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Role of Bark Color on Stem Temperature and Carbohydrate Management during Dormancy Break in Persian Walnut

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Abstract. **Temperature is assumed to be the principal regulatory signal that determines the end of dormancy and resumption of growth. Indirect evidence that stem temperature interferes with phenology comes from the common orchard practice of painting stems to protect them from disease. This work studies the effects of application of white paint to the stems of persian walnut (*Juglans regia*) trees on winter stem temperature, carbohydrate content, and spring phenology. Painting bark resulted in the delay of budbreak by several days, higher nonstructural carbohydrate (NSC) concentrations in the bark and wood of painted extension shoots and changes in the spatial gradients of NSC during budbreak. The demands of maintenance respiration exceeded mobilization from local carbon pools during bud development suggesting a potential role of carbohydrate transport during spring budbreak in persian walnut. Painting provides an exciting perspective for mitigating effects of milder winter in orchards. The effect of reducing diurnal and spatial temperature variability limits early budbreak, NSC depletion associated with intense maintenance respiration, freeze–thaw cycles and frost dehardening.**

Many woody perennials from temperate and Mediterranean climates are dormant during winter. They respond to the fall reductions in temperature and photoperiod by halting growth, shedding leaves, and acquiring cold hardiness (Charrier et al., 2015). Once dormant, temperature-regulated biological activity signals the progression of winter (summation of chilling hours) into spring (accumulation of heat), and results in the timely end of dormancy and resumption of growth (Campoy et al., 2011; Pope et al., 2014; Sánchez-Pérez et al., 2014). Temperature is assumed to be the principal regulatory signal that determines this progression, but it also has an influence on the regulation of metabolic rates in all biological systems (Heide, 2008, 2011). Thus, it can affect dormant tree physiology and can indirectly affect the timing of budbreak (Alves et al., 2004, 2007; Bonhomme et al., 2010; Charrier et al., 2015; Zwieniecki et al., 2015). However, winter temperatures are shifting. Climate models predict that Mediterranean and temperate climates will experience increase in diurnal temperature variation in the upcoming decades (Field et al., 2014; Luedeling et al., 2013). These changes would have significant implications for winter biology of trees. Warmer winters result in the reduction of chill hour accumulation, leading to erratic phenology and the loss of synchronization in flowering (Pérez et al., 2008). Indeed, the negative impacts of warming winters are already visible across both natural ecosystems and fruit or nut cropping systems as phenological shifts and reduction in yield (Améglio et al., 2000; Cleland et al., 2007; Ford et al., 2016).

Models of chill hours (Utah chill model, dynamic model) have been developed to predict budbreak based on the accumulation of cold temperatures (chilling) followed by warm temperatures (forcing). Unfortunately, these models are descriptive, requiring continuous recalibration to new locations, years, species, or even genotypes (Aslamarz et al., 2010; Luedeling et al., 2013; Pérez et al., 2008). The limitations of these models emanate from sparse knowledge about the physiological or genetic mechanisms involved in perceiving and responding to chilling. Meanwhile, functional–structural plant models have been developed to study plant reproductive and vegetative growth from the perspectives of carbohydrate

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partitioning and source–sink relationships (Allen et al., 2005; Da Silva et al., 2014; DeJong et al., 2011). In these models, winter biology is usually represented as maintenance respiration, likely undermining an entire suite of biological activities related to winter temperature which influences winter carbohydrate management and subsequent spring performance (Bonhomme et al., 2005, 2010; Lacointe et al., 2004). As persian walnut orchards are long-term investments, mediating the effects of winter temperature changes may become a major task for orchard management in the near future (Melke, 2015; Wang et al., 2016). Anchoring future models with mechanistic physiological principles of dormancy and phenology may increase their predictive power and so provide better tools for orchard management.

Interestingly, indirect evidence that stem temperature interferes with phenology comes from the common orchard practice of painting stems to protect them from disease (Karels and Boonstra, 2003; Sheppard et al., 2016). White bark is also naturally displayed in species of *Betula*, *Fraxinus*, and *Populus* among others, and it is generally assumed that white bark affects stem heat balance by reducing the effects of radiation (Campbell and Borden, 2005). Increasing the bark reflectivity by whitening it may thus provide a means of reducing daytime stem overheating (Karels and Boonstra, 2003). However, the specific effects of stem whitening on stem temperature are unknown. We can assume that they depend on multiple parameters such as exposure to direct sunlight, duration of exposure, tree water content, stem thickness, original bark color, whitened bark color, bark roughness, wind speed, and air temperature. We can also hypothesize that if stem whitening affects stem temperature, it also affects metabolic reaction rates in stems. While short-term metabolic responses to small changes in stem temperature may not be significant, NSC status may be cumulatively impacted over the course of an entire winter.

The metabolism of NSC is essential to many aspects of winter biology including frost resistance, survival throughout the dormancy period, the breaking of dormancy, and the breaking of buds (Bonhomme et al., 2005; Charrier et al., 2015). To prevent freezing stress and ice damage in tissues, trees increase intracellular soluble carbohydrate (SC) concentration to increase osmotic pressure of cells. During dormancy, maintenance respiration requires mobilization of NSC for survival (Bonhomme et al., 2005). Resumption of growth and organogenesis of new photosynthetic organs requires mobilization of NSC as building blocks for new tissues (Hartmann and Trumbore, 2016). Besides, maintenance respiration, NSC status or concentration in a particular tissue, the transport and relative content of NSCs between soluble and nonsoluble forms are all influenced by temperature (Allen et al., 2005; Zwieniecki et al., 2015).

In the presented work, we study how the application of white paint to the stems of young persian walnut trees affects winter stem temperature, carbohydrate content, and spring phenology. We hypothesize that lower temperatures in painted stems reduce maintenance respiration, preserving higher carbohydrate reserves throughout the winter and into the spring. In addition, we test if the observed changes in carbohydrate content can be explained in functional tree models with maintenance respiration alone. Finally, we discuss the potential role of temperature in spring carbohydrate mobilization and bud growth as well as the implications of delayed budbreak in painted stems.

Materials and Methods

Respiration and temperature measurements. Stem temperature and respiration were measured on the extension shoots of 4-year-old persian walnut trees ('Chandler' on *Juglans hindsii* × *J. regia* 'Paradox' hybrid rootstock) grown in an orchard at the University of California, Davis in central California (lat. 38.537197°N, long. 121.793257°W). Trees were 4 m tall with diameter at breast height around 10 cm and tree spacing was 3.7 × 4.6 m. The paint treatment was applied to three trees on 1 Mar. 2016 while buds were dormant at stage 0. One vertical nonbearing extension shoot was painted per tree on a 1-m-long segment with a latex paint (57901 light base; Valspar Corp., Minneapolis, MN) diluted 1:1 with water. Diameter of the base of the extension shoots was around 2 cm. K thermocouples (Omega Engineering, Stamford, CT) were placed on painted and control extension shoots on the north and south surfaces of the stem as well as under the bark also on both north and south surfaces. Before thermocouple deployment, all thermocouples were calibrated. A power drill with a small drill bit was used to place under bark thermocouples ≈2 mm into the stem. Thermocouples were then glued in place using cyanoacrylate glue (Loctite 505; Loctite Corp., Düsserdorf, Germany). Stem temperatures were recorded every 5 min using a data logger (CR1000; Campbell Scientific, Logan, UT) from 4 Mar. to 4 Apr. 2016. Comparative air temperature and radiation data were collected from a California Irrigation Management Information System (Durham CIMIS Station #12) station located 1.5 km from the orchard (lat. 38.535694°N, long. 121.77636°W).

Stem respiration was estimated for both painted and control extension shoots. Shoots were harvested and brought to the laboratory where respiration rate was estimated by measuring CO_2 efflux from stem segments (LI-6400; LI-COR, Lincoln, NE) at 24 °C on 10-cm-long sections (n = 6) (Sperling et al., 2015). The ends of each stem were covered with wax paper (parafilm M®; Bemis Co., Neenah, WI) to restrict gas exchange to exposed bark. CO_2 efflux was recorded every minute for at least 2 h. Efflux was expressed by both surface area of stem and volume.

Modeling stem temperature and statistical analysis. Stem temperature ($T_{S(t)}$) variability was fitted using a linear mixed-effect model including air temperature ($T_{A(t)}$), solar radiation ($R_{(t)}$), and last measured difference between stem temperature and air temperature [$(T_S - T_A)_{(t-1)}$] as independent variables, $T_{A(t)} \times [(T_S - T_A)_{(t-1)}]$ interaction and random effect for each tree studied (see Eq. [1]). Air temperature represents the main source of heat conduction, radiation provides a source of energy input, and the measured difference between stem and air temperatures allows us to approach heat transfer dynamically. Each independent variable had a significant effect on stem temperature ($P < 0.001$) as well as the interaction between $T_{A(t)}$ and $(T_S - T_A)_{(t-1)}$ [$P < 0.05$ (Table 1)]. The following model resulted in the lowest Akaike information criterion:

$$T_{S(t)} = R_{(t)} + T_{A(t)} + (T_S - T_A)_{(t-1)} + T_{A(t)} \times (T_S - T_A)_{(t-1)} + \text{random effect(tree)} \quad [1]$$

Once validated, the model was applied in Spring 2016 to an experimental walnut orchard located at California State

University, Chico (lat. 39.695641°N, long. 121.824207°W) using nearby CIMIS data for air temperature and solar radiation values (Durham CIMIS Station, lat. 39.608639°N, long. 121.824430°W). In Spring 2017, the model was applied to the orchard at the University of California, Davis (lat. 38.537197°N, long. 121.793257°W). To assess that the model was not site specific, two different sites were studied. The variable $(T_S - T_A)_{(t-1)}$ was assumed to be 0 at the end of the first night, an assumption validated by direct measurements which show that in the nightly absence of an external heat source, stem temperature equilibrates with air temperature. Cumulative respiration was then estimated from respiration measured at 25 °C in the laboratory, assuming a thermal coefficient (Q_{10}) of 2. The associated consumption of carbohydrates was estimated in glucose equivalents.

