Predicting Storage Disorders by Developing Diagnostic Toolboxes

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Depending on the variety, apple fruit are susceptible to many types of physiological disorders. These disorders can be associated with either air storage, controlled atmosphere (CA) storage, or both, and can occur early or later in storage depending on the disorder. Most storage disorders also are affected by growing season. For instance, higher risk of superficial scald development is typically associated with hot dry summers, while higher risk of 'Empire' flesh browning is typically associated with cooler summer growing conditions. Remarkably little is known about the biochemical factors that are responsible for the different disorders.

"Apple fruit are susceptible to a range of physiological disorders that develop during storage. The erratic behavior of these disorders, often due to the effects of growing season, means that the industry faces the potential loss of fruit each year. In an international project funded by the USDA Specialty Crops Research Initiative we aim to develop diagnostic tools for the industry that will predict and determine progress of several disorders."

To address this lack of information about disorders, we have assembled an international team to develop a diagnostic "toolbox" that can be used to manage postharvest necrotic disorders. The objective of the project is to use new technologies to find hidden changes in apple fruit chemistry or "biomarkers" that can be used to:

1. Predict risk of disorder development in fruit at harvest;
2. Determine disorder development during storage so that storage operators can respond by modifying storage management, or by marketing fruit before losses become severe;
3. Diagnose disorders after they occur, as many disorders can look similar and are difficult to differentiate from one another after storage.

The overall goal is develop cost effective risk assessment and diagnostic tools that will be used by industry, which could conceivably be anything from tests that can be performed by agricultural service laboratories, similar to having mineral analysis performed, to those that can be utilized in the field, packing shed, or supply chain like dip-stick tests or on-line analyzers.

The project is funded by the USDA Specialty Crops Research Initiative, with matching funds from AgroFresh, the Washington Tree Fruit Research Commission, Cornell University, the Boyce Thompson Institute, the New Zealand Institute for Plant and Food Research, and KU Leuven, Belgium.

The project also has industry stakeholders who are charged with providing an advisory role. The committee is comprised of Jeff Crist, Rod Farrow and JD Fowler in New York, and Ines Hanrahan, Brent Milne, and Mike Willet in Washington State.

At Cornell University, the scientific team is made up Chris Watkins, Nigel Gapper, Jackie Nock, Jim Giovannoni, Zhangjun Fei and Silin Zhang, who are responsible for fruit storage, transcriptomics and bioinformatics, and Brad Rickard and Lei Lei who are responsible for the economic aspects of the project. At USDA, Wenatchee, Dave Rudell leads the metabolomic team that includes Jim Mattheis, Jinwook Lee, Rachel Leisso, Dave Buchanan, and Janie Countryman. The Plant and Food Research team, led by Jason Johnston and Robert Schaffer, is also working on genomics, while Bart Nicolaï and Maarten Hertog from the KU, Leuven in Belgium are responsible for using complex mathematical routines to help transform the vast quantities of numerical data into usable information.

What disorders are we studying?

We selected five disorders because of their importance to our industries (Figure 1).

1. Superficial scald, a browning of the skin of fruit of susceptible varieties such as Granny Smith, Delicious, Cortland and McIntosh. This disorder is usually prevented commercially by the use of diphenylamine (DPA) and 1-methylcyclopropene (1-MCP; SmartFresh). It is considered to be a chilling injury that develops after prolonged storage in air or CA.
2. External carbon dioxide injury, a skin disorder associated with carbon dioxide in the storage environment. The incidence can be enhanced by 1-MCP treatment, but prevented by DPA. It is seen most commonly on Empire apples which have not been treated with DPA.
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4. Soft scald, a concern primarily for Honeycrisp and Jazz apples. Soft scald is a low temperature skin disorder; reasonably good control can be obtained by a conditioning treatment of 7 days at 50°F. 1-MCP and DPA can reduce, but not prevent, its incidence.
5. Soggy breakdown, a concern primarily for Honeycrisp apples. This disorder is located in the flesh and control factors are similar to those described for soft scald.

What are "gene expression profiling" and "metabolomics"?

