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Energetic aspects of Sugar import and Malate breakdown in the ripening berry

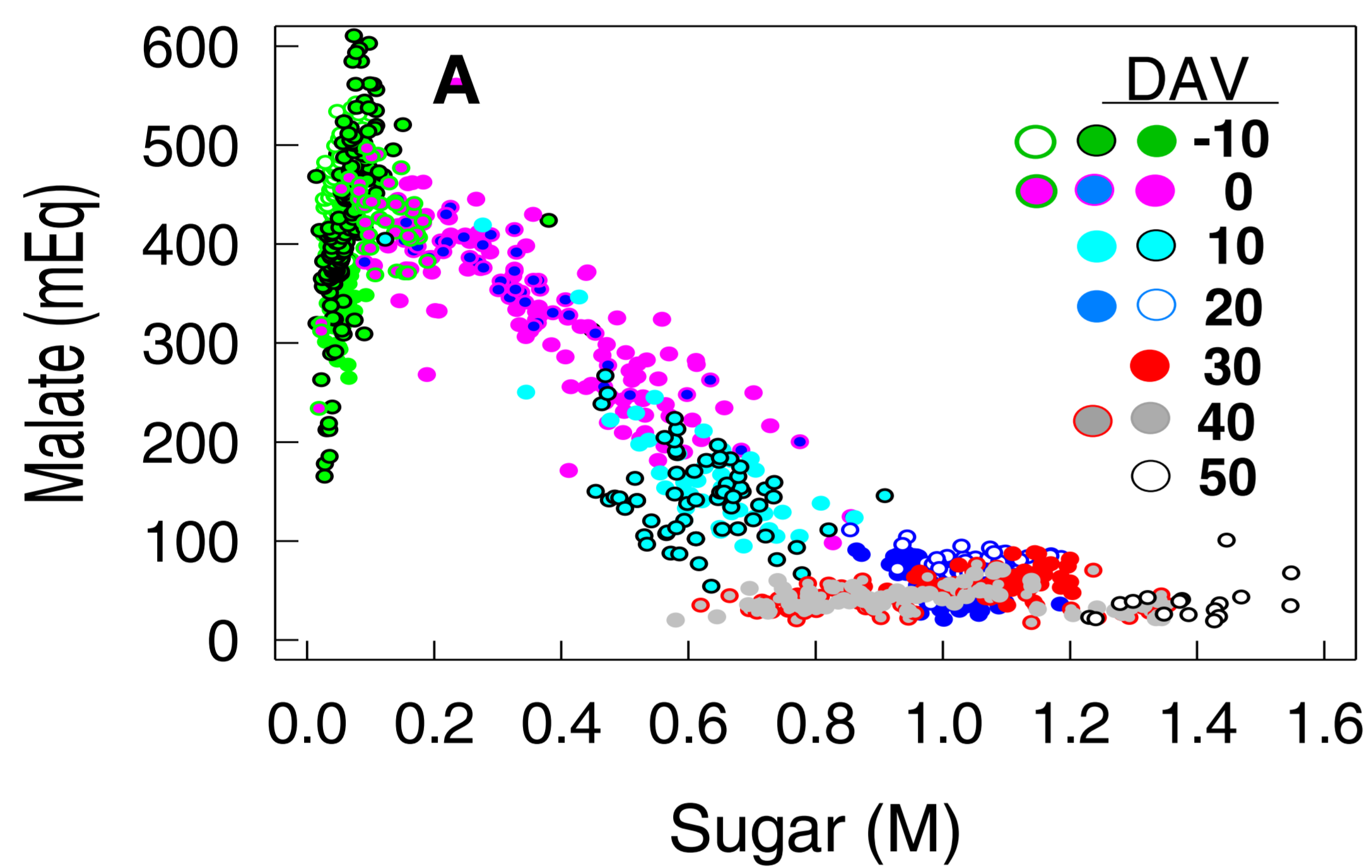
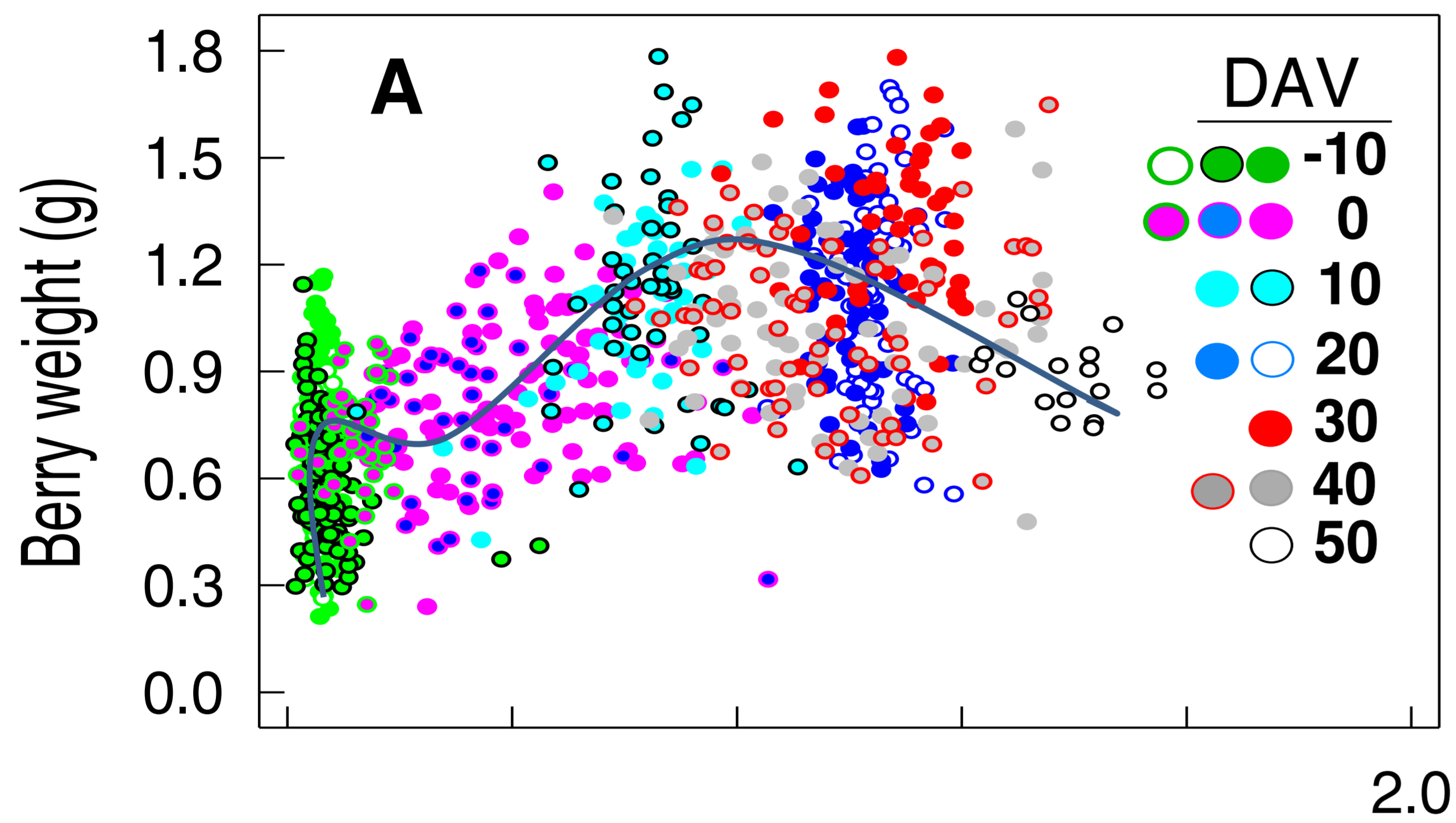
