

# Late Maturity and Excess Irrigation Trigger Kernel Darkening in ‘Howard’ English Walnut (*Juglans regia* L.) at Harvest, but not in ‘Chandler’

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**Additional index words:** packing tissue brown, kernel color, pellicle, lightness, midday stem water potential, hull split, hull bloom, cold storage

## Abstract

English walnut (*Juglans regia* L.) kernel color is an important quality factor for producers, processors, buyers and consumers because it affects marketing, sales, brand reputation, and prices. In the field and during postharvest handling, oxidation of phenolics in the pellicle (seed coat) triggers kernel darkening, resulting in amber-colored kernels. Our two-year study confirmed that ‘Howard’ is highly susceptible to dark kernel color development due to excess irrigation and harvesting at later physiological maturity, unlike ‘Chandler’. In ‘Howard’, the combination that reduced most the percentage of light-colored kernels was later physiological maturity at harvest and excess irrigation: this may account for ~40% of losses due to amber kernel color at harvest. The percentage of light-colored kernels is also reduced during cold storage, but the impact of cold storage is small (~10% loss) compared to that from incoming kernel color quality pre-determined by maturity stage at harvest and irrigation. Our data confirm the benefits of carefully monitoring proper irrigation, physiological maturity, and low-temperature storage (~0°C) for California walnut cultivars.

## Introduction

Walnuts are a highly valuable crop in California, with ~216,507 ha planted. The California industry was valued at \$1.2 billion in 2016 and accounts for > 99% of the United States walnut supply. Walnuts are California’s third most valuable export crop and the area under production has increased steadily over the last 10 years (California Walnut Board, 2019). The compensation that walnut growers receive for their crop is highly dependent on kernel quality, of which an extra-light or light color of the kernel pellicle (seed coat) is an important component (Fuller and Stafford, 1993). Kernel quality is determined according to USDA guidelines (United States Standards for Grades of Shelled Walnuts (*Juglans regia*), 2017). Walnut visual kernel color is graded into one of four categories:

extra light, light, light amber or amber. To maximize compensation for their walnut crop, highest for light-colored kernels (extra light and light), growers need to understand and manage orchard and postharvest handling factors that contribute to kernel walnut pellicle darkening.

Currently, 40% of the California industry’s plantings are ‘Chandler’ and 28% are new, lateral-bearing cultivars such as ‘Howard’ and ‘Tulare’ released from the UC Davis breeding program (McGranahan and Leslie, 2009). The remaining 12% are older cultivars such as ‘Serr’, ‘Franquette’, ‘Eureka’ and ‘Hartley’ (<https://walnuts.org/walnut-industry>). A new group of lateral-bearing cultivars from the UC Davis breeding program whose parents have a similar genetic makeup to current commercial cultivars are

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being planted across California and are being evaluated for their storage performance, including 'Ivanhoe', 'Solano' and 'Durham'. California walnuts are harvested from October to November, initially stored in-shell and then later cracked upon both domestic and international (mostly China, South Korea, Germany, Japan, Canada and Turkey) order requests and sold. Kernel pellicle darkening (amber color development) and rancidity will become more problematic as the volume of the walnut crop expands, delivery to distant international markets increases, marketing of shelled walnut kernels becomes more prevalent, and the crop carry-in supply increases (California Walnut Board Grades & Standards Committee, 2018, personal communication). Growing domestic production combined with continued planting and increasing global supply not only makes the global market competition challenging, but also emphasizes maintaining light-colored kernels to meet the rising market demand. Quality losses due to kernel color darkening are an impediment to meeting the quality standards for international and domestic markets and decrease the economic value of the industry. The rapid expansion of walnut production also requires tightly adjusted orchard and postharvest management practices that are effective and easily adopted by the industry. Orchard and postharvest factors such as harvest maturity, cultivars, processing, storage, transportation and in-store handling conditions (temperature, relative humidity, and water activity) can also affect the rate of quality loss due to kernel color darkening and rancidity. Therefore, there is interest in developing and applying postharvest technologies that reduce kernel quality deterioration during storage and postharvest handling. The first step to protect quality is to understand the role of pre-harvest factors on kernel quality, as limited information (Ramos, 1997) exists on how severe water stress (Ramos et al., 1978), harvesting date (Sibbett et al., 1974; Yoshikawa et al., 1978; Warmund et al., 2017), cultivar-rootstock com-

binations, and canopy management affect the rate of kernel color darkening.

