

THE ONSET OF GRAPEVINE BERRY RIPENING IS CHARACTERIZED BY ROS ACCUMULATION AND LIPOXYGENASE-DERIVED GALACTOLIPID PEROXIDES

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Hydrogen peroxide (H₂O₂) and singlet oxygen (¹O₂) accumulate inside Pinot Noir skin cells at softening, in cytosol and chloroplasts.

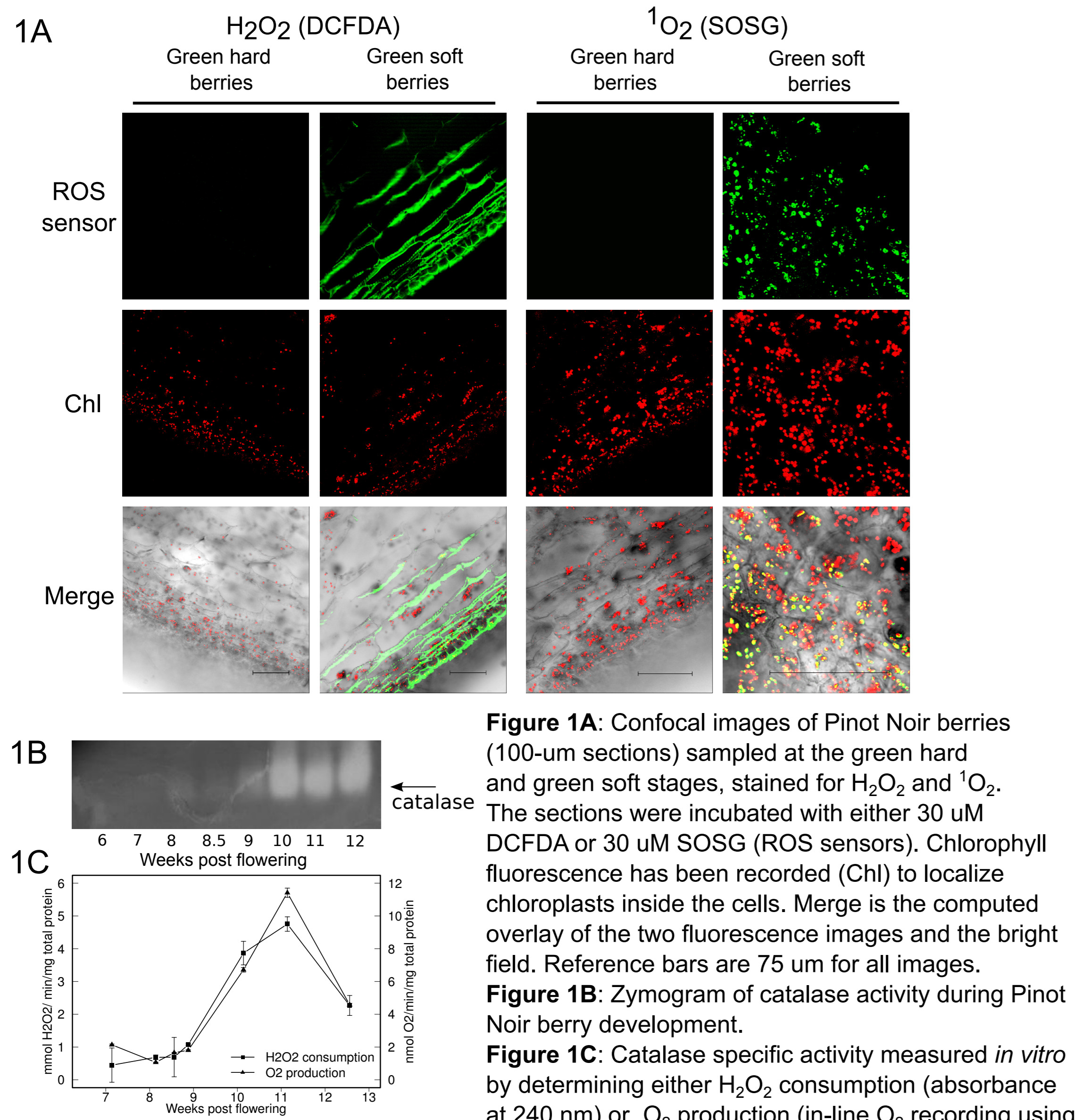
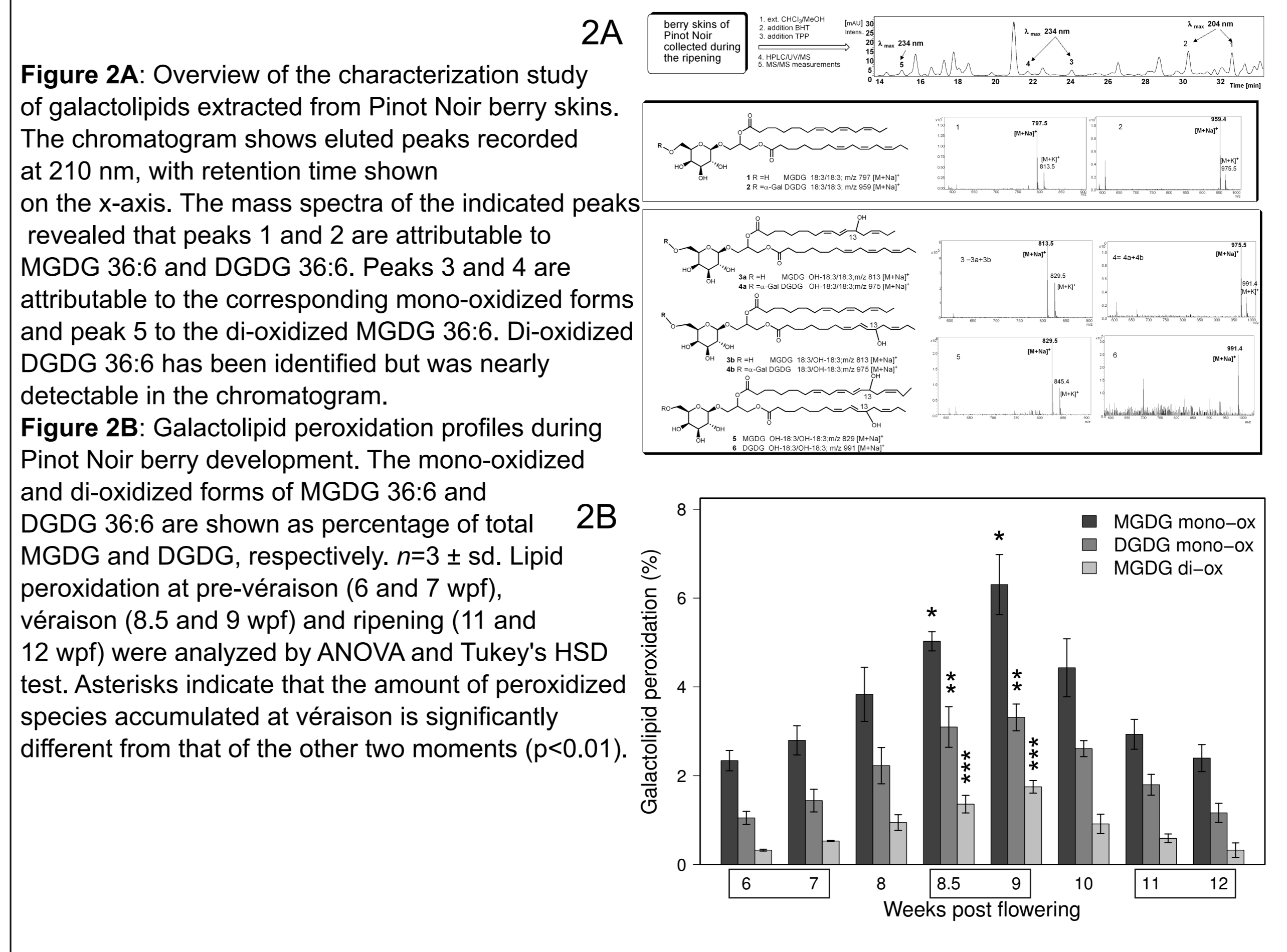


Figure 1A: Confocal images of Pinot Noir berries (100-um sections) sampled at the green hard and green soft stages, stained for H₂O₂ and ¹O₂. The sections were incubated with either 30 uM DCFDA or 30 uM SOSG (ROS sensors). Chlorophyll fluorescence has been recorded (Chl) to localize chloroplasts inside the cells. Merge is the computed overlay of the two fluorescence images and the bright field. Reference bars are 75 um for all images.

