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CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

Project title: Postharvest Physiological Disorders: Pinking in Romaine Hearts

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Objectives

Long-range objectives

Determine protocols to ensure minimal development of pinking on romaine lettuce hearts.
Determine the relative importance of different postharvest factors that contribute to development of the pinking disorder.

Immediate Objectives

1. Determine the extent of pinking disorder in relation to romaine lettuce maturity.
2. Determine the extent of pinking disorder in relation to storage temperature and time.
3. Determine the extent of pinking disorder in relation to delays to cool.
4. Determine the extent of pinking disorder in a few varieties of romaine lettuce harvested early, midseason and late season in the Salinas area.

Abstract

Pinking discoloration on romaine hearts leads to significant quality and product losses. This discoloration is mostly associated with harvest injuries and can be reproduced in the lab with controlled cutting injuries. Discoloration is linked to the activity of wound-induced PAL and other enzymes in phenolic metabolism resulting in the undesirable pink-red-brown discolorations. We evaluated discoloration in romaine hearts from different sources in relation to storage temperature, cut to cool times, and alternative treatments to the water-spray used at the time of packing. Discoloration was scored on a 5 point scale (1=none, 3=moderate, 5=severe, a score of 3 indicates end of shelf-life). Storage/distribution temperatures are extremely important to the development of discoloration. Storage at 0-2.5C (32-36F) consistently reduced the severity of discoloration and resulted in the longest shelf-life compared to storing at 5C (41F) or higher. These temperatures also insured minimal decay development. Cut to cool times greater than 4 hours resulted in more pinking discoloration even if product was then stored at 2.5C (36F). The only spray treatment that had potential to be used in place of the water-spray at packing was 10% ethanol, but results were inconsistent. Strict temperature control and minimal cut to cool times are clearly important to minimize pinking discoloration on harvest injuries of romaine hearts.

Procedures

Testing was conducted with romaine lettuces from the Salinas area. Lettuces were harvested at typical commercial maturity unless otherwise indicated. The determination of maturity was based on leaf number and stem tissue length. Product was harvested with assistance from romaine heart field crews from commercial fields. Most of the field product was obtained from D'Arrigo with coordination by Ed Mora. Product was not washed, but bags were water-sprayed for product packed in the field. Packaged romaine hearts were vacuum cooled (within 4 hours) and then transported in coolers with ice to the lab in Davis, except for the delay to cool tests, in which the lettuce was room cooled. Tests began on the day of harvest. Romaine hearts were usually stored in unsealed plastic bags in trays on carts at temperatures of 0.5, 2.5 or 5C (33, 36 or 41F). In place of the water-spray applied in the field, some testing was conducted using different spray treatments; for these tests, romaine hearts were purchased from Costco, Woodland and were within 2-3 days of the packing date.

Marketability evaluations included 3 aspects. *Pinking Discoloration* was scored on a 1 to 5 scale, where 1=none, 2=slight, 3=moderate, 4=moderately severe, and 5=maximum or severe. This was done on individual leaves (3) of each heart. *Decay or visible deterioration* was scored on a 1 to 5 scale where 1=no macroscopic decay, 2=slight decay, but product salable, 3=moderate, useable but not salable, 4=moderately severe and 5=severe, unusable. Decay was generally minimal in the testing. *Storage life* was defined as the number of days to reach a pinking defect score of 2.5-3. Digital photography was used to illustrate results.

Compositional analyses included analysis of phenolic and phenolic enzymes. *Phenolics*. Simple uncolored phenolics are the precursors to the colored polymerized products causing the pinking and discoloration. Phenolics were determined by a standard spectrophotometric procedure for free phenolics. *PAL and PPO enzyme assays*. Both enzymes were determined in lettuce by spectrophotometric procedures published by Saltveit and Cantwell. Extracts were prepared from fresh lettuce tissue and these were frozen at -80C until determination of enzyme activity.

Tests were conducted with a minimum of 3 replicates per evaluation per storage condition. 1 replicate = 2-3 romaine hearts. Data were analyzed as means ± std deviation or by ANOVA with mean separation by LSD.05.