"Gene expression profiling" and "metabolomics" are both disciplines that look at chemical changes within living organisms and

Superficial scald

External carbon dioxide injury

Flesh browning

Soft scald (external injury) and soggy breakdown (internal injury)

Figure 1. The physiological storage disorders under investigation in this project.

genes that control these changes under a given set of conditions. Put simply, in our case, we are trying to identify key changes in apple fruit under different storage conditions that are associated with development of each of the disorders. Powerful new tools, used in the medical research field for similar purposes, are being adapted for this use.

The project overview is shown in Figure 2. To find biomarkers that the industry can use, we are comparing and contrasting naturally occurring chemical changes during storage. Conditions chosen included that that increase or reduce disorder development, as well as comparison of fruit from orchards with high risk and low risk of developing disorders. Once candidate markers have been selected, validation will be performed at multiple sites. Cost-effectiveness of prospective tools will be evaluated at different levels of the apple supply chain to indicate where economically significant benefits lie. Tools will be interactively

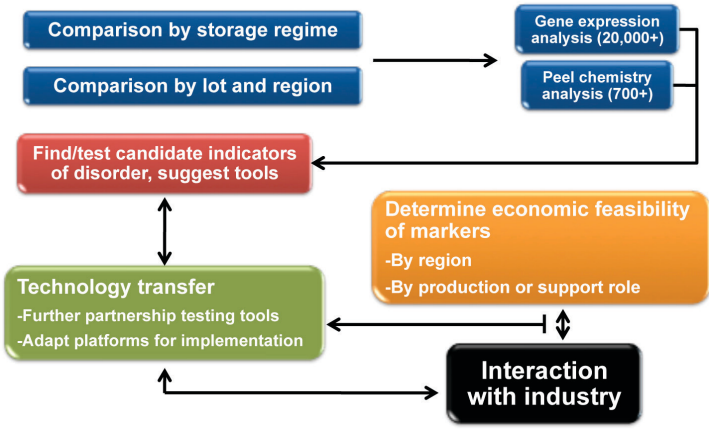

Figure 2. Overview of the project showing interrelationships between fruit sampling and treatment and analyses of gene and metabolite changes. The objective is to identify genetic and metabolite biomarkers that can be used by apple industries to predict the disorder development.

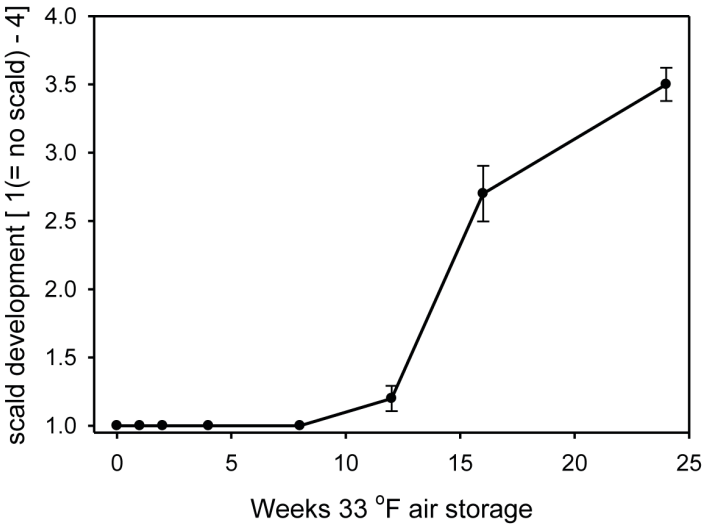

Figure 3. Superficial scald development on untreated, DPA-treated, or 1-MCP-treated Granny Smith apples stored at 33°F. Error bars represent SE (n=18).