Carbohydrates and phenology. Carbohydrate and phenology data from Spring 2016 were collected from 2-year-old persian walnut trees ('Chandler' on 'Paradox' rootstock) grown at California State University, Chico. Carbohydrate and phenology data from Spring 2017 were collected from 5-year-old persian walnut trees ('Chandler' on 'Paradox' rootstock) grown in the orchard at the University of California, Davis. Chico and Davis sites are 132 km away and show very similar weather conditions. The paint treatments were applied on 23 Feb. 2016 and on 17 Jan. 2017 to individual nonbearing extension shoots on 10 randomly selected trees (one extension shoot per tree). At this time of year, the site had already met walnut chilling requirement (Pope et al., 2014). The individual nonbearing extension shoots chosen for painting were painted bottom to top using latex paint (57901 light base) diluted 1:1 with water, following common management practice. The most apical 0.4 m of each painted extension shoot was left unpainted. Five extension shoots were harvested from both painted and paired unpainted (control) shoots from each tree on 29 Mar. 2016 and on 5 Apr. 2017, when the apical buds of controls had all reached developmental stage 3, as defined by Tixier et al. (2017). This stage is characterized by fully unfurled leaflets, but no net photosynthesis (Tixier et al., 2017). A second set of shoots was harvested for each treatment once all painted stems had reached developmental stage 3 on 4 Apr. 2016 and 10 Apr. 2017, respectively, for both years. Extension shoots were photographed and the phenological stages of buds along each shoot were recorded at each harvest. Stem samples were collected at different positions from the apical bud for NSC measurements (starch and SC). Bark was removed from the wood immediately and the samples were then dried at 70 °C for 48 h before grinding into a fine and homogeneous powder. SCs were extracted by incubating 25 mg of dry material in 1 mL of deionized water for 15 min at 70 °C followed by centrifuging for 10 min at 21,000 g_n. The supernatant was diluted 1:20 and quantified using anthrone as a reagent [0.1% (m/v) in 98% sulfuric acid] by reading absorbance at 620 nm (Leyva et al., 2008). 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 5 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, the samples were centrifuged for 5 min at 21,000 g_n, the supernatant was diluted 1:20 and quantified using the method described above.

Statistical analysis. Data presented in Fig. 1 were analyzed with mixed effect logistic regression with treatment and date as fixed factors and trees as random factor. Data presented in Figs. 2–4 were analyzed with linear mixed effect models using the nlme package from R (R Core Team, 2013), following Pinheiro and Bates (2000). For NSC distribution analysis, the experimental design was a split-block design in blocks, where the trees are blocks, shoots are main plots, and positions are subplots (Steel and Torrie, 1980). We used linear mixed effect models with date and stem segment position as fixed factors and each individual shoot measured repeatedly over distances as random factor. Analyses of variance (ANOVA) were

Table 1. Probability values from analysis of variance performed on linear mixed effect models on stem temperature of persian walnut trees ('Chandler' on 'Paradox' rootstock). Stem temperature ($T_{S(t)}$) at a given time t was fitted to mixed effect model with the explanatory variables: air temperature ($T_{A(t)}$), solar radiation ($R_{(t)}$), and last measured difference between temperature and air temperature $[(T_S - T_A)_{(t-1)}]$. Symbol "×" represents the interaction between two factors.

Effect	Control $T_{S(t)}$	Paint $T_{S(t)}$
$R_{(t)}$	<0.0001	<0.0001
$T_{A(t)}$	<0.0001	<0.0001
$(T_S - T_A)_{(t-1)}$	<0.0001	<0.0001
$T_{A(t)} \times T(T_S - T_A)_{(t-1)}$	0.0309	0.0311

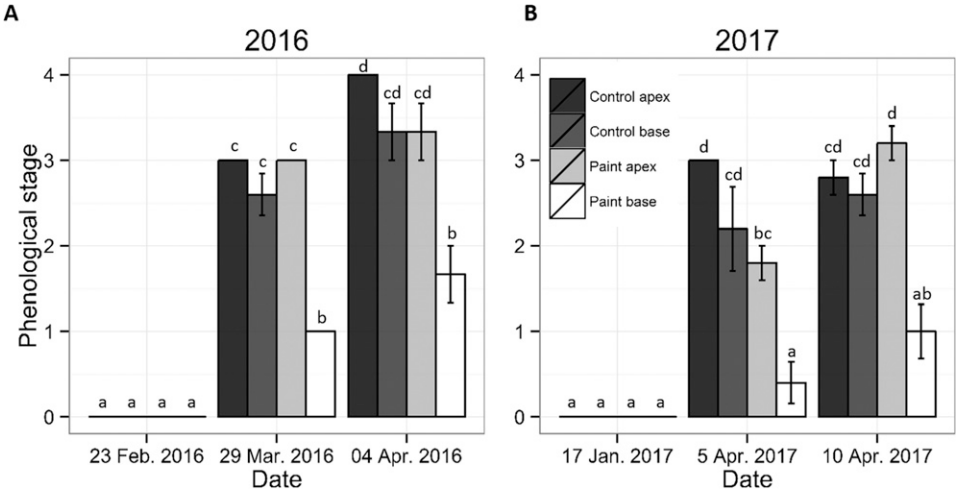

Fig. 1. Effect of painting treatment on persian walnut ('Chandler' on 'Paradox' rootstock) budbreak phenology during (**A**) Spring 2016 and (**B**) Spring 2017. Nonbearing extension shoots were painted in white whereas buds were dormant and phenology was compared with control extension shoots at stage 0 (dormant buds, 23 Feb.). Once the buds on apex reached stage 3 (nonphotosynthetically active leaves fully developed, 29 Mar. and 4 Apr.), stages were identified on the apex or at 70 cm from the branch apex (base) on five extension shoots per treatment. Average of phenological stage is plotted and error bars represent SE. Data were analyzed using mixed effect logistic regression. Data with different letters are significantly different according to a Tukey's honestly significant difference test ($P < 0.05$).

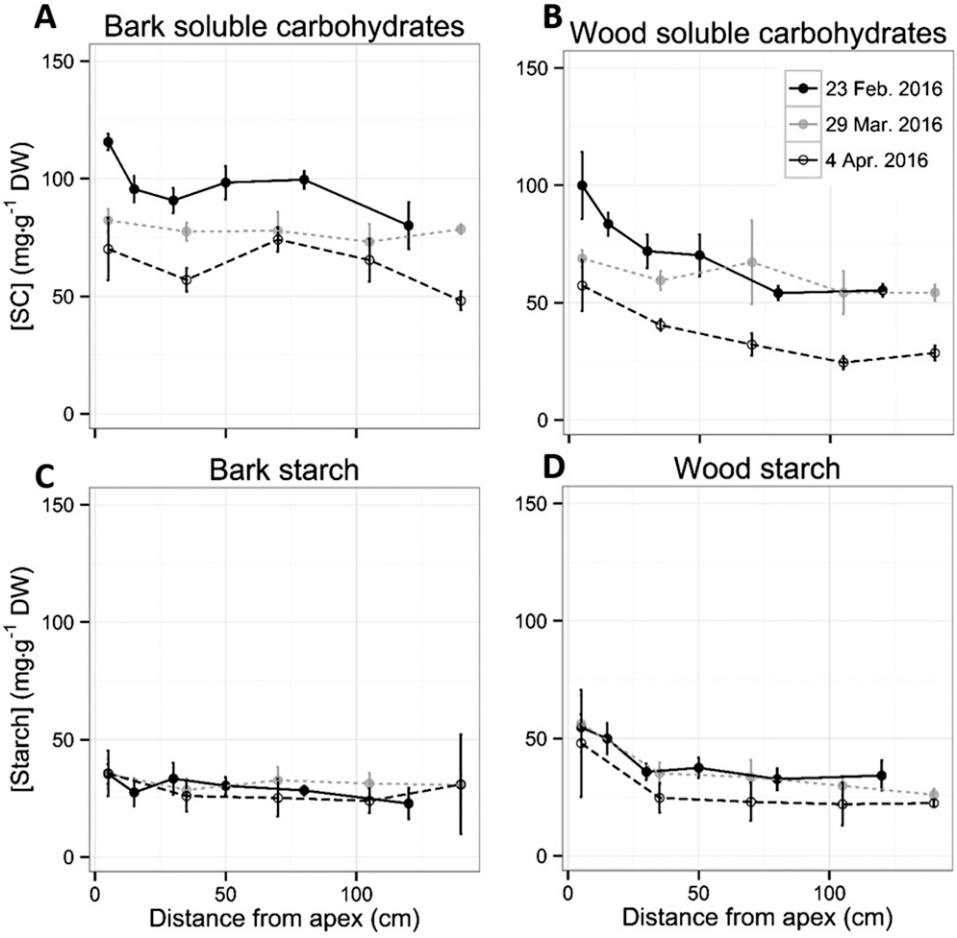

Fig. 2. Temporal and spatial distribution of carbohydrates in (**A**, **C**) bark and (**B**, **D**) wood in control persian walnut ('Chandler' on 'Paradox' rootstock) nonbearing extension shoots during budbreak. Shoots were collected at stage 0 (dormant buds, 23 Feb.), stage 3 (nonphotosynthetically active leaves fully developed, 29 Mar.) and stage 4 (photosynthetic leaves, 4 Apr.) during Spring 2016. (**A**, **B**) Soluble carbohydrate concentration (SC) and (**C**, **D**) starch concentration (Starch) were analyzed at different positions from the shoot apex. Data points represent mean values from five extension shoots and error bars represent SE. A significant effect of date was observed for SC in bark and wood ($P < 0.05$, using a linear mixed effects model). A significant effect of position on extension shoots was observed for SC in bark, SC and starch in wood ($P < 0.05$, using a linear mixed effects model).

performed on each model ($\alpha < 0.05$). Tukey's honestly significant difference (HSD) tests were performed on each model to separate means when ANOVA results were significant.

