FINAL PROJECT REPORT

Project Title: Support systems to deliver elite new cultivars for extended storage

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Other funding sources

Agency Name: WTFRC Apple Review
Amt. awarded: $642,160 (2012-2014)
Notes: "Apple scion breeding program" The foundational program on which the current WTFRC project builds

Agency Name: Winston Churchill Memorial Trust
Amt. awarded: $2,000
Notes: C. Hardner's airfares from Brisbane, Australia to Hawaii

Agency Name: Queensland Alliance for Agriculture and Food Innovation
Amt. awarded: $10,000
Notes: Two weeks of C. Hardner salary

Agency Name: USDA-CSREES, Specialty Crop Research Initiative
Amt. awarded: $7.2 mil plus same matched by universities and industry (Sep 2009 – Aug 2014)
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". RosBREED project datasets being generated will be mined further for DNA test development for storability traits

Total Project Funding: $77,724

Budget History:

Item	**Year 1:** May 2013- Apr 2014
Salaries	$58,832
Benefits	$ 3,960
Wages	$ 9,464
Benefits	$ 918
Equipment	
Supplies	
Travel	$4,550
Plot Fees	
Miscellaneous	
Total	$77,724

RECAP ORIGINAL OBJECTIVES

Focusing on extended storage, our overall goal is to deliver routinely implementable methods to the Washington Apple Breeding Program (WABP) for revealing genetic potential for commercial performance. This project is to improve the WABP's prospects for developing superior new cultivars that provide exceptional fruit quality like Honeycrisp but without the storage flaws of that cultivar.

Specific objectives:

1. Optimize resource allocation in the WABP
2. Implement software for routine prediction of genetic potential

SIGNIFICANT FINDINGS

Optimize resource allocation in the WABP:

- Opportunities were identified to optimize resource allocation in the WABP.
- The WABP's Phase 1 and Phase 2 have been thoroughly dissected for their operational activities, costs of each activity, and appropriateness of traits evaluated at that phase. Complete datasets of historical WABP data for these two Phases have been compiled. Only with such dissection and dataset preparation can efficiencies be identified and alternatives objectively compared.
- The greatest proportion of costs for Phases 1 and 2 are in harvesting and fruit processing, especially labor, indicating that any reduction in fruit processing time while achieving the same or better genetic outcomes would substantially save costs.
- An experiment is underway to identify efficiencies that might be gained in Phase 1 fruit quality evaluations. As this one-year project runs from May 2013 to Apr 2014 with extended storage of 2013 season fruit, the final datasets and their analyses are not yet complete.
- In Phase 2 trials, efficiencies were identified in identifying selections superior in their genetic potential under extended storage: fruit quality evaluations after both normal storage (2 months) and extended storage (4 months) are unnecessary; conducting only one is sufficient. After a regular 2-month storage evaluation, a supplemental 4-month duration could be used just to reveal storage disorder fatal flaws.
- DNA testing capability was advanced for storability-related traits by refining the predictiveness and technical efficiency of previously available DNA tests, developing new DNA tests, and identifying new genomic regions to target.
- Deployment of DNA tests is enhancing efficiency, accuracy, and creativity in the WABP.

Implement software for routine prediction of genetic potential:

- New software was developed, delivered, and implemented in the WABP for routine prediction of genetic potential among Phase 2 selections.
- The first routine use of this software, *Elite Advance*, in 2013 by WABP staff supported decision-making by the breeder for advancement of certain selections from P2 to P3.
- *Elite Advance* was also useful in identifying outlier data points that could unduly bias selection decisions.

RESULTS & DISCUSSION

Activity 1. Optimize resource allocation in the WABP

The efficiency of current selection methods in Phase 1 and Phase 2 trials to identify selections with elite performance under extended storage is being compared with alternative methods, using a cost-benefit ratio by incorporating economic information into models previously developed.