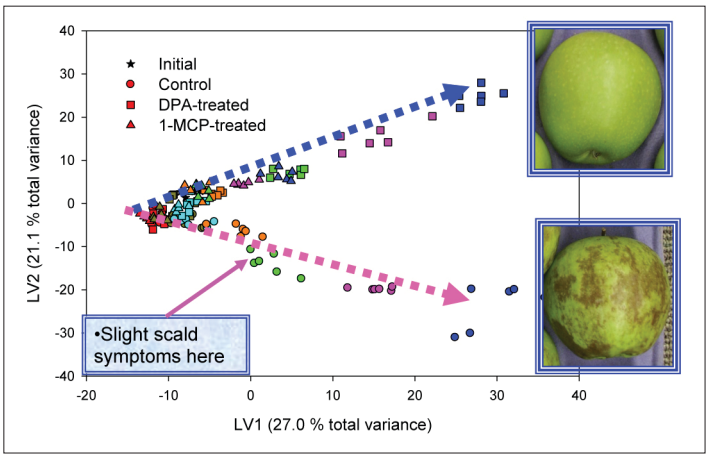

Figure 4. Metabolic profiles of metabolites extracted from peel tissues of untreated, DPA-treated, or 1-MCP-treated Granny Smith apples during air storage at 33°F. Fruit were sampled at 0 (black), 1 (red), 2 (brown), and 4 weeks (light blue) and 2 (orange), 3 (green), 4 (pink), and 6 months (blue). The blue line shows the changes in metabolite levels over time that results in fruit free of scald (DPA and 1-MCP treated), while the pink line shows changes that are associated with scald development. These changes are detectable well before visible injury can be detected. (Modified from Rudell et al. 2009.

developed with service providers and the fruit industry and will depend upon the type of biomarkers found and the easiest ways they can be measured in the field.

Preliminary results

Extensive tissue collection of fruit from different orchards, postharvest chemical treatments and storage conditions is well underway. Results from these experiments are not yet available, but preliminary experiments illustrate the approaches taken.

Superficial scald (Figure 1) is a disorder that becomes visible after many weeks in storage (Figure 3), but control methods have to be applied soon after harvest to be effective. This suggests that changes in the fruit metabolism that can result in injury occur early in the storage period. In a study of superficial scald-related