Results

Painting influences stem temperature. Stem temperatures were highly dependent on solar radiation and air temperature (Supplemental Figs. 1 and 2). This was confirmed by a strong overall correlation between air temperature and stem temperature [$P < 0.0001$ (Table 1)]. Linear mixed effects analysis also revealed significant effects of exposure in control stems (south–north), with south-facing stem sides (surface and the wood beneath combined) experiencing significantly higher temperatures than north-facing stem sides [$P < 0.05$ (Table 2)]. During the measured period, the average south-facing stem surface temperature in control trees was 14.5 ± 0.1 °C whereas the average of north-facing surfaces of the same stems was 13.8 ± 0.1 °C (Table 2). Painting significantly reduced stem temperature [$P < 0.05$ (Tukey's HSD)] on south-facing stem sides when compared with unpainted controls. On average, paint reduced temperatures by 0.7 ± 0.1 °C within south-facing wood and 0.5 ± 0.1 °C at south-facing surfaces. The effects of painting were less pronounced for north-facing stem sides with average reductions of 0.3 ± 0.0 °C and 0.2 ± 0.0 °C within and at the surface of stems, respectively. Wood temperatures beneath painted south-facing stem surfaces were not significantly different from those beneath north-facing stem surfaces (14.1 ± 0.1 °C and 13.7 ± 0.1 °C, respectively).

Time of day had a significant effect on stem temperature ($P < 0.001$), which was associated with the presence of radiation [day-radiation values > 0 and night-radiation values = 0 (Table 2)]. The daytime average temperature of control south-facing stem surfaces was 17.5 ± 0.2 °C and 10.6 ± 0.1 °C at night. Values for wood of the same south-facing control stem sides were 18.0 ± 0.2 °C during the day and 10.5 ± 0.1 °C at night. Control north-facing stem sides experienced an average stem surface temperature of 15.9 ± 0.1 °C during the day and 10.8 ± 0.1 °C at night; associated below-surface temperatures were 16.7 ± 0.2 °C during the day and 10.6 ± 0.1 °C at night. The effect of painting in south-facing stem sides was significant during the day, with no significant differences between control and painted stems at night (Table 2). The daytime average temperature of painted south-facing stem surfaces was 16.6 ± 0.2 °C and 10.6 ± 0.1 °C at night. North-facing stems showed no significant differences between painted and control stems for day or night.

Painting of bark influences phenology. On 23 Feb. 2016 and on 17 Jan. 2017, buds were dormant (stage 0). By 29 Mar. 2016, apical and basal buds from all control shoots had reached developmental stage 3 and unpainted apical buds on painted extension shoots were not delayed significantly beyond this date (Fig. 1A). In contrast, buds collected from the basal zone of painted shoots showed highly significant developmental delays. By 4 Apr. 2016, all apical control buds had advanced to stage 4, characterized by the achievement of photosynthetic independence and initiation of internode extension. Most basal control buds and most apical buds on painted shoots had advanced beyond stage 3. Basal painted buds had not yet advanced to stage 2. During Spring 2017 (Fig. 1B), apical and basal buds from painted shoots were both delayed. By 5 Apr. 2017, apical and basal buds from control shoots had reached developmental stage 3 whereas apical buds from painted shoots reached this stage by the 10 Apr. 2017. Basal painted buds had not yet advanced to stage 2 at this date.

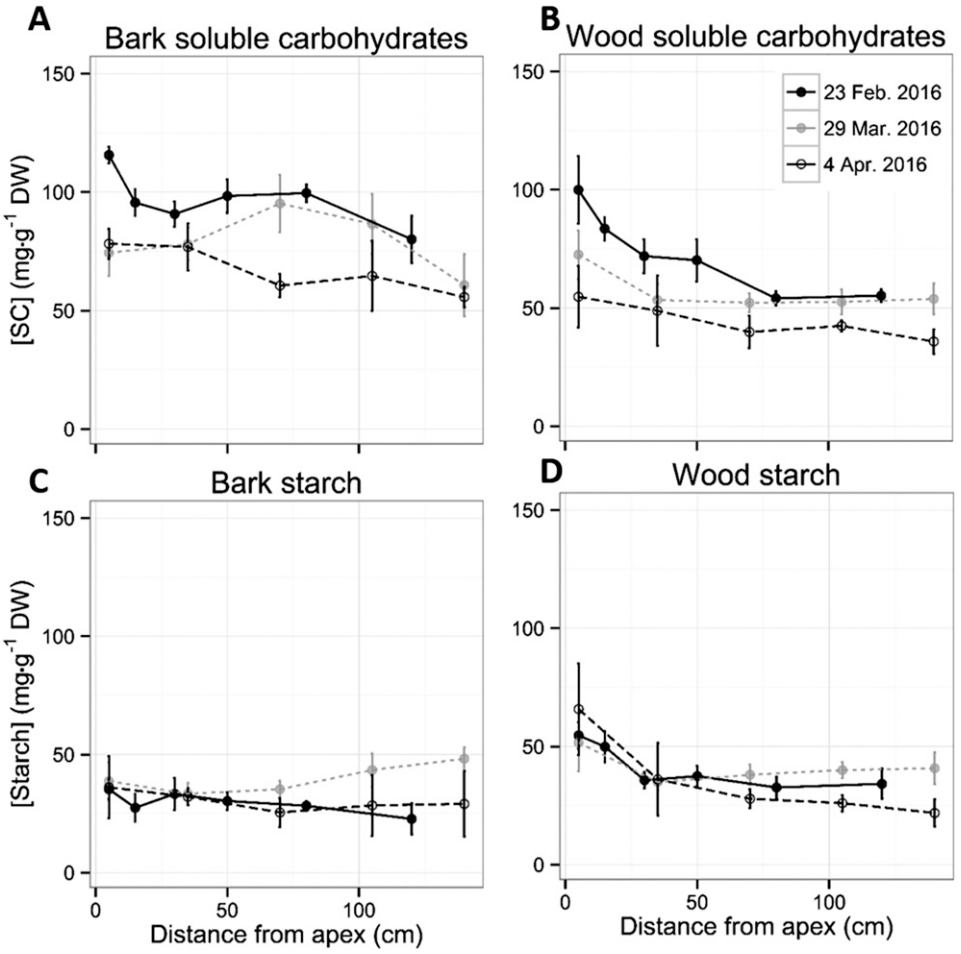

Fig. 3. Temporal and spatial distribution of carbohydrates in (**A**, **C**) bark and (**B**, **D**) wood in painted persian walnut ('Chandler' on 'Paradox' rootstock) nonbearing extension shoots during budbreak. Shoots were collected at stage 0 (dormant buds, 23 Feb.), on 29 Mar. and at stage 3 (nonphotosynthetically active leaves fully developed, 4 Apr.) during Spring 2016. (**A**, **B**) Soluble carbohydrate concentration (SC) and (**C**, **D**) starch concentration (Starch) were analyzed at different positions from the shoot apex. Data points represent mean values from five extension shoots and error bars represent SE. A significant effect of date was observed for SC in wood ($P < 0.05$, using a linear mixed effects model). A significant effect of position on extension shoots was observed for SC and starch in wood ($P < 0.05$, using a linear mixed effects model).

Budbreak is associated with NSC consumption. The date of collection throughout bud development significantly decreased the NSC concentration in all shoots during the spring of 2016 and 2017. The date of sampling during the spring seasons of 2016 and 2017 affected SC concentration in both wood and bark in control shoot, decreasing significantly over the course of sampling (Tables 3 and 4; Fig. 2; Supplemental Fig. 5). In painted shoots, date of collection significantly affected SC concentration in wood, decreasing significantly over the course of sampling as in the control shoot, for both seasons (Tables 5 and 6; Fig. 3; Supplemental Fig. 6). Bark SC concentration decreased throughout but developed during Spring 2017 but no date effect was observed during Spring 2016. Similar differences between the 2 years were observed for wood and bark starch concentrations with no significant changes in starch concentrations from 23 Feb. 2016 to 4 Apr. 2016 whereas significant changes were observed from 17 Jan. 2017 to 10 Apr. 2017. Position (distance from extension shoot apex) significantly affected SC and starch concentrations in wood in both control and painted shoots during the spring of 2016 and 2017 (Figs. 2 and 3; Supplemental Figs. 5 and 6). The overall trend was an increase in carbohydrate concentration toward the apex of extension shoots (Fig. 2). No interaction effect between date and position was observed except for starch in the wood of painted extension shoots during Spring 2016 (Table 5). The position significantly affected bark SC concentration in control shoots during Spring 2016 (Table 3).

In control shoots, a significant decrease in SC concentration was observed over the period of bud development with a significant effect of the position of sampling. During Spring 2016, average SC concentration in wood dropped from 70.46 ± 3.74 mg·g^{-1} DW on 23 Feb. to 36.08 ± 5.14 mg·g^{-1} DW on 4 Apr. [$P < 0.05$ (Fig. 2B)], and increased toward the shoot apex ($P < 0.05$). Average SC concentration in bark also dropped over the same period—from 95.94 ± 2.63 to 64.44 ± 3.67 mg·g^{-1} DW ($P < 0.05$)—and increased significantly toward the shoot apex [$P < 0.05$ (Fig. 2A)]. Starch concentration in wood and bark on the other hand did not change significantly over time with February and April values of 32.68 ± 1.58 and 47.95 ± 2.88 mg·g^{-1} DW, respectively (Fig. 2C and D). Starch concentration in wood increased significantly toward the apex.