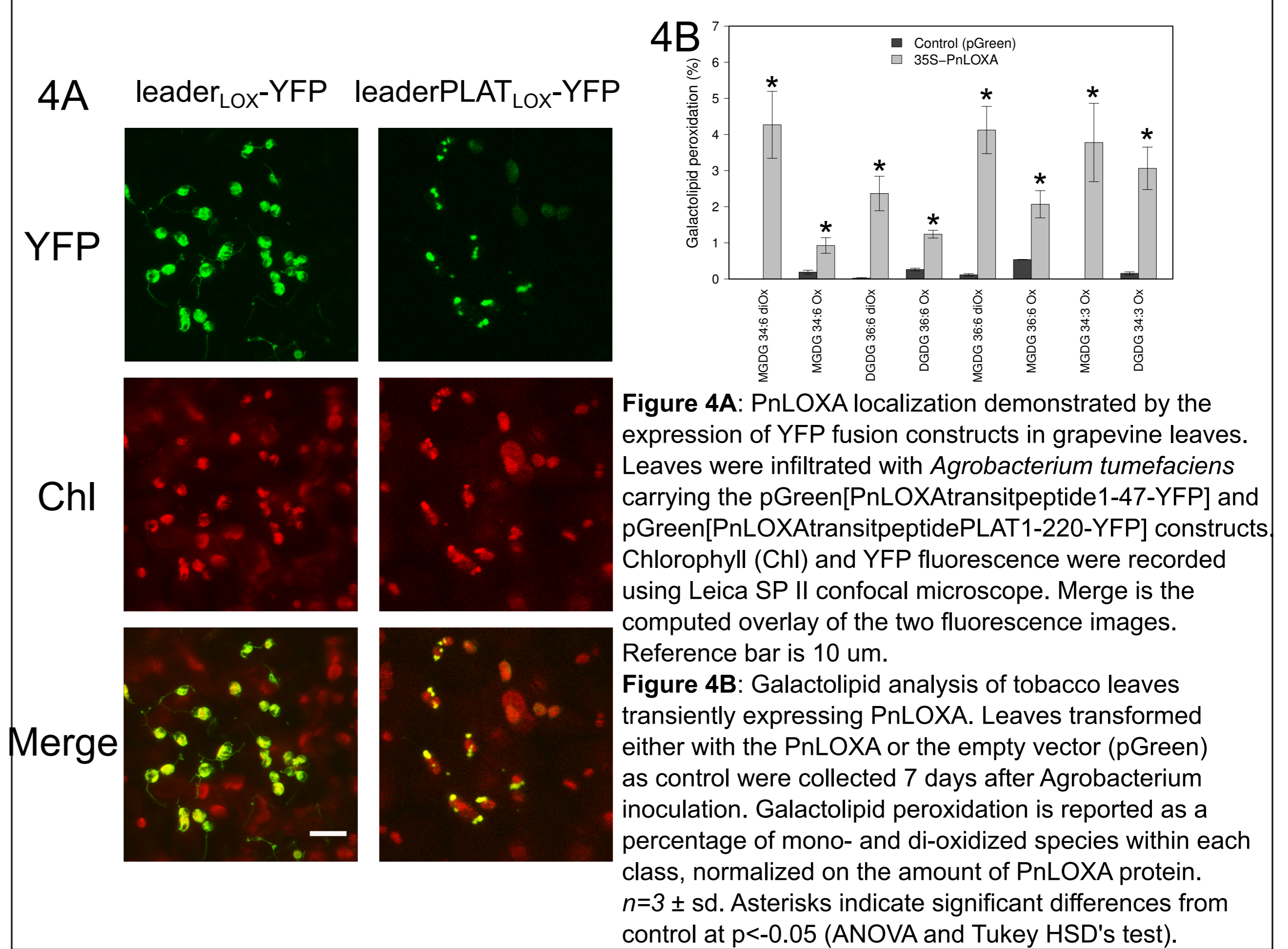
Figure 1B: Zymogram of catalase activity during Pinot Noir berry development.