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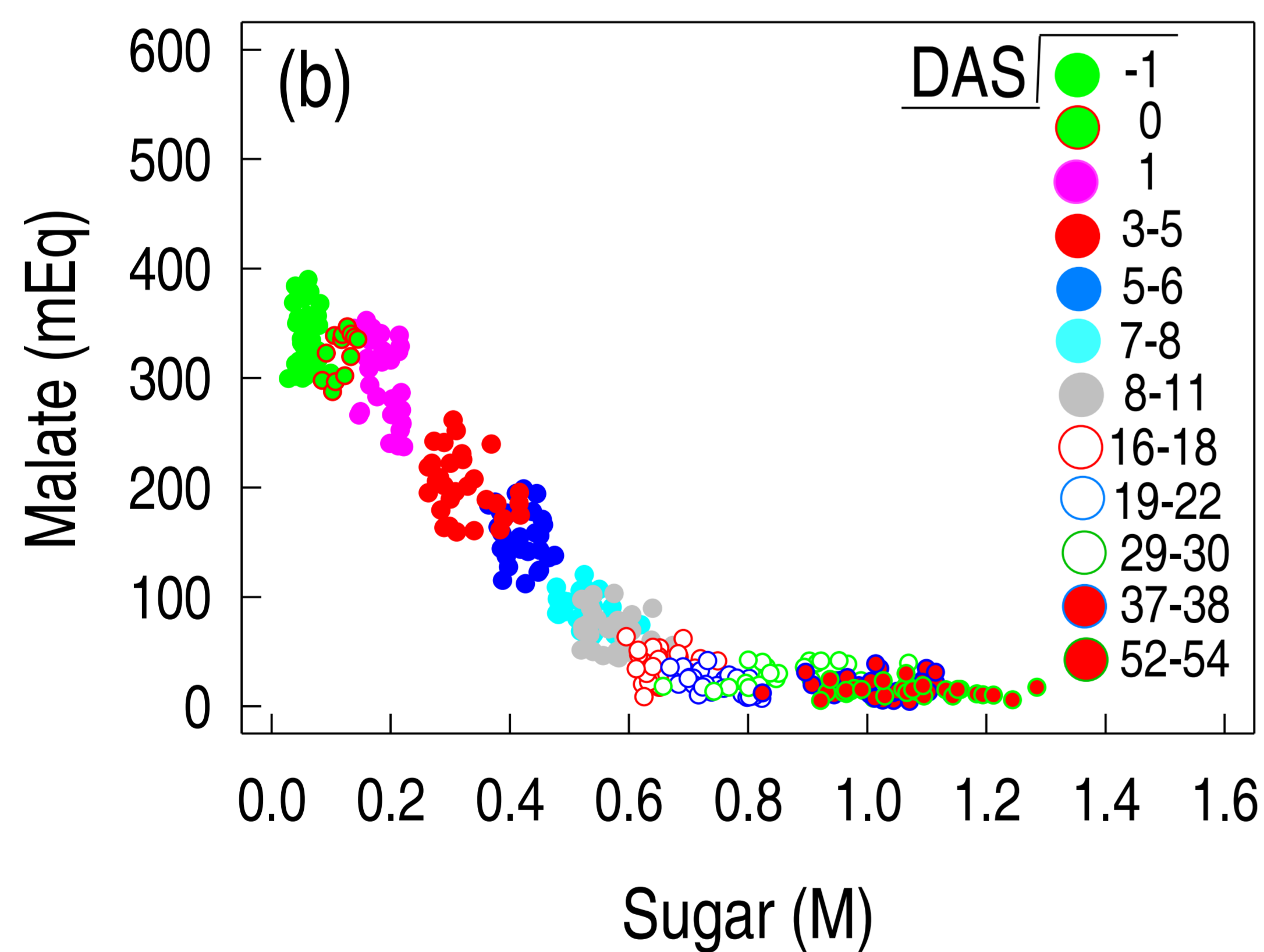
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Present knowledge on berry metabolism is limited by fruit heterogeneity and asynchrony. More than 1000 Individual Pinot berries were harvested in order to characterize berry growth, sugar and acids at 10 days intervals.