As far as we know, no previous research has addressed the effects of excess irrigation and detailed physiological maturity on kernel postharvest performance in the lateral-bearing cultivars that comprise ~80% of the current California industry. Thus, our research is a novel effort which examined the effect of maturity and excess irrigation on kernel darkening of two English walnut cultivars grown in California.

### Material and Methods

*Orchard structure, experimental design and plant material.* A long-term 'Howard' and 'Chandler' plot to quantify scion-root growth and nut quality performance under different irrigation treatments was planted in March 2013 in the UC Davis Plant Sciences experimental fields (38° 32' 42" N / 121° 44' 21" W). The experimental orchard was trained to a minimally pruned center leader and spaced at 8 X 8 meters in a square planting. For each cultivar, irrigation (standard or excess) and maturity stage (M2/hull split and M3/onset of hull bloom) treatments were randomly assigned within each of the six blocks. Within each block, the experimental layout unit for each cultivar, maturity and irrigation treatment combination consisted of a row of four trees, of which the middle two trees were designated as experimental units. A buffer row was planted on either side of the six blocks or replications. The orchard has full-coverage micro-sprinklers and ground cover, except for a 1.5-m-wide herbicide-sprayed strip in the tree row. Mid-day stem water potential (MDSWP) was measured using a pressure chamber throughout the season to monitor tree water stress in each irrigation treatment (Lampinen, et al., 2016). The standard irrigation treatment was designed to keep the trees at no less than ~0.2 MPa below the proposed walnut baseline water status while avoiding prolonged periods at or wetter than the baseline. The excess irrigation treatment was intended to simu-



**Fig. 1.** M1=Packing tissue brown (PTB) Maturity Stage.



**Fig. 2.** M2=Hull Split Maturity Stage

late conditions previously observed during wet spring seasons, when yellow ‘Howard’ canopies were observed, by keeping the trees close to or 0.1 to 0.2 MPa above baseline (Lampinen et al., 2016).

*Harvesting and handling.* During the two seasons of the study, the two walnut cultivars were harvested at two physiological maturities based on the packing tissue brown (PTB, Figure 1) date: at stage M2 (hull split, Figure 2) and at stage M3 (onset of hull bloom, Figure 3; Yoshikawa et al., 1978). In 2016, ‘Chandler’ and ‘Howard’ attained stage M2

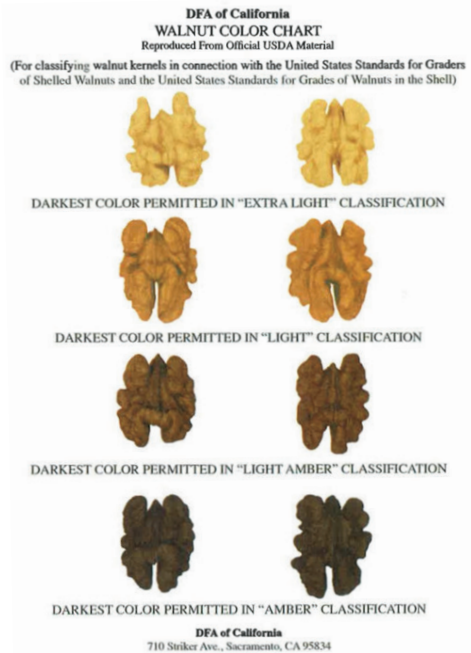
on October 3 and September 23, respectively. In 2017, ‘Chandler’ stage M2 occurred on October 2, 19 days after PTB, while ‘Howard’ stage M2 occurred 24 days after PTB on October 28. In both seasons, stage M3 was reached about seven days after stage M2. In each season, ~ 60 nuts (hull and kernel) at the same visual stage of fruit development were labeled and identified in each replication for each of the six blocks per treatment, using small tags to assure harvesting at each physiological maturity (Ortiz et al., 2019). At each harvest date, thirty nuts per treatment-replication were harvested and the hull was removed immediately prior to drying at 43 °C. Initial moisture content of ‘Chandler’ and ‘Howard’ samples varied from ~20% to 12% and it took up to eight hours to reach the 8.0% moisture content target. The end of the drying process was determined by measuring weight changes in a few samples in the lot and by moisture sensor readings. After drying, nuts were cracked carefully by hand, using small hammers and tweezers to extract undamaged halves. The first crack was at a point just above center on the non-suture side of the shell. The next crack was on the top center of the shell until it was broken enough to carefully remove the shell from one half of the nut. Then tweezers were used to break off pieces of the shell without damaging the kernel pellicle, until the kernel was entirely