In painted shoots, SC concentration decreased over time and increased toward the shoot apex in wood (Fig. 3). Average SC concentration dropped over time from 70.46 ± 3.74 mg·g^{-1} DW on 23 Feb. 2016 to 56.89 ± 3.98 mg·g^{-1} DW on Apr. 2016 [$P < 0.05$ (Fig. 4B)]. However, bark SC concentration remained constant at 73.44 ± 3.85 mg·g^{-1} on average among the different positions (Fig. 3A). As observed in control shoots, no significant change in starch concentration was found between sampling dates. Bark and wood starch remained constant at 50.03 ± 4.36 and 47.95 ± 2.88 mg·g^{-1} DW (Fig. 3C and D). A significant interaction between position and date was detected ($P < 0.05$).

Painting influences NSC distribution in extension shoots during budbreak. Extension shoots had reached stage 3 of bud development on 29 Mar. 2016 and 5 Apr. 2017 whereas painted shoots were delayed. We compared the spatial distribution of NSC in control and painted extension shoots at this time with a linear mixed effect model. Statistical analyses are summarized in Tables 7 and 8. The painting treatment significantly affected bark NSC concentrations during both seasons. In Spring 2016, the painting treatment significantly affected starch concentrations in bark but not wood (Fig. 4C and D), whereas SC concentrations in neither wood nor bark differed

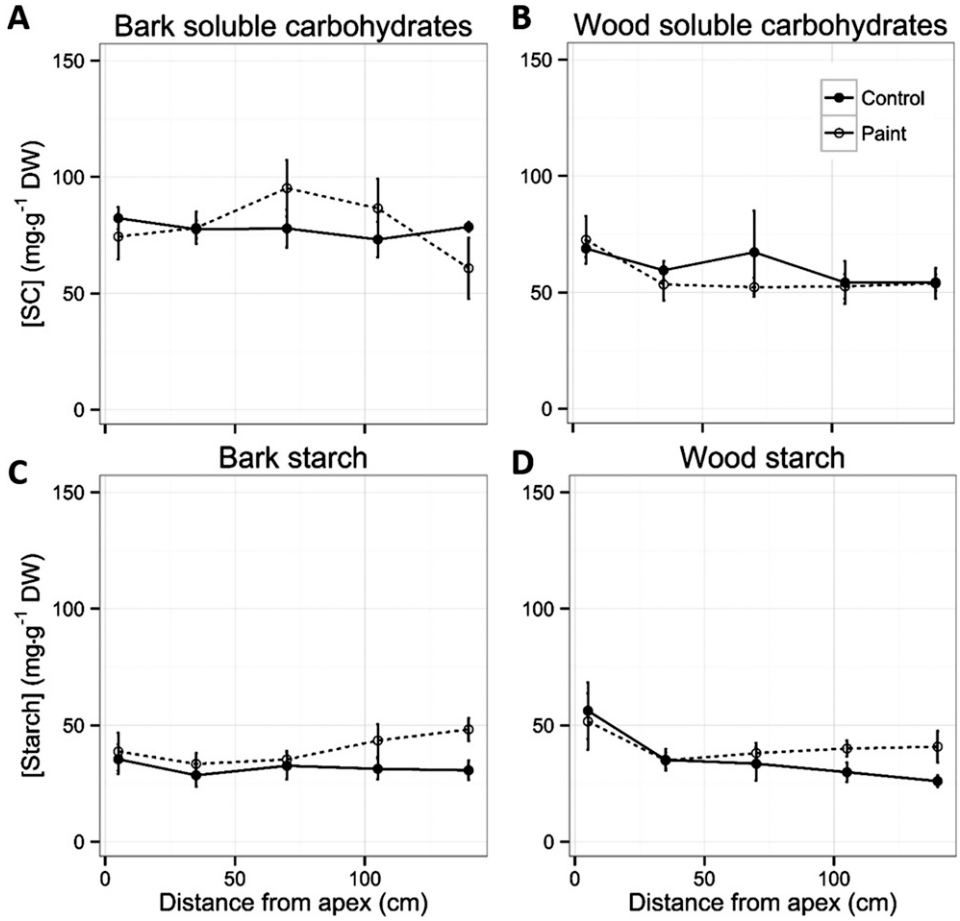

Fig. 4. Comparison of spatial distribution of carbohydrates in (**A**, **C**) bark and (**B**, **D**) wood in painted and control persian walnut ('Chandler' on 'Paradox' rootstock) nonbearing extension shoots (29 Mar. 2016). (**A**, **B**) Soluble carbohydrate concentration (SC) and (**C**, **D**) starch concentration (Starch) were analyzed at different positions from the shoot apex. Data points represent mean values from five extension shoots and error bars represent SE. A significant effect of treatment was observed for starch in bark ($P < 0.05$, using a linear mixed effects model). A significant effect of position on extension shoots was observed for starch in wood ($P < 0.05$, using a linear mixed effects model). Significant interaction between treatment and position was observed for starch in bark and wood ($P < 0.05$, using a linear mixed effects model).

significantly between treatments (Fig. 4A and B). However, a significant interaction between treatment and position was detected for starch concentration in both wood and bark (Table 7). Position effect was only observed in control extension shoots with increasing concentrations in the wood toward the apex, whereas starch concentrations were unaffected by position along the painted extension shoots [$P < 0.05$ (Fig. 4D)]. A similar effect of painting on wood starch distribution was observed during Spring 2017 with significant increase in concentration toward the apex in control shoots, whereas starch concentration was unaffected by position along the painted extension shoots (Supplemental Fig. 7).

During Spring 2016, starch concentration in the bark of painted shoots was significantly higher than in control shoots (Fig. 4C). At 5 cm from the apex, starch concentrations in bark were comparable between control and painted shoots at 35.37 ± 6.33 mg·g^{-1} DW and 38.70 ± 8.14 mg·g^{-1} DW, respectively. Maximum difference was observed at 140 cm from apex with 30.63 ± 4.18 mg·g^{-1} DW in control shoots and 48.19 ± 4.89 mg·g^{-1} DW in painted shoots. Painted extension shoot had higher levels of starch in wood and a significant change in the distribution of the starch (Fig. 4D). At 5 cm from apex, starch concentrations were similar in control and painted extension shoot (56.24 ± 12.07 and 51.60 ± 12.02 mg·g^{-1} DW, respectively). In control extension shoots, starch content decreased with increasing distances from the apex and reached a minimum of 25.96 ± 2.48 mg·g^{-1} DW at 140 cm from the apex, whereas in painted shoots, it did not significantly decrease (40.76 ± 6.70 mg·g^{-1} DW). Bark and wood SC concentrations were not significantly affected by the painting treatment. Bark SC concentrations were 81.32 ± 4.58 mg·g^{-1} DW in painted extension shoots and 77.88 ± 2.43 mg·g^{-1} DW in control extension shoots. Wood SC concentrations were 56.47 ± 2.95 mg·g^{-1} DW in painted extension shoots and 60.9 ± 4.05 mg·g^{-1} DW in control extension shoots.

By 4 Apr. 2016, painted extension shoots had reached stage 3, but control shoots were already photosynthetic (Tixier et al., 2017). Spatial distribution of carbohydrates was compared in the two treatments with a linear mixed effects model (Supplemental Fig. 4). No significant differences between treatments were observed although a significant position effect of wood SC and starch was maintained ($P < 0.05$) with increases in concentration toward the apex.

During Spring 2017, SC concentration in the bark of painted shoots was significantly higher than in control shoots (Supplemental Fig. 7A). SC concentration was on average among the different positions in control and painted shoots at 64.32 ± 1.78 mg·g^{-1} DW and 74.56 ± 4.43 mg·g^{-1} DW, respectively. Painted extension shoots had higher levels of starch in wood, and a significant change in the distribution of the starch (Supplemental Fig. 7D). At 5 cm from apex, starch concentrations were similar in control and painted extension shoots (85.78 ± 5.16 and 77.06 ± 3.47 mg·g^{-1} DW, respectively). In control extension shoots, starch content decreased with increasing distances from the apex and reached a minimum of 53.39 ± 4.52 mg·g^{-1} DW at 80 cm from the apex, whereas in painted shoots, it did not significantly decrease (72.27 ± 6.84 mg·g^{-1} DW). Bark starch and wood SC concentrations were not significantly affected by the painting treatment.

Respirational response to changes in stem temperature exceeds local mobilization of carbohydrates. The respiration of painted and control extension shoots showed no significant differences for the same temperature. At 25 °C, respiration rates were 1.35 ± 0.1 and 1.31 ± 0.1 μmol·m^{-2}·s^{-1} for painted and control treatments, respectively. To evaluate the thermal effects of painting on cumulative respiration, winter

Table 2. Comparison of average, daytime, and night stem temperature between painted and control persian walnut ('Chandler' on 'Paradox' rootstock) extension shoots, considering exposure and location in the shoot. Temperatures of the stem surfaces and wood under the bark were recorded during Spring 2016 on extension shoots north and south exposures using thermocouples. Data were analyzed with analysis of variance performed on linear mixed effect models with extension shoots as a randomized factor.

			Stem temp [mean ± SE (°C)]		
Treatment	Exposure	Location	Avg	Day	Night
Control	South	Surface	14.5 ± 0.1 cd[z]	17.5 ± 0.2 c	10.6 ± 0.1
		Wood	14.8 ± 0.1 d	18.0 ± 0.2 c	10.5 ± 0.1
	North	Surface	13.8 ± 0.1 ab	15.9 ± 0.1 a	10.8 ± 0.1
		Wood	14.1 ± 0.1 bc	16.7 ± 0.2 b	10.6 ± 0.1
Paint	South	Surface	14.0 ± 0.1 b	16.6 ± 0.2 b	10.6 ± 0.1
		Wood	14.1 ± 0.1 bc	16.6 ± 0.2 b	10.7 ± 0.1
	North	Surface	13.5 ± 0.1 a	15.7 ± 0.1 a	10.6 ± 0.1
		Wood	13.7 ± 0.1 ab	16.1 ± 0.1 ab	10.6 ± 0.1

[z]Data sharing different letters are significantly different according to Tukey's honestly significant difference test ($P < 0.05$).