Phase 1 trials

An experiment is underway to identify opportunities to enhance the design of Phase 1 trials, targeting extended storage. This experiment involves (i) economic modeling of Phase 1 operations, (ii) collating all available historical Phase 1 data, (iii) assembling a new dataset of fruit quality performance for a large set of current Phase 1 seedlings followed by statistical analyses in quantitative genetics and economics to compare cost-efficiency of alternative, logistically feasible Phase 1 designs, and (iv) developing recommendations for the 2014 harvest season. Fruit quality evaluations after extended storage will not be conducted until around Feb 2014. Therefore, subsequent statistical analyses to calculate cost-efficiency among alternative Phase 1 trial designs have not yet taken place. As originally planned, this part of the project is expected to be completed by May 2014 so that recommendations for the 2014 harvest season can be developed and subsequently implemented. Progress in steps (i) to (iii) is described below.

i) The cost structure of Phase 1 activities, developed in previous years for marker-assisted seedling selection cost-efficiency estimates, has been updated to include personnel time and a more detailed cost inventory of consumables. The collated cost structure information is in a spreadsheet with an output of cost per seedling. The spreadsheet is easily manipulated to allow comparisons of costs of different Phase 1 structures. Detailed notes have been included to make future updating or altering the sheet easier. The largest proportion of Phase 1 costs are in nursery growing (42%), then fruit harvesting and processing (34%) (Figure 1). A reduction in fruit processing time would reduce costs, such as by using a streamlined fruit sampling protocol that efficiently gathers required information to confidently identify genetic potential without redundancy (addressed in the Jan 2013 report for the "Increasing decision confidence in cultivar development and adoption" WTFRC project). WABP resources can also be more efficiently allocated by using DNA testing to cull inferior seedlings in Phase 1, especially prior to nursery budding and field planting (as addressed by Edge-Garza et al. 2010 and in various previous WTFRC projects with Peace as PI, 2007-2012).

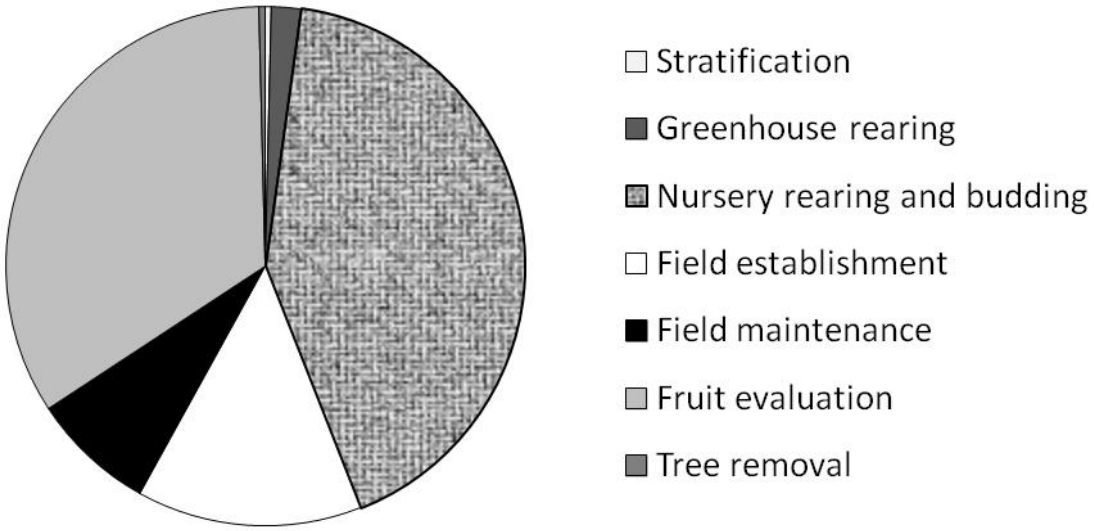

Figure 1: Cost structure of Phase 1 operations in the WABP. Phase 1 is the first stage of seedling evaluation, lasting approximately eight years from pollination.