**Fig. 3.** M3 Onset of Hull Bloom Stage

free of the shell. Fisherbrand™ Scoopula™ spatulas (Thermo Fisher Scientific, Waltham, MA) were used to separate the two halves of the kernel. Of the walnuts harvested for each replicate, nuts were cracked randomly until there were 15 sound halves, without insect damage, shrivel, or other damage unrelated to the treatment. The kernel halves were placed in plastic walnut evaluation trays obtained from the Dried Fruit Association of California. These trays have 100 slots, sized to hold kernel halves. The trays were spray-painted black to provide a uniform background color for the color evaluation. The walnuts were stored in trays inside cardboard boxes at 0°C with 50% RH and a water activity below 0.7 (Kader and Thompson, 2002; Ortiz et al., 2019) until they were evaluated for initial color, within one week of cracking.

**Kernel color evaluations.** Individual kernel color was evaluated using two methods: a subjective visual score following current Dried Fruit Association of California (DFA) guidelines (United States Standards for Grades of Shelled Walnuts, 2017) and an objective Minolta Chroma Meter CR400 (Konica Minolta, Ramsey, NJ). The DFA provides a chart for color evaluation that places the kernels into one of four categories (Figure 4): extra light (1), light (2), light amber (3) and amber (4). The percentage of extra light and light kernels in the samples was calculated from the number of kernels classified as DFA 1 and 2 from the total sample. The Minolta Chroma Meter removes human bias and error by expressing color as objective numerical values: abbreviations such as  $L^*$  (lightness),  $C$  (saturation) and  $hue^\circ$  (color shade; McGuire, 1992). We used  $L^*$ , which represents changes from white (high values) to black (low values), to express dark color development on kernels based on our previous work (Ortiz et al., 2019). The objective color of each individual kernel was measured with the Minolta Chroma Meter in the center of the outer side of the walnut kernel half (closest to the shell) at harvest and during storage at 0°C. In 2016 and 2017, color evo-



**Fig. 4.** DFA chart for color evaluation that places the kernels into one of four categories: extra light (1), light (2), light amber (3) and amber (4).

lution was measured every four months for 12 months cold storage (~0°C) under current UC-recommended best storage conditions (Kader and Thompson, 2002).

A randomized complete block design with six replications (blocks) was used for each cultivar with maturity stage (M2 and M3) and irrigation (Standard and Excess) as factors. The DFA kernel color score (1 to 4), percentage of extra-light and light kernels and  $L^*$  (0 to 100%) were arcsine-transformed prior to statistical analysis to compensate for its discrete population variability nature. The data were subjected to an analysis of variance (ANOVA) and the means were separated by Tukey's test using the R statistical program. Correlation analysis for changes in the percentage of light amber and amber kernels during cold storage were plotted as a function of time. Each line was calculated by linear regression.