Table 3. Probability values from analysis of variance performed on linear mixed effect models in temporal changes of carbohydrate content in control extension shoots of persian walnut trees ('Chandler' on 'Paradox' rootstock). Bark and wood soluble carbohydrate concentration (SC) and starch concentration (Starch) were analyzed at different positions on extension shoots for different dates of Spring 2016. Five extension shoots were studied. Symbol "×" represents the interaction between two factors.

Effect	Bark (SC)	Bark (Starch)	Wood (SC)	Wood (Starch)
Position	0.0062	0.3430	<0.0001	<0.0001
Date	<0.0001	0.1862	0.0001	0.3380
Position × date	0.2142	0.3401	0.0883	0.9216

Table 4. Probability values from analysis of variance performed on linear mixed effect models in temporal changes of carbohydrate content in control extension shoots of persian walnut ('Chandler' on 'Paradox' rootstock). Bark and wood soluble carbohydrate concentration (SC) and starch concentration (Starch) were analyzed at different positions on extension shoots for different dates of Spring 2017. Five extension shoots were studied. Symbol "×" represents the interaction between two factors.

Factors	Bark (SC)	Bark (Starch)	Wood (SC)	Wood (Starch)
Position	0.0683	0.3781	<0.0001	0.0004
Date	<0.0001	<0.0001	<0.0001	<0.0001
Position × date	0.0127	0.8493	0.1639	0.0652

stem temperature was modeled, parameterizing the model using data from 23 Feb. 2016 through 4 Apr. 2016 and from 17 Jan. through 5 Apr. 2017 (Supplemental Fig. 3). The corresponding NSC consumption due to respiration was calculated at the shoot level in glucose equivalents (Fig. 5A). Over 35 d, from pretreatment sampling on 23 Feb. 2016 until budbreak on 4 Apr. 2016, painted shoots respired 114.11 mmol of carbon and control shoots respired 133.09 mmol of carbon. An identical volume of 1.84×10^{-4} m^3 based on volume measurements from a standard extension shoot was considered, then modeled NSC consumption was compared with observed carbohydrate mobilization, considering total stem NSC as the substrate for maintenance respiration for both seasons (Fig. 5). Observed NSC values were markedly higher than those modeled. Observed NSCs in both treatments differ much less from initial values than they do from modeled values for both seasons.

Discussion

We have provided evidence that painting dormant *J. regia* extension shoots significantly reduces the daytime temperatures that shoots encounter. This effect almost certainly results from painted bark's increased reflectance of direct solar radiation (Sheppard et al., 2016) and is thus restricted to daylight hours, decreasing diurnal temperature variance (Table 2). These changes in extension shoot temperature can be linked to changes in spring phenology and the physiology of carbohydrate metabolism. Specifically, we found that painting bark resulted in the delay of budbreak by several days (Fig. 1), higher NSC concentrations in the bark and wood of painted extension shoots (Fig. 4), and changes in the spatial gradients of carbohydrates in wood during bud development (Tables 2–8). We have also provided evidence that the demands of maintenance respiration exceed mobilization from local carbon pools during budbreak (Fig. 5). Although lower stem temperatures reduced maintenance respiration and resulted in greater NSC reserves in painted extension shoots, these higher levels of NSCs cannot be explained solely by reductions in respiration rates as total carbon respired would exceed any observed reduction in even control shoots. We thus further discuss the potential role of carbohydrate transport during spring budbreak and the influence of temperature on this transport.

Stem temperature has an effect on dormancy breaking physiology. The physiology of dormancy and the timing of its end are considered to be driven in major part by the air temperature experienced by dormant trees (Charrier et al., 2015; Cleland et al., 2007; Pope et al., 2014). The collective accumulation of chill hours and heat hours throughout the winter are often cited as the stimuli behind synchronous budbreak within a given population, ensuring successful reproduction (Cleland et al., 2007). Indeed, manipulating stem temperature by just 1–2 °C with the application of white paint, we showed a delay of budbreak by several days (Fig. 1). The painting treatment affected the accumulation of heat hours but did not affect chilling hours. For both seasons, paint was applied after the sites had reached chilling requirement. Besides, for these sites, chilling hours are accumulated during the night when there is no solar radiation, thus no difference in temperature between control and paint treatment was observed (Table 2). The delays we observed in budbreak were associated with less carbohydrate consumption (Fig. 4). As new stems, flowers, and leaves in *J. regia* are not yet autotrophic, they depend upon stored reserves for their development. It follows that the spring metabolic reactivation in dormant tissue which initiates bud growth and cambial activity relies upon carbohydrate mobilization (Bazot et al., 2013; Dietze et al., 2014; Tixier et al., 2017; Witt and Sauter, 1994). This is supported by the decrease in NSC that we observed along stems over the course of budbreak in both control and painted stems during the springs of year 2016 and 2017 (Figs. 2 and 3). However, the steadiness of starch concentration over Spring 2016 seems contrary to previous work showing the high mobilization of starch from xylem parenchyma during the breaking of dormancy (Loescher et al., 1990; Maurel et al., 2004; Witt and

Table 5. Probability values from analysis of variance performed on linear mixed effect models in temporal changes of carbohydrate content in painted extension shoot of persian walnut trees ('Chandler' on 'Paradox' rootstock). Bark and wood soluble carbohydrate concentration (SC) and starch concentration (Starch) were analyzed at different positions on extension shoots for different dates of Spring 2016. Five extension shoots were studied. Symbol "×" represents the interaction between two factors.

Factors	Bark (SC)	Bark (Starch)	Wood (SC)	Wood (Starch)
Position	0.22118	0.5746	0.01971	0.00272
Date	0.08437	0.1271	0.01448	0.85338
Position × date	0.38994	0.1744	0.89184	0.03134

Table 6. Probability values from analysis of variance performed on linear mixed effect models in temporal changes of carbohydrate content in painted extension shoots of persian walnut ('Chandler' on 'Paradox' rootstock). Bark and wood soluble carbohydrate concentration (SC) and starch concentration (Starch) were analyzed at different positions on extension shoots for different dates of Spring 2017. Five extension shoots were studied. Symbol "×" represents the interaction between two factors.

Factors	Bark (SC)	Bark (Starch)	Wood (SC)	Wood (Starch)
Position	0.5283	0.3557	<0.0001	<0.0001
Date	<0.0001	<0.0001	<0.0001	<0.0001
Position × date	0.0067	0.8009	0.0918	0.1273

Table 7. Probability values from analysis of variance performed on linear mixed effect models in paint treatment effect on extension shoot carbohydrate distribution during Spring 2016 in persian walnut ('Chandler' on 'Paradox' rootstock). Bark and wood soluble carbohydrate concentration (SC) and starch concentration (Starch) were analyzed at different positions on extension shoots the 29 Mar. 2016. Five painted shoots were compared with five control shoots. Symbol "×" represents the interaction between two factors.

Factors	Bark (SC)	Bark (Starch)	Wood (SC)	Wood (Starch)
Position	0.30442	0.8406	0.0771	<0.0001
Paint	0.28241	0.0153	0.1391	0.1032
Position × paint	0.0729	0.0405	0.2457	0.0035

Table 8. Probability values from analysis of variance performed on linear mixed effect models in paint treatment effect on extension shoot carbohydrate distribution during Spring 2017 in persian walnut ('Chandler' on 'Paradox' rootstock). Bark and wood soluble carbohydrate concentration (SC) and starch concentration (Starch) were analyzed at different positions on extension shoots the 5 Apr. 2017. Five painted shoots were compared with five control shoots. Symbol "×" represents the interaction between two factors.

Factors	Bark (SC)	Bark (Starch)	Wood (SC)	Wood (Starch)
Position	0.0651	0.8339	0.0029	0.0001
Paint	0.0169	0.6545	0.5265	0.1141
Position × paint	0.6006	0.593	0.6487	0.0584

Sauter, 1994). Starch mobilization was observed during budbreak in 2015 and 2017 (Tixier et al., 2017). An explanation for this incongruity may be found in the differing climatic conditions between years. More research will improve our confidence in this interpretation, but we can propose that because higher winter temperatures promote higher respiration rates, they generated higher levels of NSC consumption (DeJong et al., 2011) or that higher temperatures otherwise impeded spring carbohydrate reallocation from remote locations by decreasing starch degradation activity (Zwieniecki et al., 2015).

Overall, painted shoots showed higher levels of total NSCs, especially starch, when compared with controls (Fig. 4). However, it is difficult to decipher if the higher level of carbohydrates in the painted branches caused by lower temperature is a consequence of the delayed budbreak or of the lower respiration rate, or both. Lower temperatures could delay the dormancy-signaling pathway in the bud, thus slowing NSC mobilization for bud growth resulting in higher starch concentration (Streb and Zeeman, 2012). However, whereas starch concentrations were different between control and paint treatment, there was no significant change in starch concentration during bud development within both control and painted treatments during Spring 2016. Since SC concentration drop was associated with budbreak in both painted and control extension shoots, it seems that the temperature effect on budbreak is not directly linked to local carbohydrate consumption. One alternative hypothesis is that decreases in stem temperature reduce the activity of starch-degrading enzymes and so the induction of starch hydrolysis (Zwieniecki et al., 2015). Interestingly, differences in starch content between painted and unpainted stems were more pronounced with increased distance from apical buds. Wood starch concentrations increased significantly toward the apex in control extension shoots whereas remaining relatively constant in painted extension shoots (Fig. 4). We recently showed the importance of NSC transport during bud development in *J. regia* and its influence on spatial gradients of NSC (Tixier et al., 2017). The paint effect on the spatial distribution of starch may be associated with carbohydrate transport and remobilization from trunk and roots during the breaking of dormancy (Hartmann and Trumbore, 2016; Lacointe et al., 2004; Loescher et al., 1990; Tixier et al., 2017). Carbohydrate reallocation and transport may also explain why respiration modeled on the actual temperatures of our sampled trees exceeded observed NSC consumption (Fig. 5).