ii) Existing Phase 1 data has been collated into a single spreadsheet to facilitate subsequent statistical analyses.
iii) 750 Phase 1 seedlings from 29 families were harvested in 2013 from both WSU Sunrise and Columbia View orchards using the typical WABP protocol of starch/iodine assessment of maturity (Cornell stage 3-5). Evaluations of appearance and maturity were completed at harvest. Texture components, soluble solids content, titratable acidity, appearance components, and disorder incidence evaluations after two months of regular atmosphere storage at 36 °F (2-3 °C) plus one week at room temperature have been completed. Fruit quality evaluations after four months of storage under the same conditions plus one week at room temperature are currently underway (i.e., fruit are in storage as of early Jan 2013).

Phase 2 trials

Similar to the economic dissection of Phase 1 trials above, an experiment is underway to identify opportunities to enhance the design of Phase 2 trials, especially for their ability to identify elite selections with superior genetic potential for fruit quality performance after extended storage. This experiment involves (i) economic modeling of Phase 2 operations, (ii) collating all available historical Phase 2 data and analyzing that data with the new software of *Elite Advance* (from Activity 2) to determine the effect of extended storage on identifying superior genetic potential for various fruit quality traits, (iii) combining the previous two elements into a single model that enables comparison of alternative Phase 2 trial designs, (iv) developing recommendations for improved Phase 2 evaluation protocols following the 2013 harvest season, and (v) identifying knowledge gaps and collecting additional Phase 2 trial data to refine Phase 2 evaluation methods for performance after extended storage. Progress in these steps is described below.

i) Phase 2 costs were collected and include personnel costs. The collated spreadsheet has a current output of cost per selection, although this can be easily manipulated to provide other outputs. Harvesting and fruit processing account of the largest proportion of costs (74% together) in Phase 2, the majority of this being labor (Figure 2).

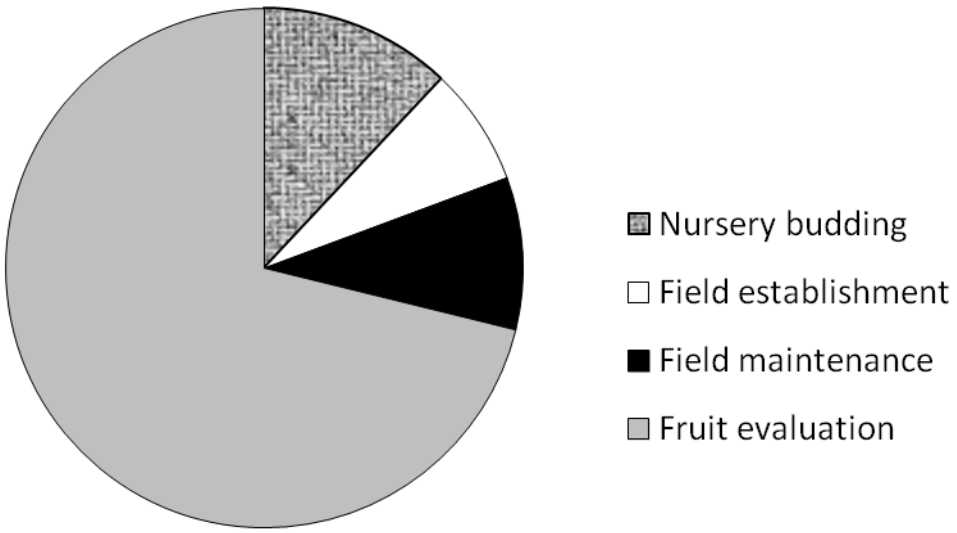

Figure 2: Cost structure of Phase 2 operations in the WABP. Phase 2 is the first evaluation of replicated selections, with multiple trees planted at multiple locations and lasting approximately six years.