Figure 1C: Catalase specific activity measured *in vitro* by determining either H₂O₂ consumption (absorbance at 240 nm) or O₂ production (in-line O₂ recording using direct injection MS). n=2 ± se.

13-S Peroxidized membrane galactolipids accumulate inside Pinot Noir skin cells at the onset of ripening.



PnLoxA is a chloroplastic lipoygenase able to peroxidize membrane galactolipids.



A 13- lipoygenase is expressed at the onset of ripening in the skin of Pinot Noir berries.

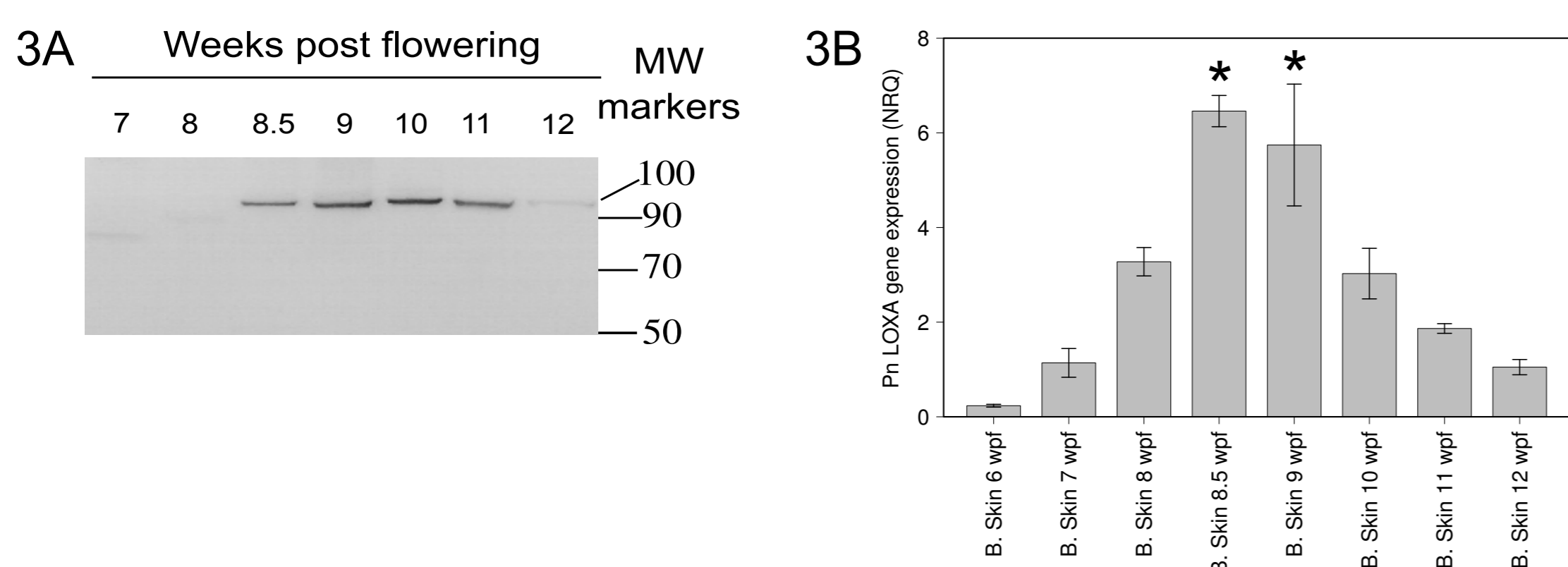


Figure 3A: Western blot analysis of lipoygenase expression in Pinot Noir berry skin extracts using a commercial antibody against Arabidopsis LOX2 and 10 ug of total protein extracts per lane. The band has been sequenced (nanolC-MC) and identified as Vv06s0004g01510, named PnLoxA.