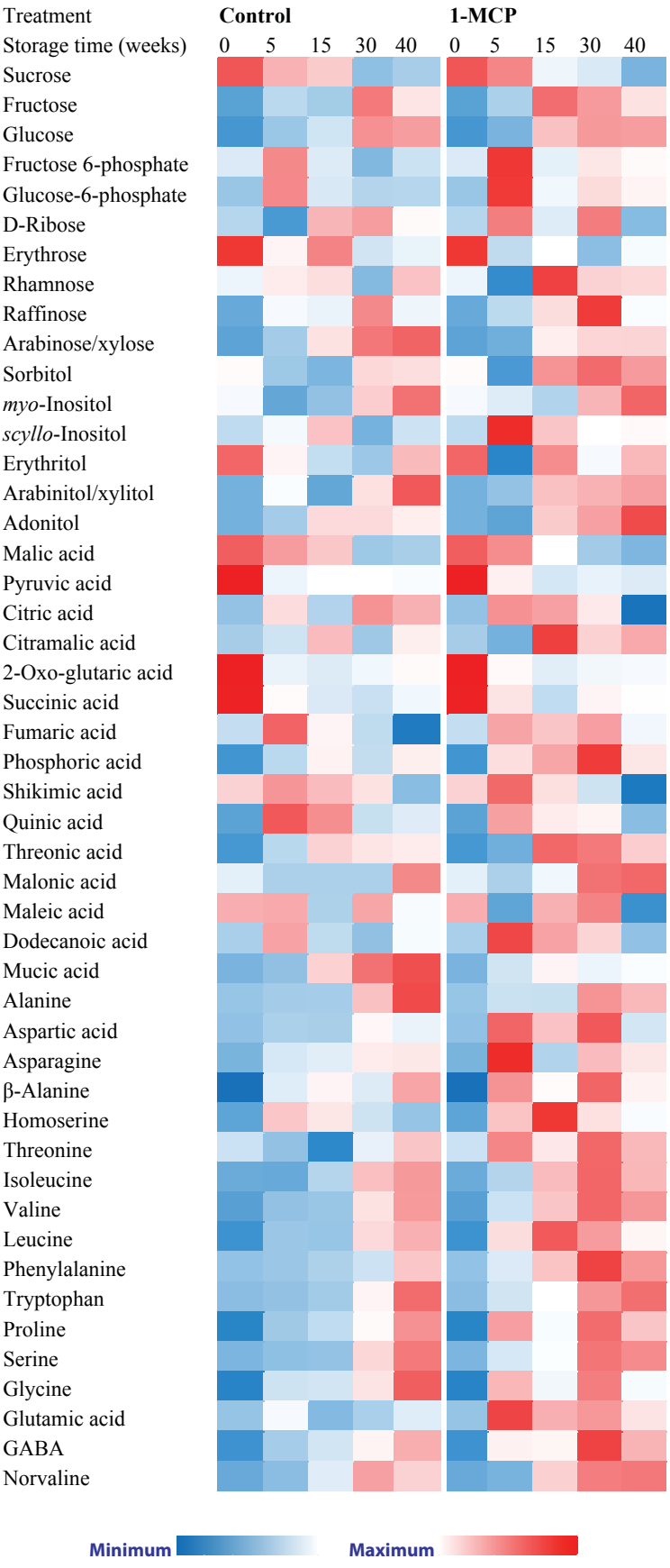

Figure 5. Heatmaps of carbohydrates, organic acids, and amino acids of Empire apple fruit untreated, or treated with 1-MCP at harvest, and then stored at 2% O_2/2% CO_2 for up to 40 weeks at 38°F. Metabolite levels correspond to the color temperature. Lower temperature (blue) indicates reduced levels of the respective metabolites and vice versa (modified from Lee et al., 2012).

metabolism, metabolomic evaluation of over 600 distinct primary and secondary metabolites was used to contrast peel metabolomes of control, DPA-treated or 1-MCP-treated apples in conjunction with superficial scald development (Rudell et al., 2009). Results revealed metabolomic divergence among different treatments within 1 week after harvest as indicted by the blue and pink lines (Figure 4). This divergence continued during storage and preceded scald symptoms by 10 weeks. Metabolic divergence over the 6-month storage period was associated with multiple metabolic pathways including those affecting fruit quality, superficial scald-associated α-farnesene oxidation, as well as hundreds of other prospective biomarkers associated with multiple metabolic pathways.

Flesh browning A similar approach was taken for Empire apples. Fruit were stored at 33°F or 38°F in CA storage either with or without 1-MCP treatment. We know from previous research reported in the New York Fruit Quarterly (James et al., 2010a,b) that browning incidence can be exacerbated by 1-MCP treatment at warmer storage temperatures. Flesh browning, like superficial scald, is a long-term storage disorder, often not evident until May/June.

In a study of metabolites in 1-MCP treated fruit stored at 38°F, we were able to find major differences in metabolite composition in the flesh (Lee et al., 2012). Figure 5 shows some of these changes in the form of 'heatmaps'. Heatmaps show the increase and decrease in the amount of a given compound in red and blue, respectively. The extent of change is indicated by the intensity of the color. For example, one compound, GABA, has low levels at harvest as indicted by the darker blue color. In the untreated fruit the concentration increases over time – progressively lighter blue and then light red. In the 1-MCP fruit, GABA increases more markedly to a peak on week 30 of storage as indicated by the darker red color.

Clearly, treatment of fruit with 1-MCP affects the metabolic composition. However, these changes reflect the many effects of treatment on ripening, and the changes that are associated with increased browning remain to be identified.

These preliminary results for superficial scald and flesh browning highlight the potential of our broad-based metabolic profiling technique for discovering predictive, diagnostic, and distinguishing biomarkers that can be employed in next generation storage disorder risk management and forensic tools for the apple fruit industry. Corresponding genomic analysis will highlight genetic processes that provoke metabolomic changes associated with disorder development or avoidance.

Summary

Apple postharvest physiological disorders, characterized by peel or flesh necrosis, result in significant yearly financial losses. The ineffectiveness or lack of availability of tools to manage these disorders in many instances makes their continued development and study of these disorders crucial. Also, current control systems provide little assurance that apples will not develop disorders in storage or elsewhere in the supply chain. An alternative control strategy, based on diagnostic biomarkers, will provide storage managers with effective tools that predict, diagnose, and distinguish these disorders to efficiently target treatments, guide storage management and marketing decisions and improve quality

assurance throughout the supply chain. Our goal is to develop economically-feasible metabolite and gene product biomarker-based tools for our apple industries by 1) improving prediction and diagnosis of identified as well as novel storage disorders to direct treatment and storage management decisions; 2) improving fruit quality assurance; and 3) integrating biomarker-based diagnostic protocols with existing protocols. Meeting project goals will impact current understanding and provide real-time information about the disorders so that more informed storage and marketing decisions can be made.

