**Introduction**

When evaluating the severity of the pinking response in lettuce ribs, it is common to use a visual observation-based system (Monaghan et al., 2016; Paillart et al., 2017; Saltveit, 2004). In this type of system, an observer makes a judgement of the severity of the pinking response based on their perception of discoloration on the rib. However, as the accuracy of these methods is dependent upon the ability of an observer to reliably quantify color and the area occupied by a color based on visual observation alone, there is potential for human-based error. RGB imaging has been applied extensively in plant phenomics to measure a variety of phenotypes, including plant color (Yang et al., 2020). Photos taken by a calibrated RGB camera in consistent lighting conditions can provide a quantitative measure of the color of the subject. In our study, we propose an RGB imaging-based method for quantifying the severity of the pinking response in lettuce ribs which relies only on open-source software, and generates results suitable for downstream statistical analysis.

**Method**

The degree of pinking was measured quantitatively with an RGB imaging-based method. Images of wounded ribs were taken at 0, 2, and 4 days after wounding in the sRGB color space. Lighting conditions and camera settings were carefully controlled to ensure consistent imaging. The white balance of the camera was set with a gray card prior to each imaging session. GNU Image Manipulation Program (GIMP, version 2.10.14; https ://www.gimp.org/) was used to crop out background pixels from each rib, and extract pixels from an area 1 mm in height centered at the cut site, for each cut site in an image. Extracted pixels for each cut site were then exported into separate images. To allow for direct comparison of each cut site to its state at previous time points, each cut site was assigned a label at day 0 based on the identity of the rib and its position on the rib.

R (version 4.0.0; R Core Team, 2020) was used to convert cut site images from the sRGB to the L\*a\*b\* color space and calculate the mean a\* for each cut site image. Illuminant D65 was specified as the reference white for the RGB to L\*a\*b\* conversion. In the L\*a\*b\* color space, a\* represents the degree of redness or greenness. A pixel with a smaller value of a\* will appear more green, while a pixel with a larger value will appear more red. Therefore, the mean value of a\* at each cut site can be used to quantify the severity of the pinking response.

R was also applied to subset the data, which was necessary to ensure that the cut sites chosen for further analysis had similar initial color. Subsetting was accomplished by defining a range of values which the mean a\* of the cut site at 0 days after wounding must fall within to be considered. For iceberg, the range chosen was -3 to 0. For romaine, -6 to -3. To equalize the number of cut sites considered from each treatment group, cut sites with an initial mean a\* differing the most from the average initial mean a\* of the smallest group were recursively removed until the number of cut sites in each treatment group were equal. For iceberg, this left 5 cut sites from each treatment method. For romaine, 3. All R code used in this analysis can be downloaded from the repository: https://github.com/bryceaskey/woundingResponse.

**Results and discussion**

To analyze the results generated by the RGB imaging-method, an unpaired t-test was used to determine if the mean a\* of the treatment groups at each time point differed significantly from that of the control group at the same time point. The mean a\* of cut sites on melatonin-treated iceberg ribs significantly differed from that of the control iceberg ribs at 2 days (p=0.00222) and 4 days (p=0.00059) after wounding (Figure …A). The mean a\* of cut sites on melatonin-treated romaine ribs significantly differed from that of the control romaine ribs only at 2 days (p=0.0242) after wounding (Figure …B). Cysteine treatment did not result in a significant difference in mean a\* from the control group for romaine or iceberg ribs at either time point. These results suggest that melatonin treatment is able very effectively inhibit the pinking response in iceberg ribs for at minimum 4 days after wounding, and effectively inhibit the pinking response in romaine ribs for at minimum 2 days after wounding.

**Figure legend**

**Figure #.** Mean a\* of cut sites measured via digital imaging method from (A) iceberg and (B) romaine ribs over 4 day experimental period. Iceberg data was generated from 5 cut sites with similar initial color, and romaine data from 3. Error bars on plot represent standard error.

**Works cited**

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I have the git repository set as private right now, but can make it public once the paper is submitted