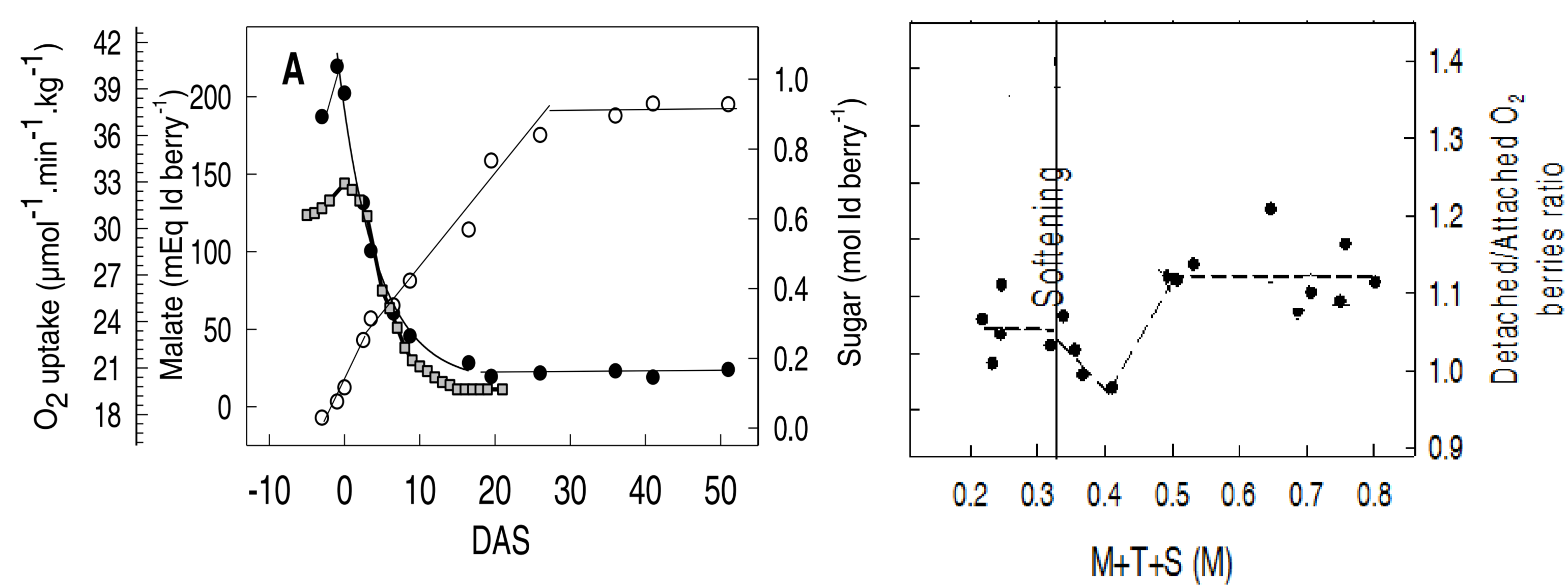
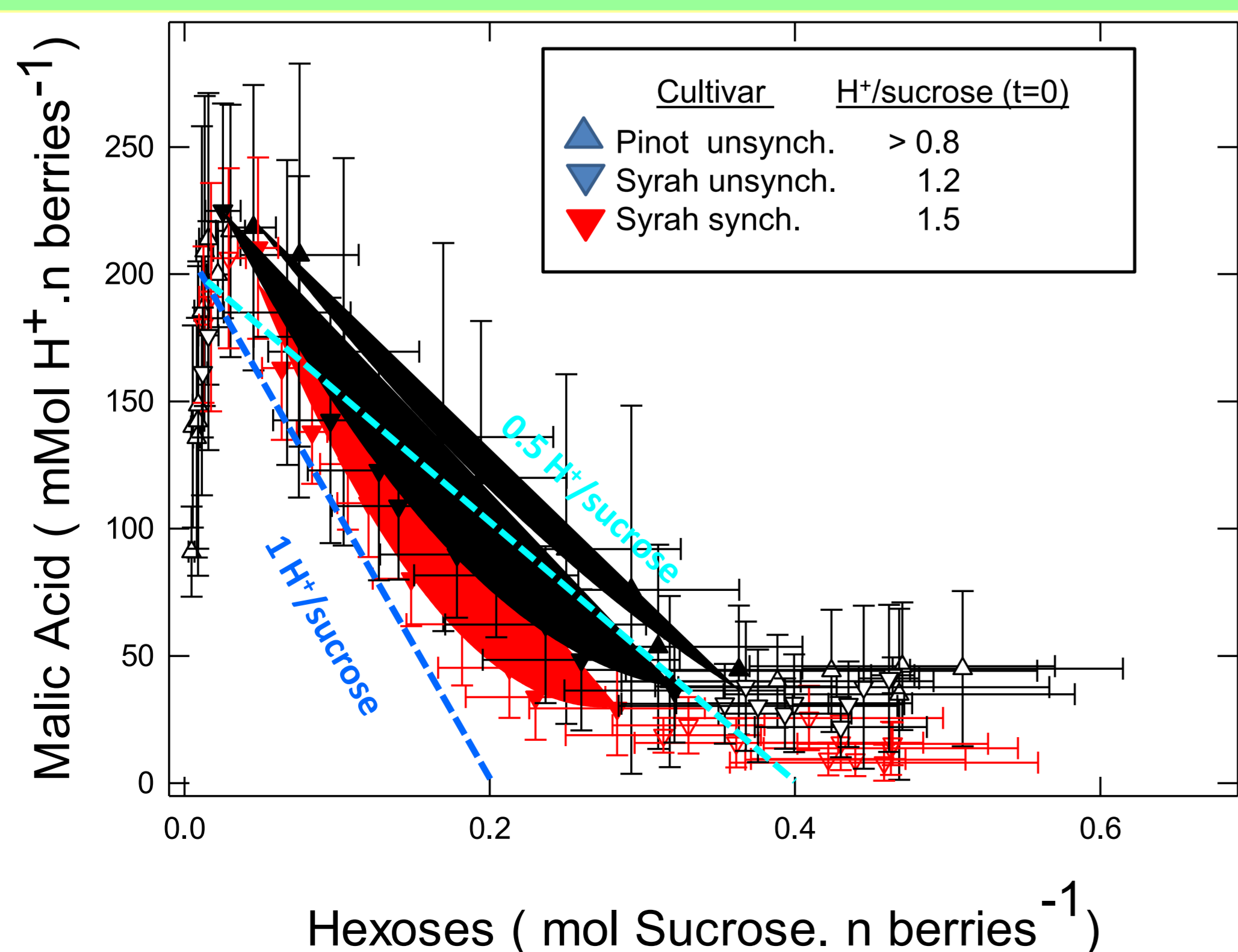
Hexose accumulated 6 times faster than they can be oxidized by berry. *In planta* measurements showed that oxygen reduction was unaffected by the rate of phloem unloading, as confirmed upon detaching the berries. By contrast, respiration diminished simultaneously with the malic acid content.



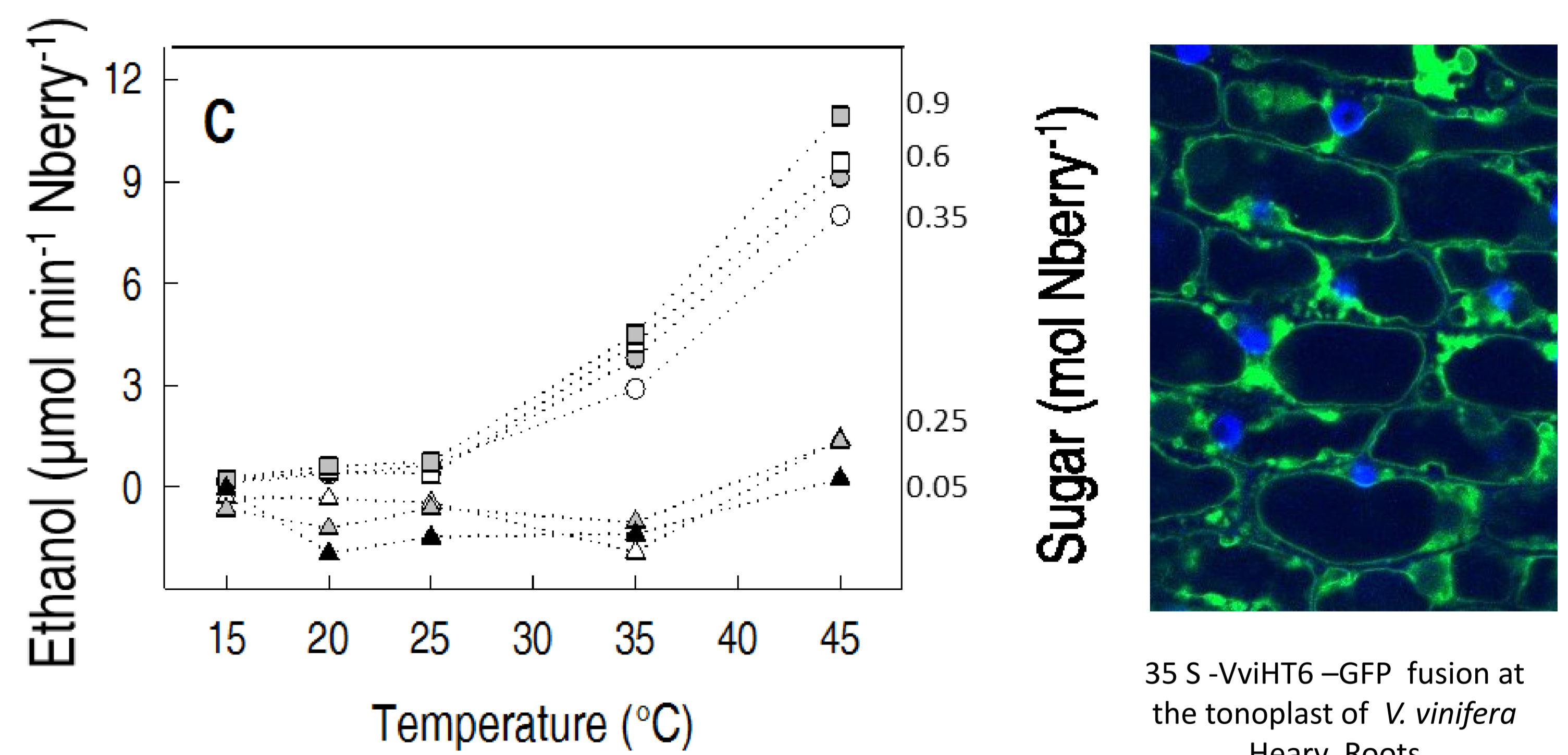
Heterogeneity in major solutes almost vanished when individual softening dates were considered. Malic acid was nearly consumed by Syrah in 10 days only, largely before technological maturity was reached



Metabolic fluxes inferred from volume and concentration data initially fitted with net sucrose/H⁺ exchange at the tonoplast, but hexose virtually doubled after the completion of malate breakdown.



Aerobic fermentation triggered by temperature developed as malate breakdown was impaired and the net H⁺/sucrose exchange was quenched.



35 S-VvHT6-GFP fusion at the tonoplast of *V. vinifera* Heary Roots