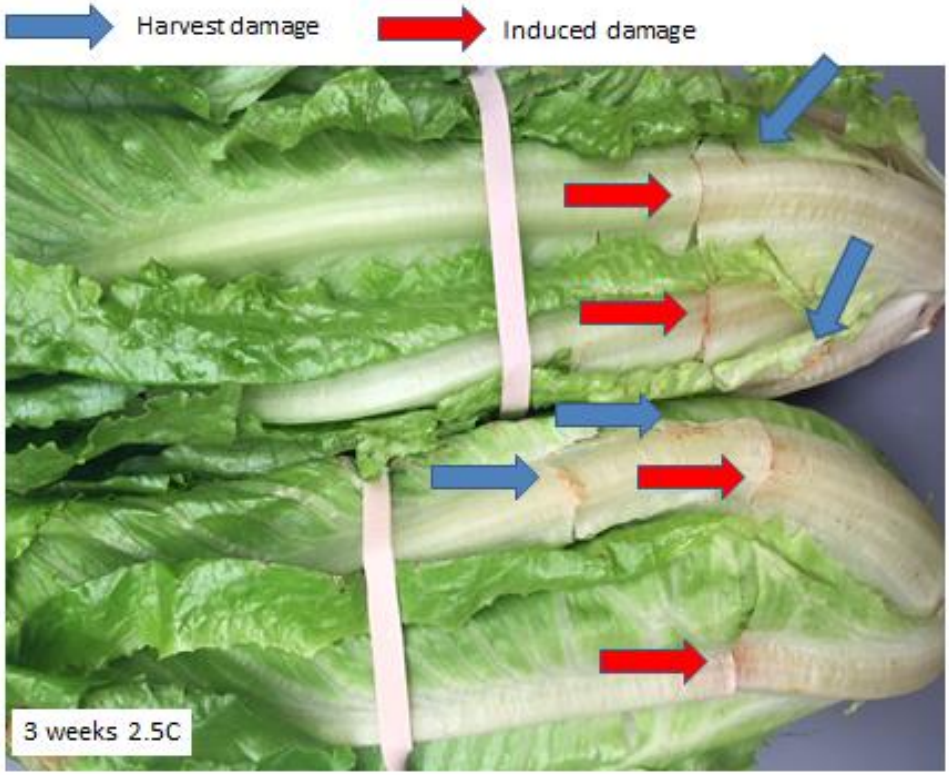
Other relevant experimental details are included in results and discussion where useful.

Results and Discussion

Work was conducted mainly on objectives 2 and 3, and work on objectives 1 and 4 is being conducted in the 2016-2017 research. An addition to the project was the evaluation of potentially useful treatments to use in place of the water spray currently used at packing.

Figure 1. Examples of pinking discoloration in damaged areas of romaine hearts. Photos provided courtesy of Ed Mora, D'Arrigo.

Figure 2. Example of typical pinking discoloration on romaine hearts after 3 weeks at 2.5C (36F). The discoloration due to harvest damage is indicated by the blue arrows and the lab cut-induced damage is indicated by red arrows.



Pinking discoloration is an important problem in romaine hearts, especially those packed as 3 or 6 hearts per bag for retail sales (**Figure 1**). Postharvest discoloration is mostly related to injuries. Harvest injuries during field packing and lab-induced injuries result in the same discoloration defect (**Figure 2**). We evaluated discoloration on both types of injury in the first year of the project. Results evaluating harvest injuries generally caused more experimental variation, while controlled lab-induced injuries provided more consistency because of a uniform number of injuries to evaluate.

Discoloration was always scored on a 5 point scale, where 1=none, 2=slight, 3=moderate, 4=moderately severe and 5=severe. **Figure 3** shows discoloration in the controlled cut injuries done in the lab to reproduce injuries observed in commercial handling of romaine hearts.

Figure 3. Rating scale for lab-induced damage and pinking discoloration on romaine lettuce midribs. A score of 1 is no discoloration (not shown), 2=slight (left), 3=moderate discoloration (middle), 4=moderately severe (middle) and 5=severe discoloration (right). Half-scores are used as appropriate.

Two tests were conducted to confirm that phenolic metabolism is associated with the pinking discoloration in romaine hearts. PAL (phenylalanine ammonia lyase) activity and total phenolics were measured. PAL is a key enzyme in lettuce phenolic metabolism and the synthesis of phenolic compounds. As phenolics are synthesized and then polymerized, they form the colored pink-red-brown pigments associated with visible discoloration in lettuces and other products.