Winter maintenance respiration requires supply exceeding shoot local reserves. The difference between modeled estimates of cumulative respiration and observed changes in NSC values allows us to estimate the carbohydrate mobilization from local maintenance respiration over the course of our experiment. Modeled carbohydrate depletion was much higher than observed carbohydrate depletion. Previous studies suggest that the transport of remote carbohydrates is required during budbreak (Lacointe et al., 2004; Maurel et al., 2004), and the present study on *J. regia* supports this idea. The total NSC levels observed on 29 Mar. 2016 were twice the levels expected based on modeled respiration (Fig. 5A). This suggests that, as NSC pools were depleted, remote carbohydrates replaced them and so provides evidence that mechanistic models of trees should not limit winter carbohydrate management to respiration (Allen et al., 2005; DeJong et al., 2011). Further evidence comes from the effects of stem temperature on the spatial gradient of starch concentration; the effect of position (distance from tip) on starch concentration that we observed in control extension shoots is not significant in painted shoots (Fig. 4). If bud growth relies on remote resources, the lack of depletion in remote storage of painted stems could be

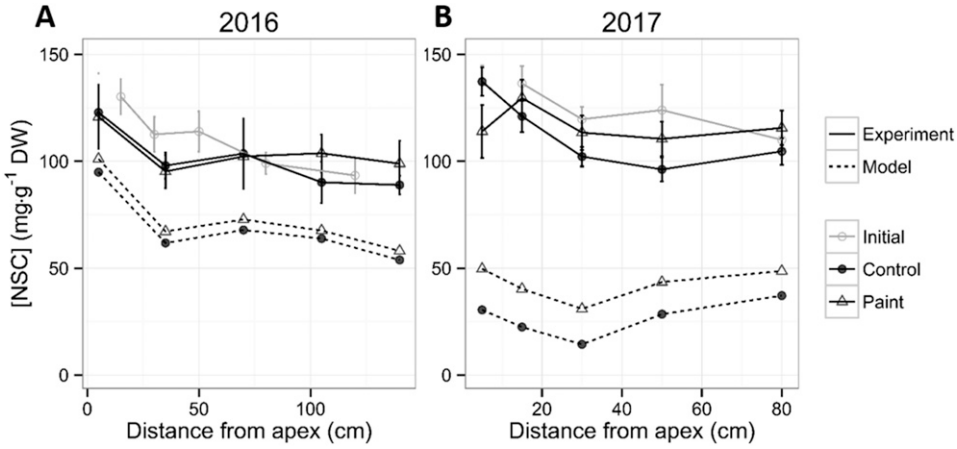

Fig. 5. Comparison of modeled nonstructural carbohydrate (NSC) depletion caused by respiration in painted and control persian walnut ('Chandler' on 'Paradox' rootstock) nonbearing extension shoots with experimental NSC concentrations during (**A**) Spring 2016 and (**B**) Spring 2017 while the buds are developing and non-photosynthetically active. During Spring 2016, extension shoots were collected on 23 Feb. (Initial) and on 29 Mar. (Control and Paint). During Spring 2017, extension shoots were collected on 17 Jan. (Initial) and on 5 Apr. Soluble carbohydrates and starch concentrations were analyzed and summed to represent NSC. A relative ratio of bark and wood weight was estimated on three extension shoots and applied to estimate total NSC. Data points represent mean values from five extension shoots and error bars represent SE. Modeled NSC consumption for control and painted extension shoots was estimated according to the method described previously and subtracted from the initial NSC concentrations.

due to decrease in temperature. Thus, the influence of temperature on NSC transport during dormancy should be studied (Sperling et al., 2017). The comparison between bearing and nonbearing shoots in relation with their source–sink relationship should be addressed to fully understand the carbohydrate management during winter season in the future research. Another consideration is that bark seems to be more affected by stem temperature than wood, reinforcing the idea that temperature affects patterns of early spring carbohydrate distribution as well as consumption; the lower the temperature, the slower the release of SCs from their starch counterparts in colder parts of the stem. A study of the expression of sucrose transporters in response to temperature would provide insight into the molecular basis of the reallocation of carbohydrates during budbreak (Bonhomme et al., 2005; Decourteix et al., 2008). Hormones such as gibberellic acid have also been correlated with modulation of dormancy and represent interesting candidates for the control of NSC metabolism (Dietze et al., 2014; Loescher et al., 1990).

Painting decreases the variance of diurnal and spatial stem temperature. Increases in daily temperature variance due to climate change are a potential source of frost damage in flushing buds (Charrier et al., 2015; Field et al., 2014). Our paint treatment decreased the variance by reducing the temperature maximums experienced by stems. South-facing stem sides experienced a drop in maximum temperature of as much as 7 to 10 °C on sunny days thus limiting the accumulation of heating hours. The increased bark reflectance provided by paint treatments may thus represent an orchard practice that prevents spring frost damage by delaying bud development. By limiting diurnal temperature and diurnal variation, painting the bark would limit effects of freeze–thaw cycles. Painting stems also reduced the temperature difference between south- and north-facing sides, a difference which has also been suggested to damage trees; branch splitting and sunscald occur as the two sides freeze and thaw on divergent cycles (Karels and Boonstra, 2003). The thawing of internal water on south-facing stem sides while north-facing stem sides remaining frozen inevitably induces divergent tension forces and subsequent structural damage (Pearce, 2001). Sunscald is linked to elevated bark temperatures from exposure to sun during winter. Karels and Boonstra (2003) suggest that exposure to sun during winter dehardens plant tissue, thereby reducing its freezing tolerance. They further suggest that sunscald may be an important selective mechanism favoring light-colored species like paper birch (*Betula papyrifera*) and quaking aspen (*Populus tremuloides*). Indeed, the prevalence of light-colored bark in deciduous tree species of northern latitudes suggests that it may serve as an essential adaptation to cold environments (Harvey, 1923). The use of paint has thus been proposed for mitigating sun damage during winter in orchard species (Sheppard et al., 2016). We provide evidence for the additional mitigating effects of paint in reducing diurnal and spatial temperature variability in young persian walnut extension shoots. Painting treatments offer multiple benefits including delaying budbreak, limiting NSC depletion associated with intense maintenance respiration during mild winter, freeze–thaw cycles, and frost dehardening.

Partial application of white paint increases asynchrony of budbreak across the painted extension shoots which experienced different thermal regimes. This finding suggests that budbreak is triggered by relatively local signals (Vitasse et al., 2014). However, general redistribution of the carbohydrates across the whole tree occurring before or during budbreak seems to be often overlooked. Understanding the relative importance of the local environment around the bud and whole tree physiology on spring budbreak might be necessary to developed management practices to mitigate climate change effect on spring tree biology.