Discussion/ Conclusions

Sugar loading, malate breakdown and respiration have received considerable attention in ripening berries. Understanding the degrees of freedom of the underlying mechanisms would help to design the ideotypes of future grape varieties more resilient to global warming regarding berry acidity. In depth revisit of fundamental metabolic fluxes in individual berries led us to reveal new sub phases in the ripening process that were out of reach on conventional unsynchronized fruits samples. We show that despite its huge intensity, phloem unloading in the ripening berry essentially escapes respiratory energy coupling, which indicates in turn that H⁺ recirculation through sugar/H⁺ transporters and ATPases at the plasma and vacuolar membranes should be globally bypassed, therefore placing SWEETS as major contributors of berry ripening (Chong *et al.*, 2014). VvHT6, induced at véraison (Terrier *et al.*, 2001) appears as the privileged candidate for the net H⁺/sugar exchange induced at the tonoplast at the onset of ripening. In this respect, contrary to our previous interpretation, the initial release of vacuolar acidity is not the trigger of aerobic fermentation as a fail-safe mechanism against cytoplasmic acidosis, so the outgoing malic acid flow must be tightly adjusted to the oxidative capacity and neoglucogenesis during early ripening. Aerobic fermentation is induced precisely when net H⁺/sucrose exchange at the tonoplast becomes limited by malate availability, so H⁺ recirculation needs to be energized. Energy coupling was documented in mitochondria from mature grape (Romieu et Flanzky, 1988), but the decrease in respiration, accompanied with greater propensity to aerobic fermentation, now raises question on respiratory complexes present in green stage, their evolution during ripening, and interaction with vacuolar function. Genetic variation in the malic acid/sugar pathway is under present investigation (Bigard *et al.*, 2018)