PAL activity is induced by wounding and warmer temperatures. **Figure 3** shows the expected time course of PAL activity changes in chopped romaine lettuce midribs (0.5 x 0.5 cm). PAL increased rapidly in tissue stored at 5 and 10C and much less slowly in tissue stored at 0C. Correspondingly, the subsequent increase in total phenolics was much more rapid at 10C and delayed at 0C, with total phenolics at 5C at intermediate concentrations. These results are illustrated visually by the appearance of the midribs after 4 days (**Figure 4**). The time courses of PAL activity and total phenolics are consistent with previous work on lettuces, and the pinking discoloration in romaine lettuce hearts can be attributed to phenolic metabolism. While these measurements of PAL and phenolics represent extremes in romaine injury, they demonstrate the basis for use of low temperatures to retard discoloration.

Figure 3. Changes in PAL activity and total phenolics in chopped romaine midrib tissue (0.5 x 0.5 cm) held at 0, 5 or 10C (32, 41 or 50F). Data are averages of 3 replicates ± std deviation.

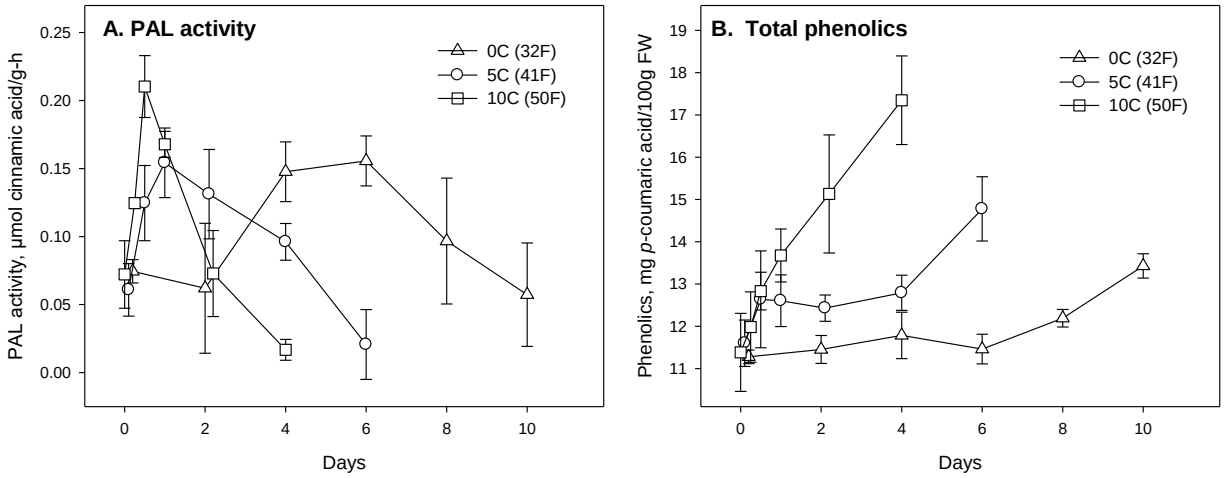


Figure 4. The appearance of chopped romaine midrib tissue after 4 days. Pieces were held at 0C or 32F (left), 5C or 41F (middle), and 10C or 50F (right).

The response of harvest damaged romaine hearts should be consistent with the chopped midrib results, in that temperature should play a crucial role in the development of the disorder. The importance of temperature is shown in **Figure 5.** Discoloration was delayed at 0 and 2.5C (32 and 36F), pinking was worse in the romaine hearts held at 7.5 and 10C (45 and 50F), and results were intermediate when hearts were held at 5C (41F). A visual quality score of about 6 or a discoloration score of 2.5-3.0 would indicate the end of shelf-life. Three tests showed that discoloration was the same when the romaine hearts were stored at 0 and 2.5C (32 and 36F).

Figure 5. Visual quality and pinking of commercially packaged romaine hearts (cv Green Forest, June 2015) stored at a range of temperatures after an initial storage period of 5 days at 32F (0C). Pinking was assessed on areas caused by harvest injuries. Data are averages of 3 replicates of 3 heads each.