ii) Extended storage data was available and collated for 2010-2012 Phase 2 trials, for the following traits:

17 sensory traits: starch rating, ground color, type of color, proportion of red color, extent of lenticels, extent of russeting, shape, size, appearance summary, crispness, hardness, juiciness, aromatic taste, sweetness, tartness, eating quality, and overall quality

8 instrumental traits: weight, diameter, soluble solids content, titratable acidity, pH, and Digi-Test texture measures of M1, M2, and Cn)

Analysis of this Phase 2 data indicated that on average there was a significant difference between 2 months storage and 4 months extended storage for many traits (Table 1).

Table 1. General effects of storage duration in Phase 2 trials on fruit quality traits. Analyses were conducted on Phase 2 selections each evaluated for 1-3 years over the 2010-2012 seasons.

Trait category	Significant differences observed between 2 and 4 months storage evaluations	
	Higher at 4 months extended storage	Lower at 4 months extended storage
Sensory	Starch rating, ground color, extent of lenticels	Size, crispness, hardness, aromatic taste, tartness, eating quality, overall quality
Instrumental	None	Weight, diameter, pH, titratable acidity, M1, M2, Cn

Between the storage conditions of 2 months and 4 months, there was no significant change in ranking of Phase 2 selections for almost all fruit quality traits (Table 2). These results suggest that there is no differential response among Phase 2 selections to extended storage. Therefore, both normal storage and extended storage treatments may not be necessary because performance under extended storage can be predicted from performance under 2 month storage and vice versa. However, extended storage has a better ability to reveal fatal flaws in the form of too-high incidences of storage disorders.

Table 2. Effect of storage duration in Phase 2 trials on identifying Phase 2 selections with superior genetic potential for fruit quality traits. Dataset was the same as used for Table 1.

Trait category	Significant re-ranking of Phase 2 selections observed between evaluations	
	Harvest and 2 months storage	2 months storage and 4 months storage
Sensory	Starch rating, hardness, eating quality	Starch rating, aromatic taste
Instrumental	M1, M2	None

For just a few texture traits (hardness, eating quality, M1, and M2), significant re-ranking of some selections was observed between harvest and 2 months storage evaluations (Table 2), indicating that evaluations at harvest for those traits cannot be used to predict performance after 2 months storage. We may have found the DNA information explaining some of this phenomenon (see *DNA information* – Ma-indel section below) which would allow us to exploit DNA tests in Phase 2 evaluations for increased efficiency.

iii) Alternative designs were modeled. Metrics for comparing alternative designs were chosen – the two methods being pursued are “acceptance interval” and “advance/discard errors”. The

acceptance interval metric measures the effect of the design on the genetic potential of the seedling and the confidence with which one could separate a seedling from a standard. The advance/discard errors measure the probability of an error of advancing (to P3) or discarding a Phase 2 selection. Nested within the equations for these two metrics is an equation to maximize the design of Phase 2 trials. Due to the nature of modeling, both of these metrics are written in the programming language of R.

iv) Evaluation of alternative Phase 2 designs is still underway.
v) Identification and filling of knowledge gaps to refine Phase 2 evaluation methods is still underway.

DNA information

Refinement of current DNA tests: Three existing DNA tests for storability-related traits were refined in 2013. These DNA tests were "Md-ACS1-indel" and "Md-ACO1-indel" for general storability (as these tests target differences in two genes involved in ethylene biosynthesis) and *Ma* locus markers for the "fresh sensation" traits of crispness, juiciness, and storability. The three DNA tests described above were deployed in 2013 in the WABP in parent selection and seedling selection. Use of all three tests, and others, in 2013 helped guide crossing decisions for better outcomes and help avoid wasteful crosses. In addition, Md-ACO1 and Ma-indel were used in 2013 to discard thousands of young seedlings predicted to be genetically inferior, thereby enriching new generations with superior genetic potential. Furthermore, such trait-predictive DNA information was also obtained in 2013 for all selections advancing into Phase 2.