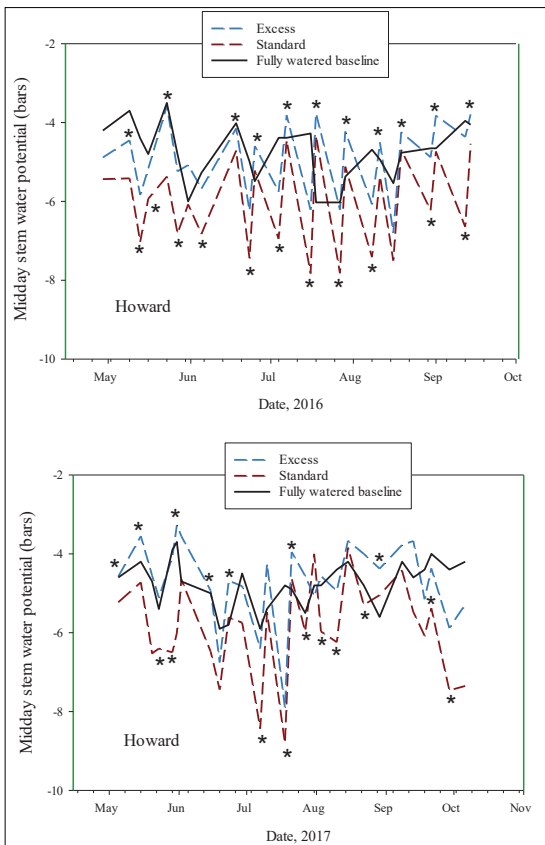
## Results and Discussion

*Effects of irrigation treatment on tree health.* In 2016, trees under the standard irrigation treatment received 830.6 mm water and trees under excess irrigation, 1,369.1 mm. In 2017, the standard irrigation treatment received 530.9 mm water and the excess irrigation treatment, 1,529.1 mm. In 2016 and 2017, the standard irrigation treatment had a MDSWP between the baseline and  $\sim 0.2$  MPa below the baseline, while the excess water treatment was 0.1 MPa wetter (less stress) than the baseline to 0.1 MPa below (more stress) the baseline (Figure 5). This is a relatively small difference in MD-

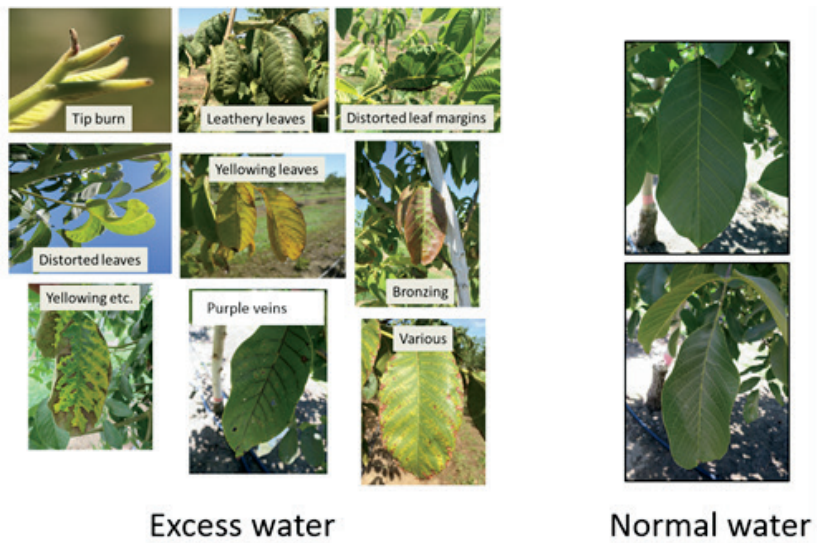
SWP, given the large difference in water applied. Trees irrigated to near or wetter than the baseline through the early part of summer developed leaf damage symptoms, including yellow leaves, leaf tip burn, marginal browning of leaves and defoliation up to a month earlier than normally-watered trees (Figure 6). Under excessively wet soil conditions, the roots may not be able to access nutrients, so these symptoms could be caused by deficiencies due to nonfunctional roots under wet conditions. Shoots that elongated during excessively wet soil conditions (i.e. MDSWP wetter than the baseline) had blank zones ('poodle tail') in these same regions the following year (Figure 7). This 'poodle

tail' condition (Figure 8) is often seen in commercial orchards, but this is the first time its cause has been confirmed as excessively wet conditions.