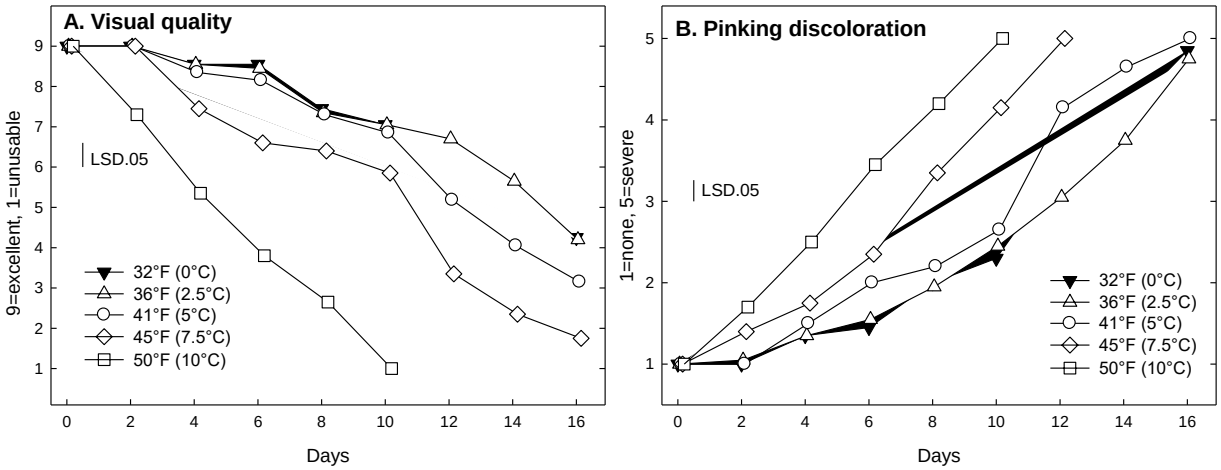
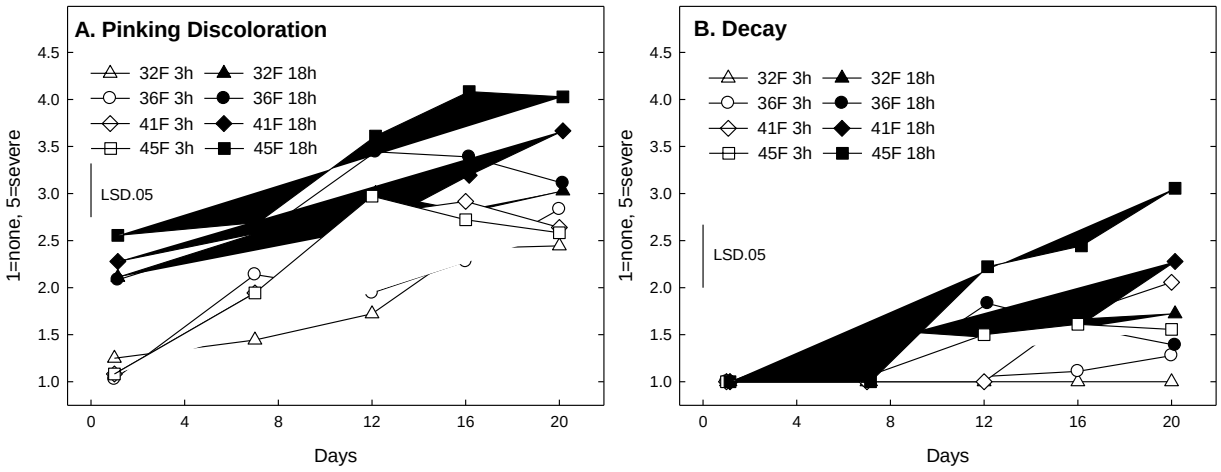