Md-ACS1-indel: Statistical analyses of RosBREED data in 2013 to refine our understanding of the relative effects of the two variants (alleles) of the *Md-ACS1* gene, as determined by running the Md-ACS1-indel DNA test, confirmed our previous understanding. However, while individuals carrying the "best" genotype for this DNA test are on average firmer than those with the "middle" genotype, differences were more pronounced at harvest than after storage. (Not enough individuals with the "worst" genotype were available in the RosBREED dataset for analysis.) Also, the effects of this DNA test are relatively small in general compared to newer DNA tests (such as Ma-indel and Md-PG1-SSR-10kd). Nevertheless, when used in selection, this DNA test is expected to improve the chances of obtaining superior genetic potential for extended storage.

Md-ACO1-indel: Analyses conducted in 2013 confirmed a small trend in the same direction as previous reports and more pronounced at harvest than after storage, similar to the DNA test above. However, differences between genotypes of this DNA test were even smaller than for Md-ACS1-indel, although there were not enough representatives of the "best" genotype to determine its contrast with the "middle" and "worst."

Ma-indel: In 2013, a previous DNA test for multi-trait *Ma* locus was successfully converted to more reliable test, "Ma-indel." Analyses of this new DNA test with the RosBREED dataset identified some great results for the WABP. Not only were the effects on acidity and crispness confirmed, but also detected were large effects on firmness (both sensory and all instrumental measures) and indeed all textural measures (including the Digi-Test's Cn, instrumental crispness, and Co, instrumental "mealiness"). The most interesting and useful contrasts were between the two alleles at this locus carried by 'Honeycrisp.' One of the alleles is associated with higher firmness at harvest and over storage than the other 'Honeycrisp' allele (Figure 3). The same valuable allele is associated with high crispness that doesn't lose as much crispness over storage as the other 'Honeycrisp' allele and all other alleles from non-'Honeycrisp' lineages (Figure 3). This "jewel in the genome" may explain why some P2 selections perform relatively differently to their peers after storage than at harvest – it may be those with the valuable Ma-indel allele from

the ‘Honeycrisp’ lineage whose fruit don’t lose so much of their crispness and firmness as storage progress – thus better maintaining a “fresh sensation,” the moniker given to the suite of traits associated with the *Ma* locus.