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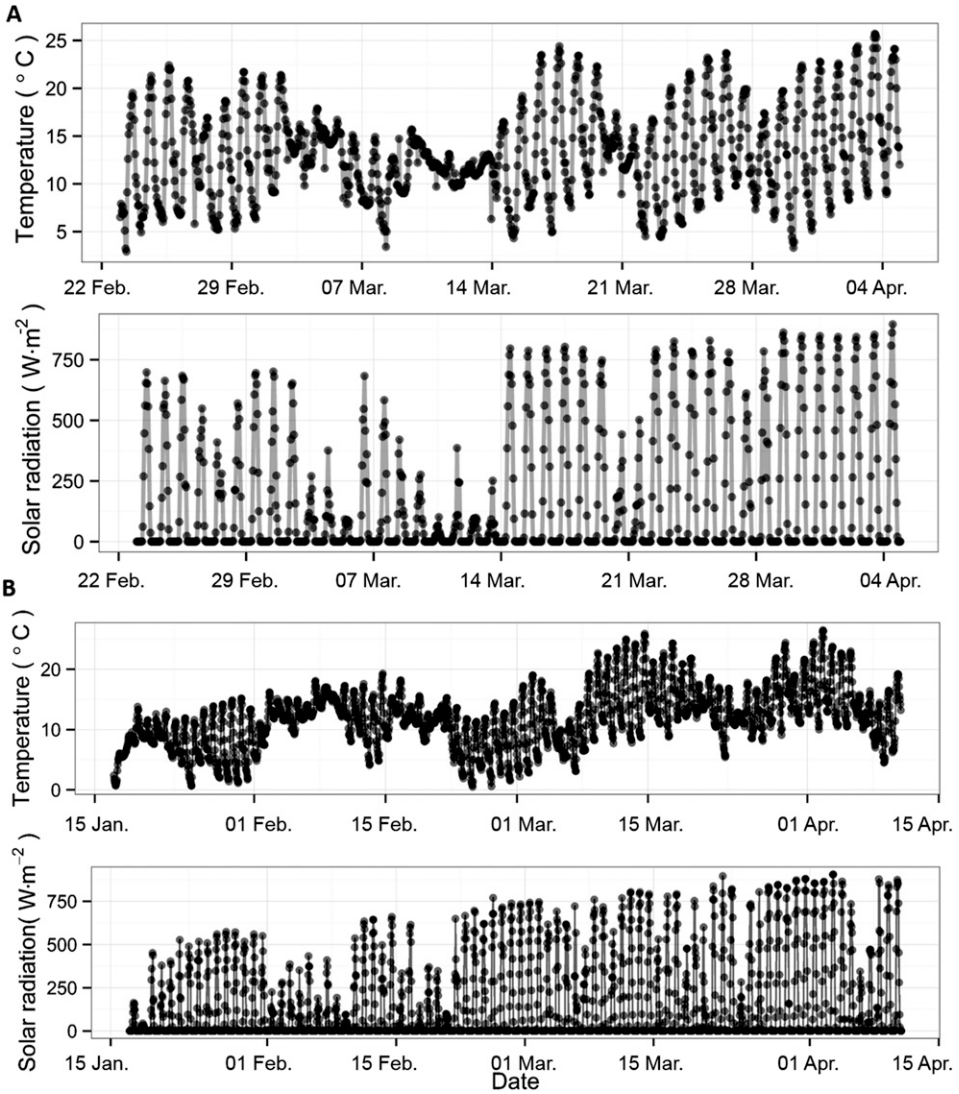
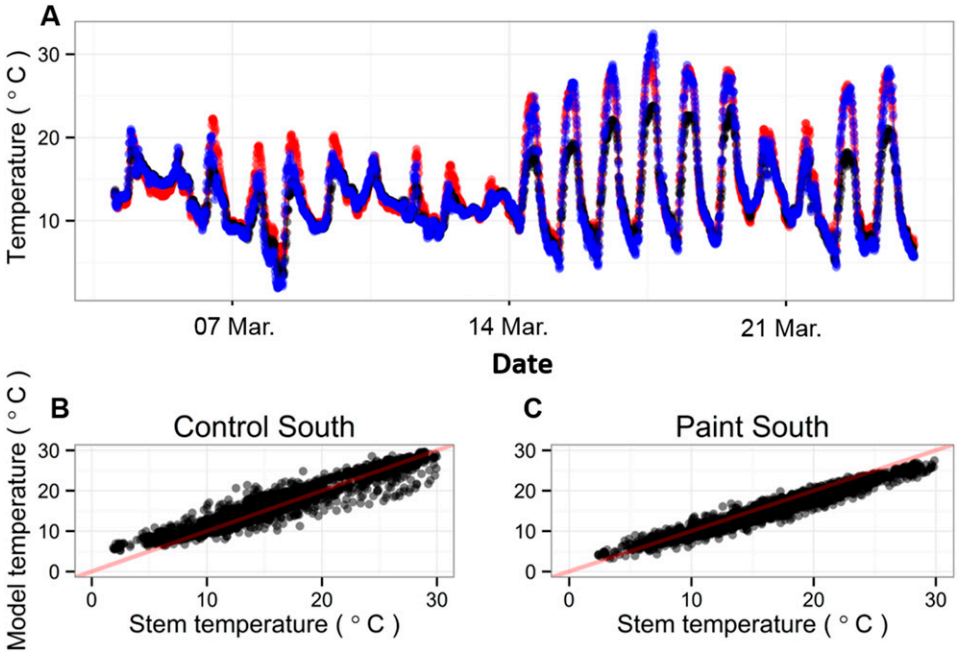
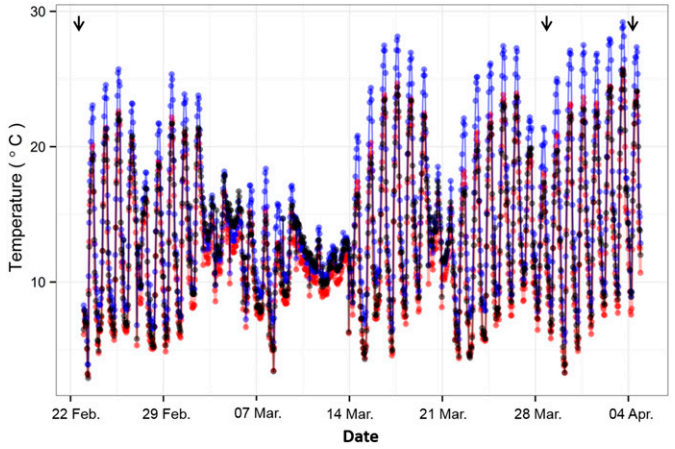


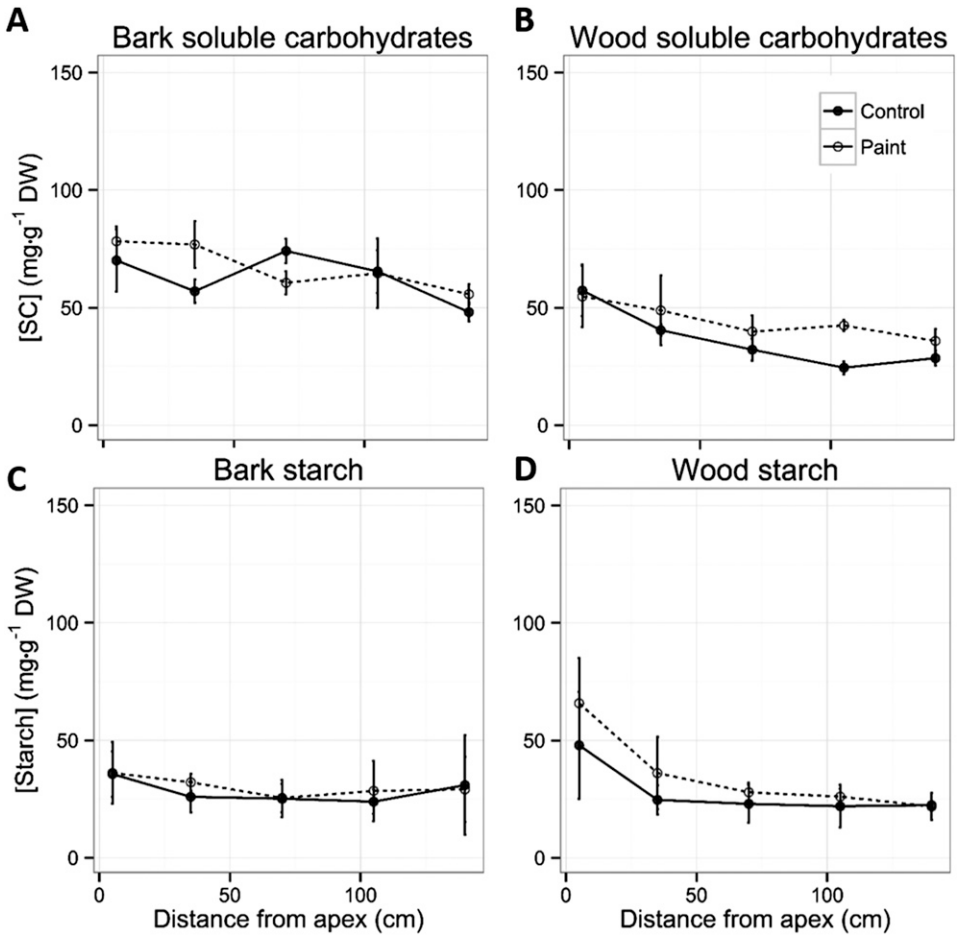
Supplemental Fig. 1. Temperature and radiation conditions at (**A**) Chico and (**B**) Davis, CA sites. (**A**) Temperature and radiation data were collected from the nearby Durham CIMIS Station #12 (California Irrigation Management Information System) (lat. 39.608639°N, long. 121.8244°W). Temperature and radiation data were collected from the Davis CIMIS Station #6 located 1.5 km from the orchard (lat. 38.535694°N, long. 121.77636°W).



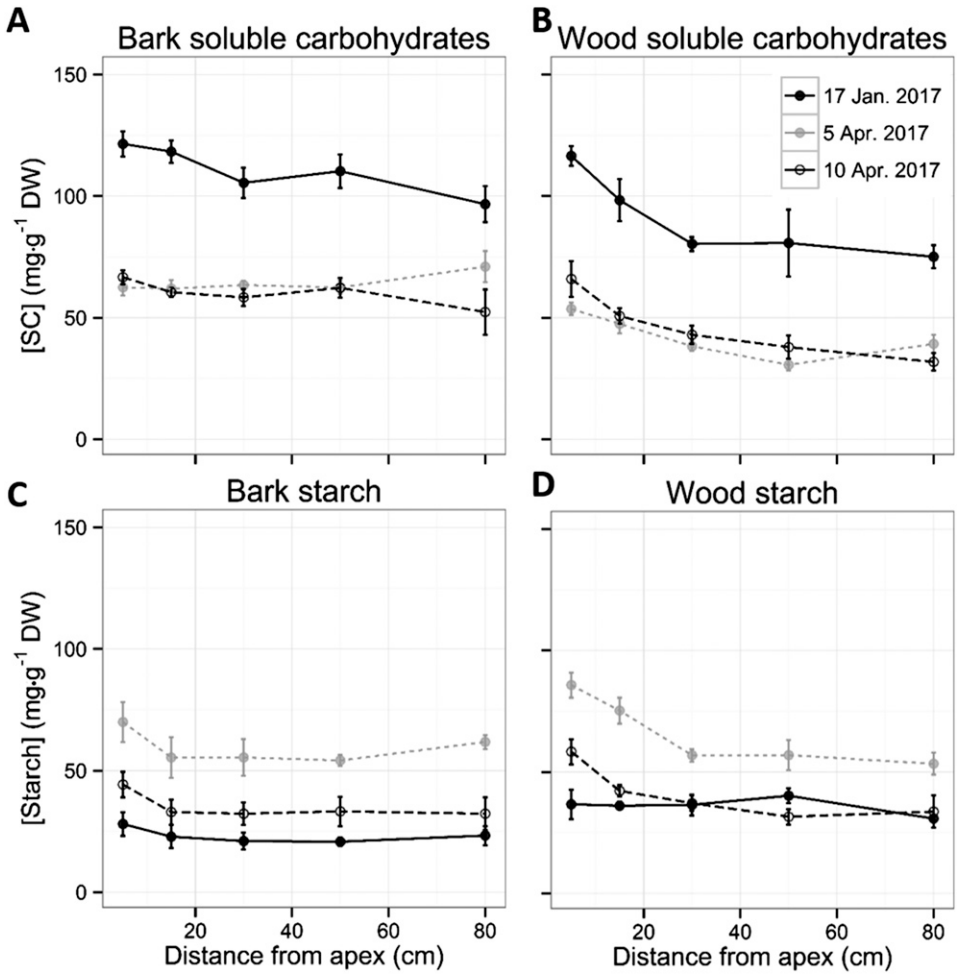
Supplemental Fig. 2. Validation of stem temperature model. In the control stem, modeled stem temperature (red) using a linear mixed effects model was compared with measured stem temperature (blue) and air temperature (black, **A**). Model temperatures were compared with stem temperatures for the control (**B**) and painted stems (**C**) ($P < 0.0001$).