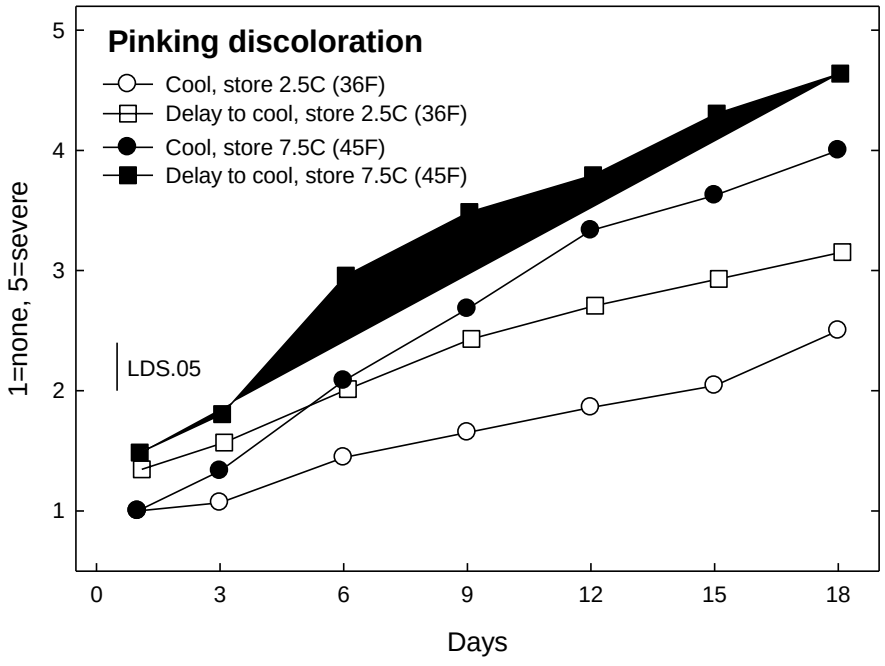
Figure 6. Pinking discoloration and decay of commercially packaged romaine hearts (cv River Road) stored at 0, 2.5, 5, or 7.5C (32, 36, 41, or 45F) after a 3 hour or 18 hour delay to cool (at 15-19C; 59-67F)). Product was vacuum cooled after 3 hours and room cooled after 18 hours (bags removed from boxes to facilitate cooling). Discoloration was scored on injuries from harvest operations. Data are averages of 3 replicates of 3 heads each.



Increasing delays to cool (increasing cut to cool times) are expected to increase the pinking discoloration. **Figure 6** shows one test in which very high pinking discoloration was observed 1 day after harvest with an 18 hour delay to cool. While this is an extreme case, it does illustrate that cooling delays will be expected to impact the pinking problem.

In a similar test, romaine hearts were cooled within 4 hours or had a delay of 18 hours. The hearts were stored at 2.5 or 7.5C (**Figure 7**). The 18 hour delay to cool resulted in visible discoloration at the first evaluation (day 1). The least pinking discoloration was found in the hearts cooled after 4 hours and stored at 2.5C, with a shelf-life greater than 18 days. The 18 hour delay to cool increased discoloration even if the hearts were stored at 2.5C, with a shelf-life of about 12-14 days (discoloration score of 2.5-3). A long cut to cool period consistently increases pinking discoloration in romaine hearts.

Figure 7. Pinking discoloration of commercially packaged romaine hearts (cv Green Forest) stored at 2.5 or 7.5C (36 or 45F) after cooling within 4 hours or after an 18 hour delay (at 18-21C). Product was vacuum cooled after 4 hours and room cooled after 18 hours (bags removed from boxes to facilitate cooling). Discoloration was scored on harvest injuries. Data are averages of 6 replicates of 3 heads each.



Another larger experiment was conducted to confirm the importance of cut to cool time and also storage temperatures. The temperature during the cut to cool period ranged from 17 to 21C (63 to 70F). Commercially harvested and packaged romaine hearts were room air-cooled after 4, 8, 13 or 18 hours to 0-0.5C (32-33F), stored for 7 days at 0-0.5C and then stored at three temperatures to simulate a transportation and distribution period of 15 days

The cooling delay clearly affected the discoloration scores (**Figure 8**), due to harvest injuries. The lowest discoloration scores, independent of storage temperature, were obtained when cooling occurred 4 hours after harvest. The highest discoloration scores were obtained when cooling was delayed to 18 hours and discoloration scores for 8 and 13 hours cooling decay were intermediate. Product stored at the lowest temperature (2.5C) developed less discoloration on damaged areas of the hearts compared to product stored at 5 or 7.5C (41 or 45F).