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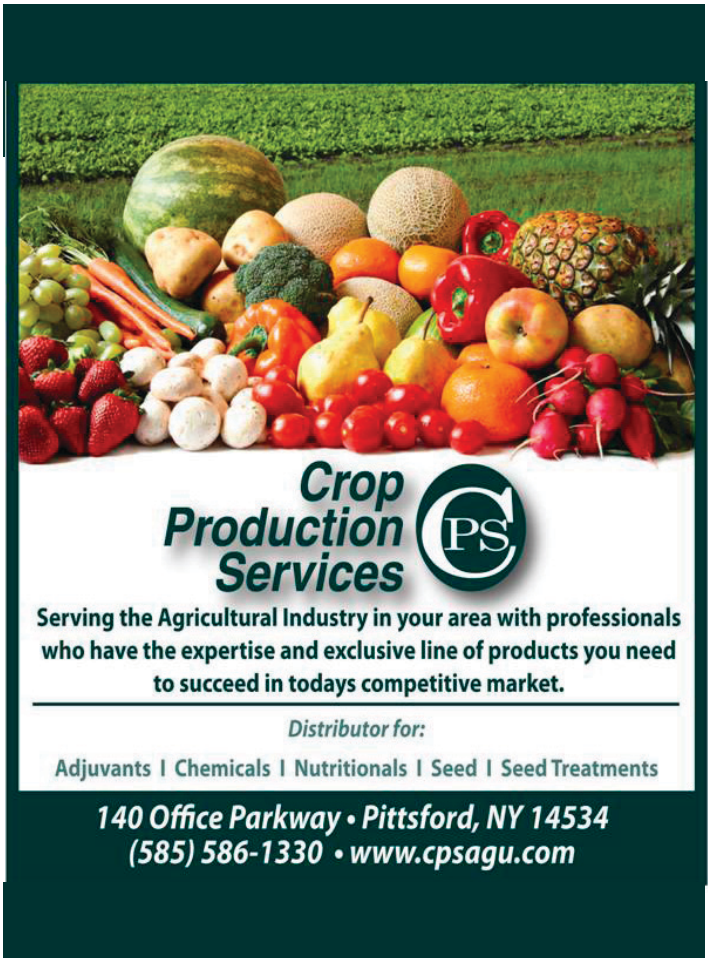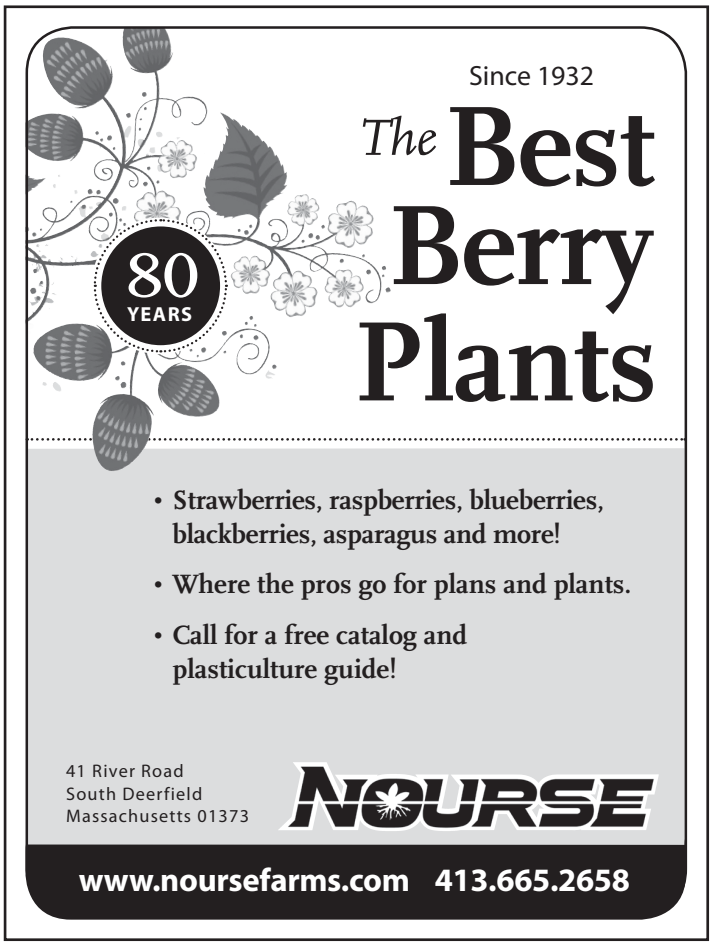
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