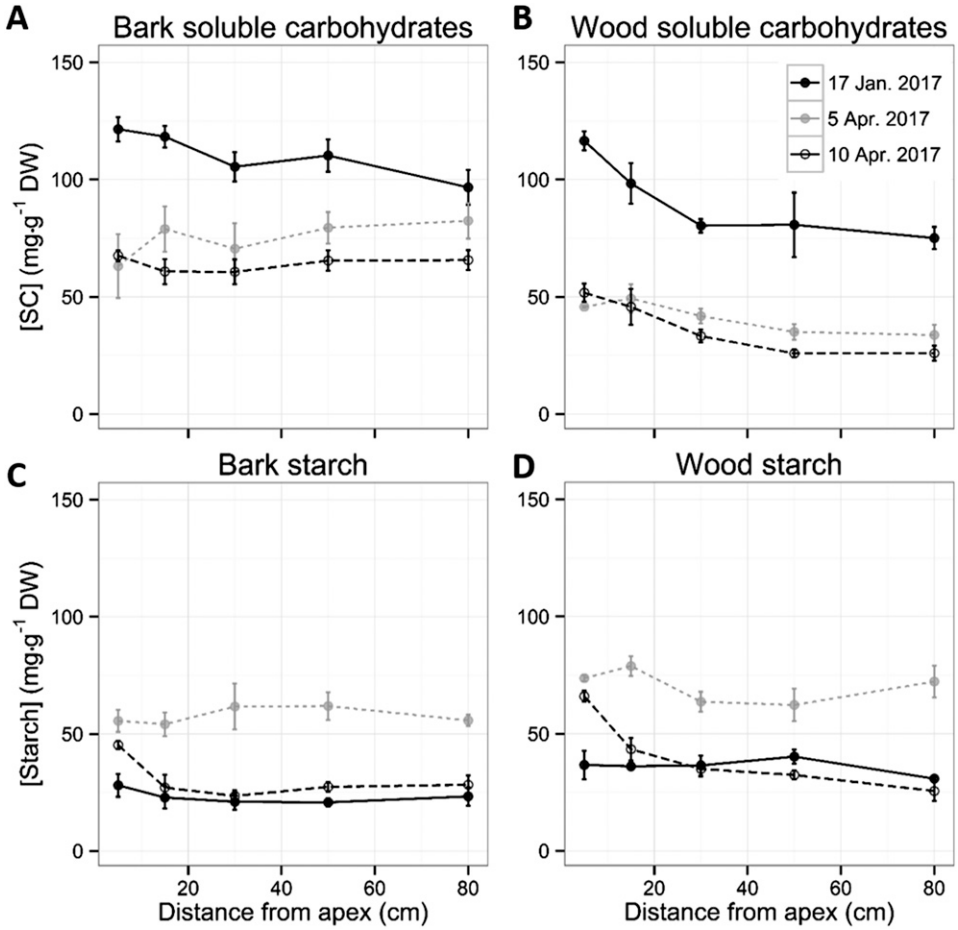
Supplemental Fig. 3. Comparison of air temperature (black), modeled control stem temperature (blue), and modeled painted stem temperature (red) on Chico site. Arrows represent sampling dates.



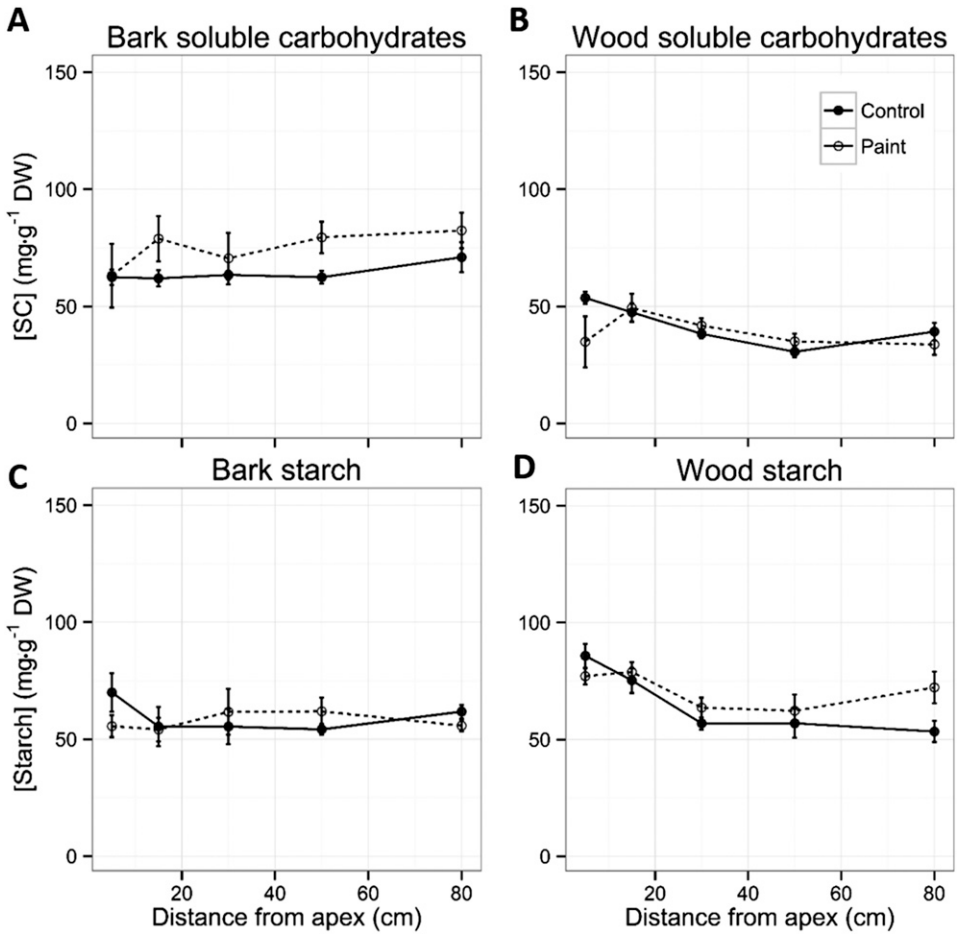
Supplemental Fig. 4. Comparison of spatial distribution of carbohydrates in bark (**A**, **C**) and wood (**B**, **D**) in painted and control persian walnut ('Chandler' on 'Paradox' rootstock) nonbearing extension shoots (4 Apr. 2016). Soluble carbohydrate concentration (SC) (**A**, **B**) and starch concentration (Starch) (**C**, **D**) were analyzed at different positions from the shoot apex. Data points represent mean values from five extension shoots and error bars represent SE. A significant effect of treatment was observed for starch in bark ($P < 0.05$, using a linear mixed effects model). A significant effect of position on extension shoots was observed for SC and Starch in wood ($P < 0.05$, using a linear mixed effects model). No significant effect of treatment was observed ($P < 0.05$, using a linear mixed effects model).



Supplemental Fig. 5. Temporal and spatial distribution of carbohydrates in bark (**A**, **C**) and wood (**B**, **D**) in control persian walnut ('Chandler' on 'Paradox' rootstock) nonbearing extension shoots during budbreak. Shoots were collected at stage 0 (dormant buds, 17 Jan.), stage 3 (nonphotosynthetically active leaves fully developed, 5 Apr.) and stage 4 (photosynthetic leaves, 10 Apr.) during Spring 2017. Soluble carbohydrate concentration (SC) (**A**, **B**) and starch concentration (Starch) (**C**, **D**) were analyzed at different positions from the shoot apex. Data points represent mean values from five extension shoots and error bars represent SE. A significant effect of date was observed for SC and Starch in bark and wood ($P < 0.05$, using a linear mixed effects model). A significant effect of position on extension shoots was observed for SC and starch in wood ($P < 0.05$, using a linear mixed effects model).



Supplemental Fig. 6. Temporal and spatial distribution of carbohydrates in bark (**A**, **C**) and wood (**B**, **D**) in painted persian walnut ('Chandler' on 'Paradox' rootstock) nonbearing extension shoots during budbreak. Shoots were collected at stage 0 (dormant buds, 17 Jan.), on 5 Apr. and at stage 3 (nonphotosynthetically active leaves fully developed, 10 Apr.) during Spring 2017. Soluble carbohydrate concentration (SC) (**A**, **B**) and starch concentration (Starch) (**C**, **D**) were analyzed at different positions from the shoot apex. Data points represent mean values from five extension shoots and error bars represent SE. A significant effect of date was observed for SC and starch in bark and wood ($P < 0.05$, using a linear mixed effects model). A significant effect of position on extension shoots was observed for SC and starch in wood ($P < 0.05$, using a linear mixed effects model).



Supplemental Fig. 7. Comparison of spatial distribution of carbohydrates in bark (**A**, **C**) and wood (**B**, **D**) in painted and control persian walnut ('Chandler' on 'Paradox' rootstock) nonbearing extension shoots (5 Apr. 2017). Soluble carbohydrate concentration (SC) (**A**, **B**) and starch concentration (Starch) (**C**, **D**) were analyzed at different positions from the shoot apex. Data points represent mean values from five extension shoots and error bars represent SE. A significant effect of treatment was observed for SC in bark ($P < 0.05$, using a linear mixed effects model). A significant effect of position on extension shoots was observed for SC and starch in wood ($P < 0.05$, using a linear mixed effects model).