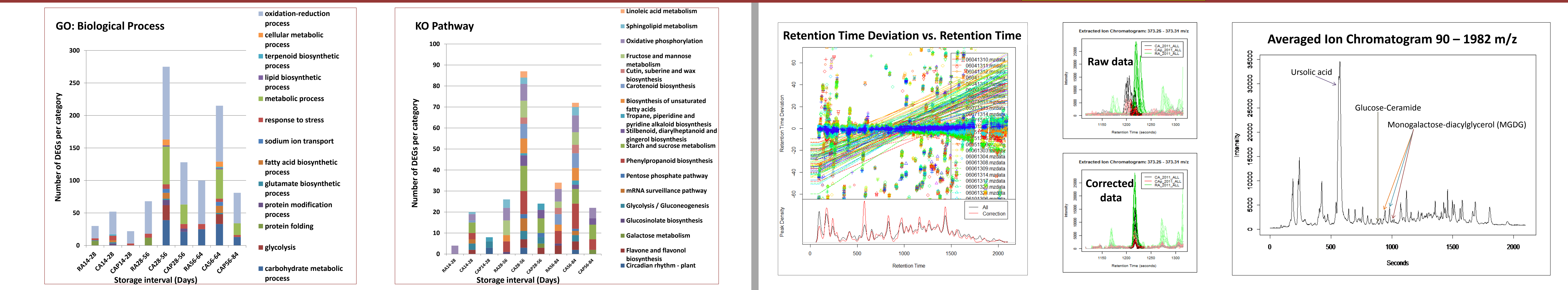
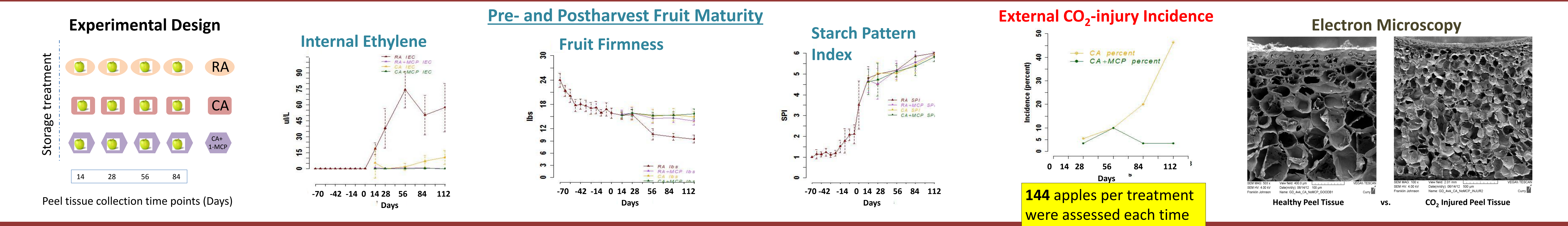
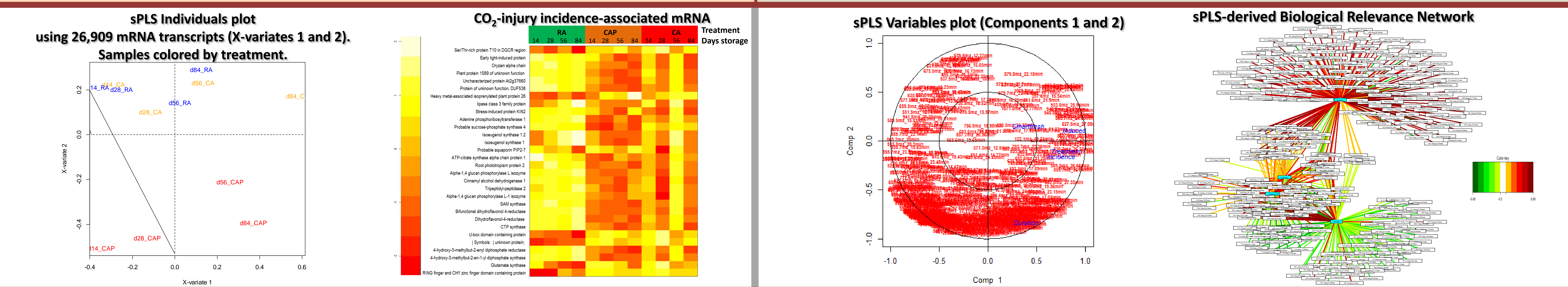


