy/smokey days, or cold days with low photosynthetic activity). After harvest, starch levels appear to begin to accumulate rapidly. Low cropping 'Gala' trees had notably higher starch concentrations throughout the season. While not all samples have been analyzed for starch, those analyzed so far show consistently lower starch levels in high cropping trees. Comparing 'Gala' to 'Honeycrisp', 'Gala' shows much greater differentiation between high and low crop loads. This highlights a consistent relationship between crop load and starch concentration in 'Gala' which is less biennial. A stronger response occurred from June 23 to June 30 in high cropping trees, possibly due to fruits having greater demand and putting greater strain on leaf starch levels when crop levels are higher. Significant reductions in starch concentrations were observed on June 30 and August 3. After harvest, starch levels appear to accumulate at a slower pace, although data from high cropping trees shows a rapid increase on Sept 14.

Following up on this research, we conducted experiments where we looked at the impact of girdling and crop load on carbohydrate and hormone movement between branches as the rest of the tree. This research was conducted with support from the WSU Tree Fruit Endowment Funds to Lee Kalcsits and results will be published in addition to this report.

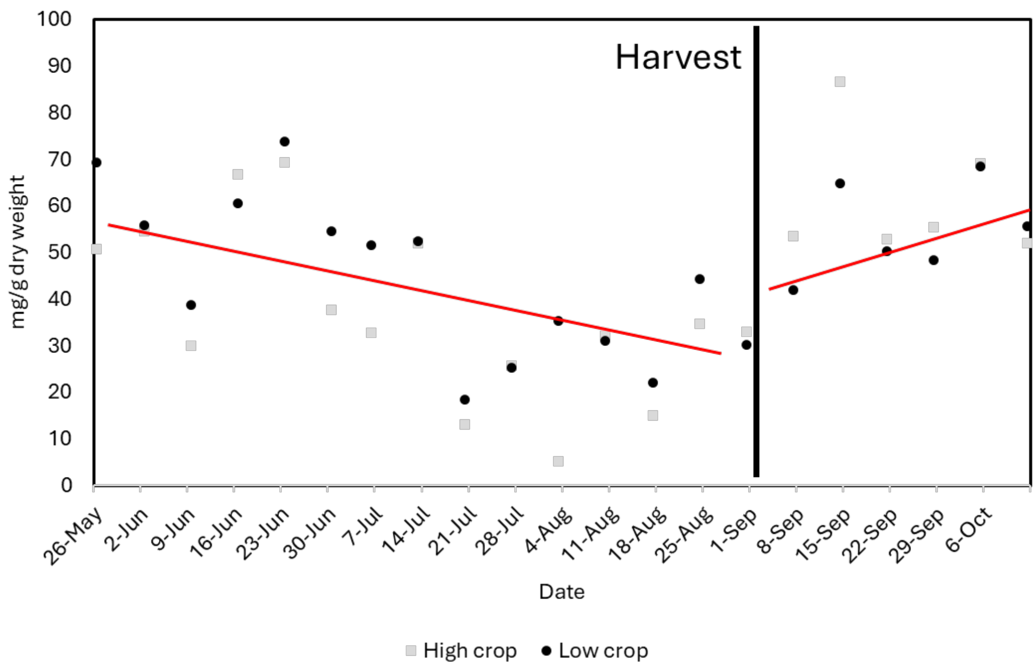

Figure 9. Leaf starch content of 'Honeycrisp' in 2023 at low (black circles) and high (grey squares) crop loads. Red lines are not statistical and are simply for illustrative purposes.