*Effect of maturity and irrigation on kernel color development.* During these two seasons, there was no interaction between maturity stage and irrigation for 'Howard' and 'Chandler' kernel color, whether measured as a DFA score, percentage of light-colored kernels, or lightness ( $L^*$ ) chroma meter readings (Tables 1 and 2). During this study, development of amber kernel color was triggered by physiological maturity stage at harvest and excess irrigation in 'Howard', but not in 'Chandler' (Table 1 and 2). In both seasons, there was no significant difference in pellicle color, measured as a DFA kernel color score, percentage of light-colored kernels, or lightness ( $L^*$ ) chroma meter reading, between 'Chandler' harvested at stage M2 and M3 (Table 1). 'Chandler' had  $\sim 91$  to 98% of kernels in the light-colored categories (extra light and light). A similar lack of physiological impact on kernel quality was observed when color was described by using  $L^*$  ( $\sim 50\%$ ). Unlike 'Chandler', 'Howard' maturity stage significantly af-



**Fig. 5.** Midday stem water potential by standard and excess irrigation treatments for 'Howard' in the 2016 (upper) and 2017 (lower) seasons; 1 MPa=10 bars.



**Fig. 6.** Trees irrigated to near or wetter than the baseline through the early part of summer developed leaf damage symptoms including yellow leaves, leaf tip burn, and marginal browning of leaves.



**Fig. 7.** Shoots that elongated during excessively wet soil conditions (i.e. midday stem water potential wetter than the baseline) had blank zones (‘poodle tail’) in these same regions the following year.

fect kernel color development at harvest. In both seasons, walnuts harvested at stage M2 had a higher percentage of (~87%) light-colored kernels than those harvested at stage M3 (~55%). There was a large significant difference (~34%) in the percentage of light-colored ‘Howard’ kernels harvested at stage M2 rather than at stage M3 in 2016, and a slightly smaller difference of ~27% in 2017 (Table 2). These significant differences were confirmed by the objective L\* values. In both seasons, ‘Howard’ kernels of nuts harvested at stage M2 were significantly lighter (~45.4%) than those harvested at stage M3 (41.3%).

As with maturity, irrigation did not affect kernel color at harvest for ‘Chandler’, measured as light-colored kernel percentages (~94%) and L\* value of ~50 in both seasons (Table 1). In 2016, for ‘Howard’ in which there was a lower percentage of light-colored kernels at harvest than in 2017, based on DFA and L\* values, excess irrigation did not significantly diminish the proportion of ‘Howard’ light-colored kernels or the lightness (~6%). However, ‘Howard’ had higher L\*

**Table 1.** Impact of maturity and irrigation on ‘Chandler’ walnut kernel browning at harvest in 2016 and 2017. DFA: Dried Fruit Association of California kernel score; Extra light and light percentage is the percentage of light-colored kernels, L\*: lightness.

2016 Season		DFA	Extra light and light	L*
Treatment		(1-4)	(%)	
Maturity	M2	1.5	97.5	51.97
	M3	1.6	93.8	50.79
	<i>P-value</i>	<i>0.5267</i>	<i>0.1111</i>	<i>0.1295</i>
Irrigation	Standard	1.5	97.1	51.81
	Excess	1.6	94.2	50.95
	<i>P-value</i>	<i>0.1541</i>	<i>0.2126</i>	<i>0.2639</i>
Maturity*irrigation	M2 Standard	1.5	97.5	52.30
	M2 Excess	1.6	97.5	51.64
	M3 Standard	1.5	96.7	51.33
	M3 Excess	1.6	90.8	50.25
	<i>P-value</i>	<i>0.9540</i>	<i>0.2126</i>	<i>0.7862</i>
2017 Season		DFA	Extra light and light	L*
Treatment		(1-4)	(%)	
Maturity	M2	1.8	96.5	49.75
	M3	2.0	90.6	49.38
	<i>P-value</i>	<i>0.1100</i>	<i>0.1290</i>	<i>0.5028</i>
Irrigation	Standard	1.9	95.4	50.00
	Excess	1.9	91.7	49.13
	<i>P-value</i>	<i>0.6699</i>	<i>0.1023</i>	<i>0.1182</i>
Maturity*irrigation	M2 Standard	1.8	96.7	49.97
	M2 Excess	1.8	96.3	49.54
	M3 Standard	2.0	94.2	50.04
	M3 Excess	2.1	87.1	48.72
	<i>P-value</i>	<i>0.4542</i>	<i>0.1449</i>	<i>0.4211</i>