Figure 3B: RT-PCR analysis of PnLoxA gene expression in the berry skin along development. Normalized relative quantities ± se were calculated using three reference genes; n=3. PnLoxA expression at véraison (marked by asterisks) was significantly different from pre-véraison (6-7 wpf) and ripening (11-12 wpf) as assessed by ANOVA and Tukey HSD test (p<0.01).

Is PnLoxA the homologue of Tomato Lipoygenase C? Are they involved in fleshy fruits aroma production or in ripening regulation, or both?

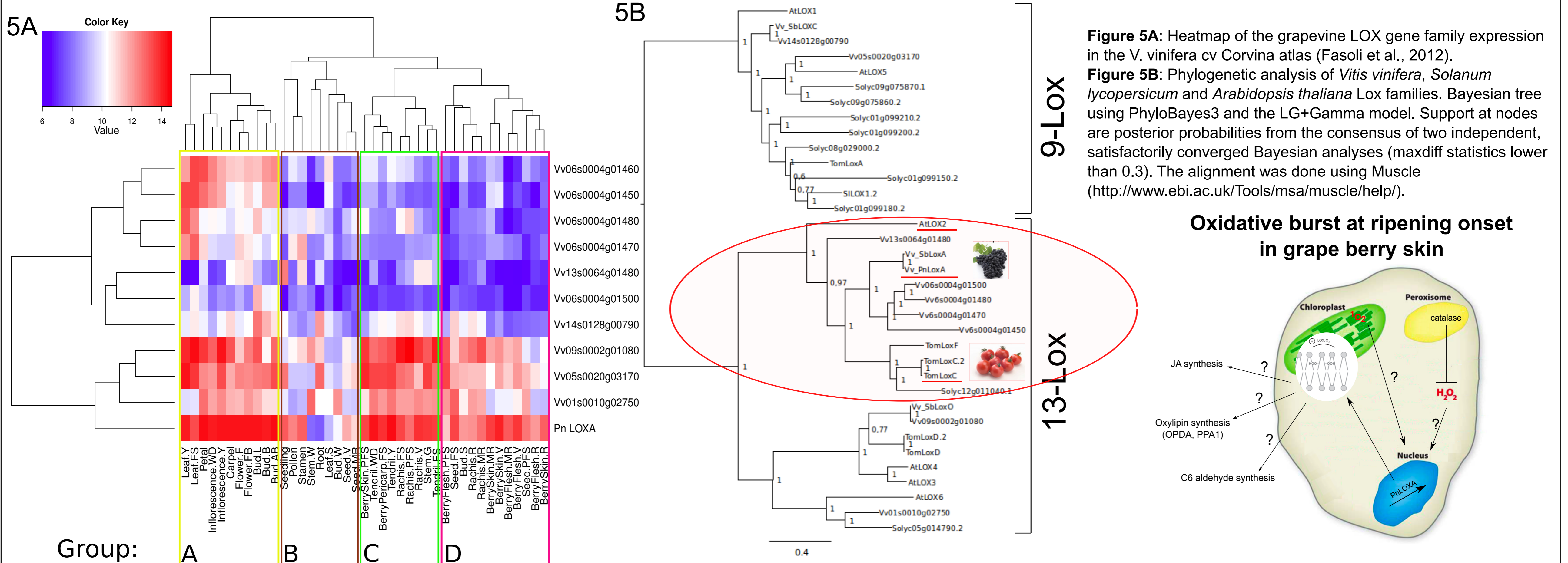


Figure 5A: Heatmap of the grapevine LOX gene family expression in the *V. vinifera* cv Corvina atlas (Fasoli et al., 2012).

Figure 5B: Phylogenetic analysis of *Vitis vinifera*, *Solanum lycopersicum* and *Arabidopsis thaliana* Lox families. Bayesian tree using PhyloBayes3 and the LG+Gamma model. Support at nodes are posterior probabilities from the consensus of two independent, satisfactorily converged Bayesian analyses (maxdiff statistics lower than 0.3). The alignment was done using Muscle (<http://www.ebi.ac.uk/Tools/msa/muscle/help/>).

Oxidative burst at ripening onset in grape berry skin