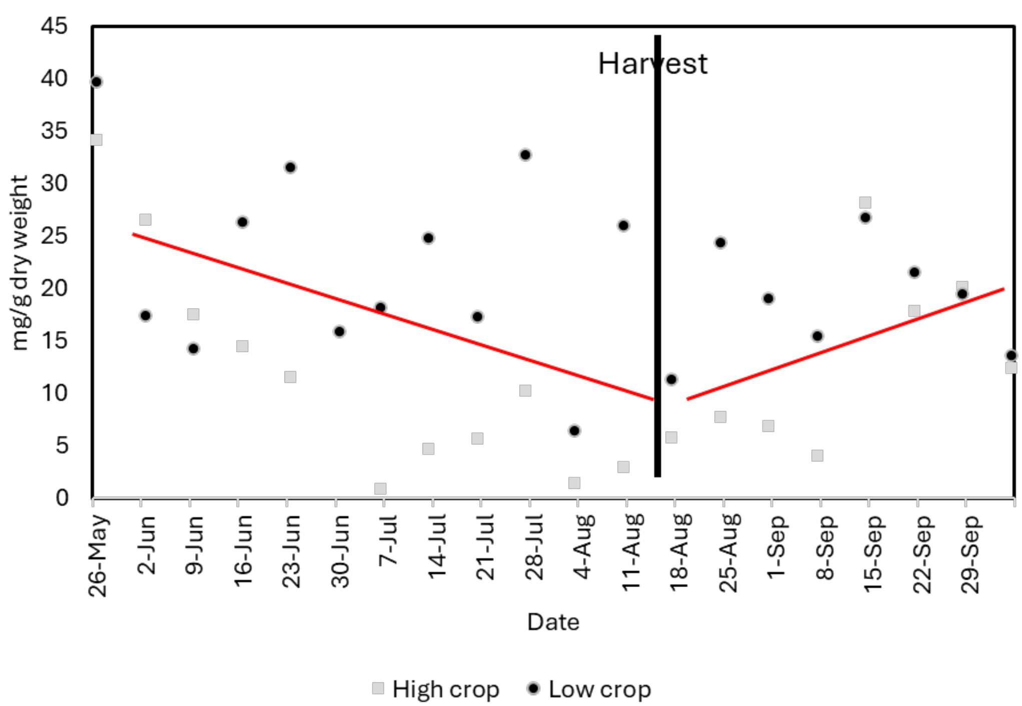

Figure 10. Leaf starch content of 'Gala' in 2023 at low (black circles) and high (grey squares) crop loads. Red lines are not statistical and are simply for illustrative purposes.

ADDITIONAL ITEMS

Timing of carbohydrate analysis procedure:

Spurs, bourse shoots, terminal shoots (1-year-old wood), and apical buds were collected for sugar and starch measurement. Samples were microwaved for 180 seconds at 800W to deactivate NSC-modifying enzymes (Quentin et al., 2015) and dried in a freeze-dryer (Labconco, Kansas City, MO). Samples were then homogenized with a Powergen High Throughput Homogenizer (Fisher Scientific, Waltham, MA). Protocols from Landhäusser et al. (2018) were followed for non-structural carbohydrate extraction. Samples were placed in 80% hot ethanol (EtOH) at 90°C for 10 minutes. The supernatant was extracted for soluble sugar quantification, while the remaining starch pellet was washed twice before being left to dry. Soluble sugars were determined and analyzed using an anthrone-sulfuric acid assay (Leyva et al., 2008), then read in a multi-detection microplate reader (Bio-Tek Instruments, Winooski, VT). Starch was quantified using a glucose hexokinase-6-phosphate (GHK) enzymatic assay (Landhäusser et al., 2018), then read in a multi-detection microplate reader (Bio-Tek Instruments, Winooski, VT).

The method of measuring starch and sugar concentrations can be time-consuming, although practicing the procedure allows for more efficient processing. There are 5-6 main components of the process: (1) microwaving, freeze-drying, and grinding samples; (2) weighing samples into tubes; (3) sugar extraction and starch wash; (4) starch extraction for absorbance reading; (5) absorbance reading of starch; and (6) absorbance reading of sugar. Here is the breakdown:

(1) 2.12 minutes per sample (freeze-drying takes 1-2 days, but is passive process)
(2) 1.7 minutes per sample (efficient speed after running many samples)
(3) 4.3 minutes per sample
(4) 2.3 minutes per sample
(5) 5.63 minutes per sample (plenty of passive time built into the process while microplate is shaking on device)
(6) 1.88 minutes per sample

In total, the average time is from start to finish is 17.93 minutes per sample. This process is most efficient with a 96-well micro-centrifuge tube holder. Running less than 96 samples becomes less efficient because of steps where samples can be run together, such as the hot water bath. Utilization of an electronic pipette dispenser also allows for quick dispensing of materials into sample tubes.

References

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EXECUTIVE SUMMARY

Project Title: Measuring storage reserves to assess the severity of biennial bearing

Keywords: carbohydrates, nitrogen, starch concentration, crop load

Abstract: Biennial bearing is not a novel concept for fruit trees, likely accompanying them since before their domestication. Biennial bearing, or alternate bearing, is characterized by a heavy crop one year, the "on" year, accompanied by little to no crop the following year, the "off" year. While environmental factors such as spring frosts and drought can initiate a biennial bearing cycle in fruit trees, genetics play an important role, with certain cultivars having a greater likelihood of bearing biennially. In apples, 'Honeycrisp' is prone to biennial bearing, even when environmental factors remain constant and in good supply. Regenerative buds can make up more than 90 percent of buds in an "on" year, while the "off" year maybe 20 percent or less of the buds being regenerative. Nonstructural carbohydrates fulfill distinct roles within plants, influencing flower bud initiation and fruit bud formation. Moreover, nitrogen reserves play a key role in fueling spring growth and flowering. We sought to quantify nonstructural carbohydrate and nitrogen levels of various plant tissues in both "on" and "off" trees of 'Honeycrisp' and 'Gala'. Crop load from each tree was used to allow for greater distinction between "on" and "off" trees. Carbohydrate levels increased linearly with crop load, although results showed greater separation in 2022 than 2023. We theorize that abnormal events in 2022, when leaf senescence did not occur going into winter, could have disrupted carbohydrate and nitrogen translocation patterns in the trees, causing data to become more significant. Crop load was more significant in affecting carbohydrate and nitrogen levels of 'Honeycrisp' than 'Gala'. Weekly leaf sampling indicated a clear impact of crop load and fruit development on sugar and starch concentrations of trees throughout the growing season. Carbohydrate and nitrogen levels of trees impact biennial bearing, although the strength and influence of this relationship are currently unclear. Follow up studies on the interactions between storage reserves and hormone movement during floral bud initiation will help support observations in this report.