values for kernels from the standard irrigation than from the excess irrigation treatment (p-value 0.055). Nevertheless, in 2016, L\* values showed “too close to call” significant differences in ‘Howard’ kernel color between irrigation treatments that were not expressed during visual evaluation, confirming our previous work that colorimeter numerical values

are more sensitive than visual observations. In 2017, for ‘Howard’ in which there was a higher percentage of light-colored kernels at harvest than in 2016, excess irrigation significantly reduced the percentage of desirable light-colored kernels at harvest from ~81% (standard irrigation) to 66%. These percentages were confirmed by L values declining

**Table 2.** Impact of maturity and irrigation on ‘Howard’ walnut kernel browning at harvest in 2016 and 2017. DFA: Dried Fruit Association of California kernel score; Extra light and light percentage is the percentage of light-colored kernels, L\*: lightness.

2016 Season		DFA	Extra light and light	L*
<b>Treatment</b>		(1-4)	(%)	
<b>Maturity</b>	M2	1.8	87.4	42.53
	M3	2.4	53.6	38.14
	<i>P-value</i>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0002</b>
<b>Irrigation</b>	Standard	2.1	73.6	41.44
	Excess	2.2	67.5	39.31
	<i>P-value</i>	<b>0.1943</b>	<b>0.2682</b>	<b>0.0548</b>
<b>Maturity*irrigation</b>	M2 Standard	1.8	91.4	43.54
	M2 Excess	1.9	83.9	41.60
	M3 Standard	2.3	56.4	39.35
	M3 Excess	2.5	51.1	37.02
	<i>P-value</i>	<b>0.9758</b>	<b>0.8476</b>	<b>0.8591</b>
2017 Season		DFA	Extra light and light	L*
<b>Treatment</b>		(1-4)	(%)	
<b>Maturity</b>	M2	2.1	87.0	48.30
	M3	2.4	60.4	44.40
	<i>P-value</i>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
<b>Irrigation</b>	Standard	2.1	81.4	47.90
	Excess	2.3	66.0	44.80
	<i>P-value</i>	<b>0.0025</b>	<b>0.0011</b>	<b>0.0001</b>
<b>Maturity*irrigation</b>	M2 Standard	2.0	90.7	49.54
	M2 Excess	2.1	83.3	47.13
	M3 Standard	2.2	72.2	46.24
	M3 Excess	2.5	48.7	42.48
	<i>P-value</i>	<b>0.1249</b>	<b>0.0734</b>	<b>0.3600</b>

from 48.3% to 44.8%.

*Kernel color evolution during 12 months cold storage.* In both cultivars harvested at different maturity stages and grown under different irrigation treatments, kernel color darkened slowly during cold storage at 0°C. In ‘Howard’, the percentage of light-colored kernels was reduced during this storage pe-

riod, reaching ~10% loss with 12 months of cold storage. Like the maturity and irrigation factors, cold storage did not affect ‘Chandler’ kernel color, data not shown. In ‘Howard’, the slopes of amber color development during the two cold storage seasons were not significantly different among treatments (Figure 9). This indicates that the percentage