Decay scores were all low when romaine hearts were stored at 2.5C (**Figure 9**), regardless of the period of delay to cool. At 5C (41F), hearts began to show decay between 11 and 13 days, regardless of delay to cool period. For product stored at 7.5C (45F), the longer periods of delay to cool impacted the decay scores, significantly reducing shelf-life.

Estimates of shelf-life (**Table 1, Table 2**) are based on days to reach critical scores (3 or moderate for discoloration and 1.5 or very slight for decay) or extrapolations of the scores if values were not reached during the evaluation period. For a 4 hour delay to cool, shelf-life was similar among the 3 temperatures for both discoloration and decay. For an 8 hour delay to cool, however, shelf-life was reduced by discoloration more than by decay in this test. With 13 and 18 hour delays to cool, shelf-life was higher in product at 2.5C. It is expected that if product were stored at 0C (32F), shelf-life would have been longer in all cases.

Table 1. Estimate of the shelf-life of romaine hearts (cv River Road) in relation to delays to cool. Product was room cooled after 4, 8, 13 or 18 hour delays. Shelf-life includes storage and distribution periods. Shelf-life ended when the discoloration score reached 3.

	Days total shelf-life based on Discoloration			
Distrib. Temp	4 h delay	8 h delay	13 h delay	18 h delay
2.5C (36F)	>23	23	19	15
5C (41F)	21	15	14	15
7.5C (45F)	20	15	14	12

Table 2. Estimate of the shelf-life of romaine hearts (cv River Road) in relation to delays to cool. Product was room cooled after 4, 8, 13 or 18 hour delays. Shelf-life includes storage and distribution periods. Shelf-life ended when the decay score reached 1.5.

	Days total shelf-life based on Decay			
Distrib. Temp	4 h delay	8 h delay	13 h delay	18 h delay
2.5C (36F)	>23	>23	>23	>23
5C (41F)	19	21	15	19
7.5C (45F)	20	20	15	13

Figure 8. Discoloration scores of romaine hearts (cv River Road) that were air-cooled to 0-0.5C (32-33F) 4, 8, 13 or 18 hours after harvest. After cooling, packaged hearts were stored for 7 days at 0-0.5C (32-33F), and then transferred to distribution temperatures for 15 days at 2.5, 5 or 7.5C (36, 41, 45F). Discoloration was due to harvest injuries and a score of 3 (moderate) corresponds to the end of shelf-life. Data are averages of 3 replicates or bags with 3 heads each. Discoloration was scored on injuries resulting from commercial harvest operations.