Introduction: In 2012, the estimate for the U.S. apple production was valued at \$3.2 billion. Understanding the molecular mechanisms of pome fruit ripening and maturation and response to postharvest storage are of great importance for the improvement of fruit storability, and for postharvest physiological disorder prevention. While CA storage offers a vehicle to maintain apple fruit quality during periods of low demand for fresh fruit, carbon dioxide (CO₂) is known to incite a peel tissue disorder recognized as CO₂-injury (Picture (left; above): examples of external CO₂-injury on ‘Golden Delicious’ fruit peel tissue), which appears as highly lignified and rough textured bronze-colored patches. Healthy and external CO₂-injured ‘Golden Delicious’ fruit messenger RNA (mRNA) and non-polar lipids (NPLs) were used to discern molecular pathways important for postharvest fruit storage and CO₂-injury incidence. *This research was funded by: Agrofresh LLC*



mRNA transcript profiling: functional bioinformatics and annotation: Blast2GO provided the assortment of GO Cellular Components (CC), Molecular Function (MF), Biological Processes (BP, above (left)) and KO Pathway (P, above (right)) terms associated to each obtained hit and returned an evaluated annotation for the queried Illumina mRNA sequences (CLCbio, www.clcbio.com) from Regular Air (RA), CA, CA+1-MCP (CAP) storage in 2011. The ‘MixOmics’ library was loaded in R, and was used for sparse Partial Least Squares (sPLS) relevance network analysis (below (left)).

Metabolomic profiling: chromatography and spectra analysis and annotation: ‘XCMS’ and ‘CAMERA’ libraries loaded in R corrected the retention time and mass spectral data from Regular Air (RA), CA, CA+1-MCP (CAP) storage obtained by LCMS/APCI in ‘Golden Delicious’ peel tissue (above). A chemical standard was purchased or Metlin Metabolite Database (metlin.scripps.edu) MS data was used to identify NPL metabolites. Peak intensity values were used to analyze differences between storage types. ‘MixOmics’ package loaded in R was used for sparse Partial Least Squares (sPLS) relevance network analysis (below).



Results: MF (not shown) was the most gene enriched category of differentially-expressed genes (DEGs) followed by BP>P>CC. The number of DEGs in CA was higher than RA and CAP fruit over the full storage duration. 1-MCP obviated the transcriptional surge of apple peel mRNA demonstrated in CA storage. CA and CAP fruit were more different than similar in all GO categories. Among treatments, the difference in CC (not shown) were mRNA for the endoplasmic reticulum, mitochondria and cell wall. 1-MCP strongly inhibited mRNA transcription for genes found in these GO sub-categories. Using RTqPCR, differences are being explored for genes responsible for external CO₂-injury incidence (above (right)). This gene set represents brassinosteroid, non-mevalonate and protein degradation pathways.

Results: Differentially-biosynthesized NPLs associated with duration (i.e. postharvest storability) were galactolipids (e.g. MGDG, DGDG), diacylglycerol and phosphatidic acid species, and a putative C40 isoprenoid. Several p-coumaryl acyl conjugates, a campesterol ester, beta-sitosteryl linolenate, beta-sitosteryl linoleate, and a glucose-ceramide associated with response to CA storage. NPLs associated with duration were also found associated with the CO₂-injury incidence variable. These were MGDG, DGDG, C40 isoprenoid, and several DG and PA species. Other lipids associated with the incidence variable and not duration included two acylated steryl glucosides (ASG), and zeaxanthin and quinone and pheophytin and chlorophyll-like species. NPLs relevant to duration and response to CA storage mentioned above were affected by 1-MCP in apple fruit peel tissue.

The deeper bottom line: The tendency of gene enrichment in RA supports the ontological claim that fruit developmental processes and maturation during postharvest cold air storage invariably acts so as to avoid discontinuities; and ‘Golden Delicious’ didn’t succumb to any postharvest physiological disorder over the full storage duration. The vast transcriptome expression induced by CA suggests to reject the hypothesis that it is unreasonable that unwarranted mRNA transcription results in a discontinuous change of growth; This action may direct the cell physiological susceptibility to CO₂-injury. Through metabolic profiling, NPLs associated with the cell wall and cell signalling pathways were shown. Depending on the cultivar, 1-MCP action may interact differently with molecular components relevant to mRNA translation and CO₂-injury.