**Fig. 8.** Trees in the excess water treatment defoliated up to a month earlier than normally-water trees.

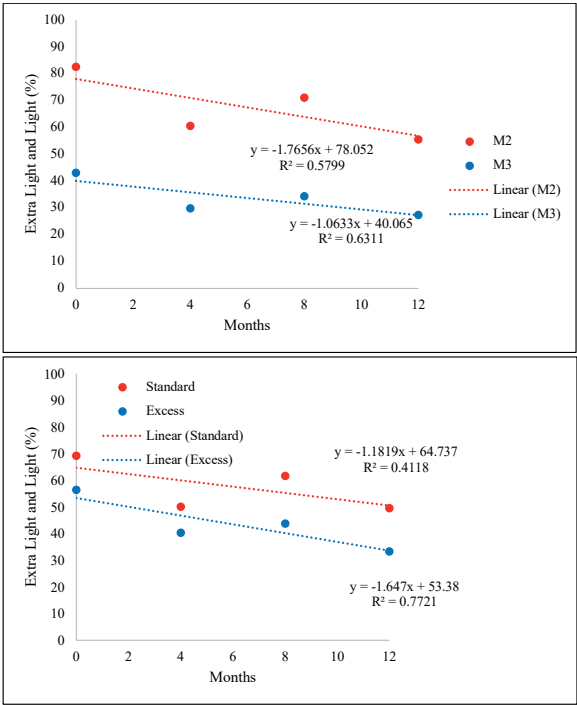
of light-colored kernels during cold storage was determined by the percentage of kernels in the light-colored categories (extra light and light) at harvest. Our unpublished work indicated that warmer temperatures increase kernel color darkening and some cultivars can be more susceptible to dark color development than others. The beneficial effect of cold storage on reducing kernel deterioration has been recommended by several researchers (Kader and Thompson, 2002; and Labavitch, 2004).

A primary mechanism of tissue browning is oxidation of phenolic compounds by the enzyme polyphenol oxidase (PPO). In undamaged plant and fruit tissues, PPOs are usually stored in the chloroplast, while phenolic substrates are stored in the vacuole (Araji, 2014). During walnut maturation and postharvest handling, the organelle membranes in the pellicle between the cellular compartments breakdown, allowing PPOs to encounter phenolic substrates (Escobar et al., 2008; Araji, et al., 2014). PPOs convert phenolic substrates to o-quinones via two oxidation reactions. The o-quinones then undergo

secondary reactions to forms of melanin, resulting in the brown-amber color (Taranto et al., 2017). Because walnut pellicles have more total phenolics content than other nuts (Zhang et al., 2009), we hypothesize that late physiological maturity and excess irrigation are causing membrane damage and triggering oxidation of phenols by polyphenol oxidase. A proteomic change profile during maturation of ‘Howard’, ‘Tulare’ and ‘Chandler’ walnuts (Crisosto personal communication) is being generated currently to reveal the mechanism of kernel-pellicle browning potential.

### Conclusions

For ‘Howard’ and ‘Chandler’, physiological maturity and irrigation affected kernel (pellicle) color without showing significant interactions. Late maturity and excess irrigation did not affect ‘Chandler’ kernel color. However, ‘Howard’ walnuts had a loss of light kernel color due to both late maturity at harvest and excess irrigation. The worst combination to reduce the percentage of light-colored ‘Howard’ kernels was late maturity



**Fig. 9.** Impact of physiological maturity (upper) at harvest and irrigation (lower) on the percentage of light-colored (extra light and light) ‘Howard’ kernels during cold storage at 0° C with a 40% relative humidity and <0.7% water activity for the 2016 crop.

plus excess irrigation with ~40% losses due to amber kernel color measured at harvest. The percentage of light-colored kernels was also reduced during low temperature storage, but the impact of cold storage was small (~10% loss) compared with the pre-determined impacts of physiological maturity and irrigation at harvest.

Our data confirm the benefits of carefully monitoring physiological maturity, proper irrigation, and low-temperature storage for walnut cultivars. Also, it points out the importance of screening recently released cultivars and breeding lines for postharvest storage life. These results should provide growers with new information to improve water management and time harvest for the

optimum maturity to reduce losses due to dark kernel color.

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