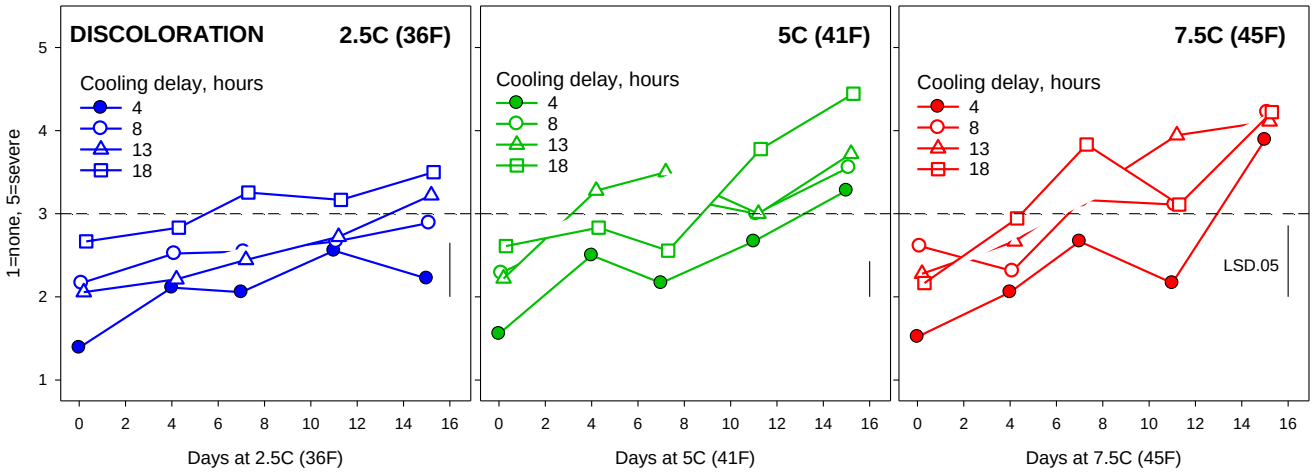
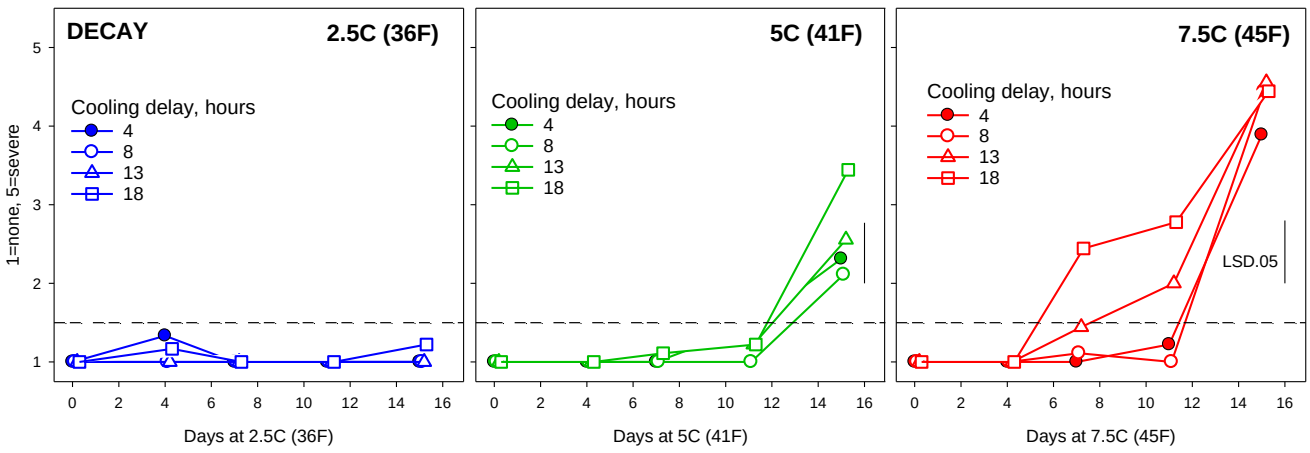


Figure 9. Decay scores of romaine hearts (cv River Road) that were air-cooled to 0-0.5C (32-33F) 4, 8, 13 or 18 hours after harvest. After cooling, packaged hearts were stored for 7 days at 0-0.5C (32-33F), and then transferred to distribution temperatures for 15 days at 2.5, 5 or 7.5C (36, 41, 45F). A decay score of 1.5 (slight) would correspond to the end of shelf-life. Data are averages of 3 replicates or bags with 3 heads each. Decay was scored if it was present on any part of the heart.



Since the field-pack operations for romaine hearts include a water spray step to provide 'slip' of the three hearts into the bags, several treatments were tested with potential to reduce discoloration in 3 experiments. Outer leaves of romaine hearts were separated and placed on trays covered with unsealed plastic bags to prevent water loss; 3 cuts were given with a stainless steel knife, leaves were spray-treated and then stored at 5C (41F) for periodic assessment of discoloration as shown by the rating scale in Figure 3.

The water-spray sometimes resulted in more discoloration than if leaves were cut and received no treatment. Chlorinated water (100 or 200 ppm NaOCl) was not better than potable water. Various ascorbic acid and citric acids sprays resulted in a higher degree of discoloration. The only treatment that provided reduced discoloration was a 10% ethanol spray solution (**Figure 10**), and while the results are encouraging, the degree of benefit was not consistent from one test to another (variety or some other factor involved?). Increasing the concentration to 20% ethanol reduced discoloration in the cut areas but increased surface discoloration, producing spots similar to those of russet spotting disorder.

In practice, the coverage and the length of exposure to the spray treatments would be limited. Compared with the consistent and important effects of temperature control, this approach does not appear to be useful to control pinking discoloration on romaine hearts.

Figure 10. Discoloration on water-sprayed and 10% ethanol sprayed romaine leaves. Leaves were cut-damaged with a stainless steel knife before spraying. Leaves were stored at 5C (41F) for 9 and 18 days. These illustrate the best results from the alternative spray treatments.