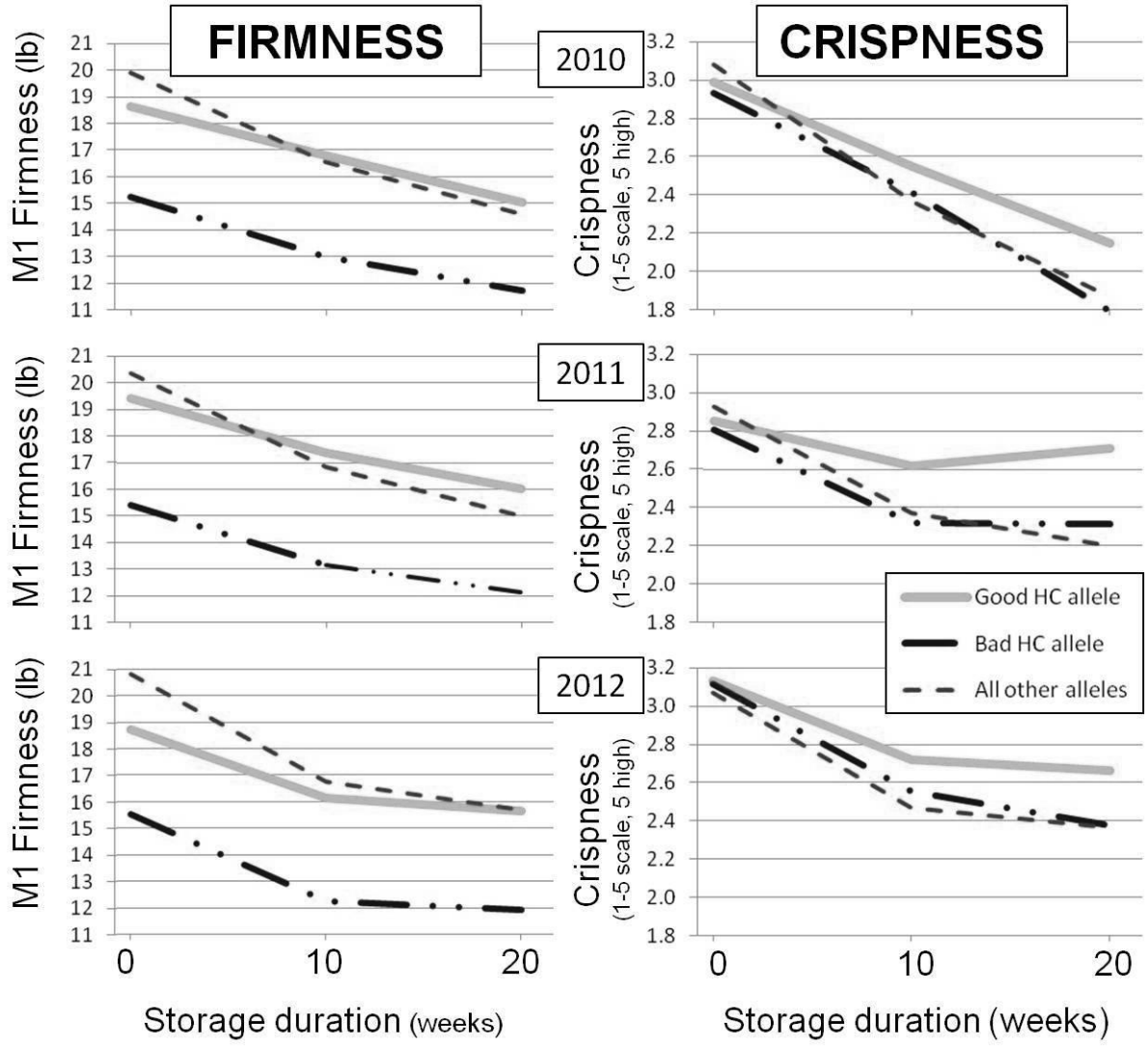

Figure 3: Contrasts among alleles distinguished by the Ma-indel DNA test among seedlings of the WABP in the RosBREED project. Each of the ‘Honeycrisp’ (HC) alleles is represented by ~40 seedlings while all other alleles are represented by a total of ~125 seedlings. Fruit quality evaluations after storage were over 10 and 20 weeks, each with an additional week of ripening at room temperature (as described by Evans et al. 2012 and Schmitz et al. 2013). Standard deviations are ~3.4-3.9 for M1 firmness and ~0.4-0.8 for sensory crispness.

New DNA tests developed: Two new DNA tests were developed, based on promising RosBREED results previously described in the Jan 2013 report for the “Increasing decision confidence in cultivar development and adoption” WTFRC project. These two DNA tests, “Bp16-indel” and “Bp13-SSR,” help predict bitter pit incidence in WABP germplasm. These tests have yet to be used in the WABP

but are expected to be valuable tools in the arsenal against bitter pit. Bp16-indel also provides a useful backup to Ma-indel as it is in the same genomic region.

Future DNA tests: Several additional genomic regions influencing fruit quality after storage were also investigated to determine whether they were promising enough to be developed into new DNA tests for future deployment in the WABP. Three genomic regions were deemed worthy of advancement: the *Md-PG1* gene for firmness, the "*LG8A*" locus for acidity, the "*LG16C*" locus for crispness, and the "*LG1Fru*" locus for sweetness. Other genomic regions are beginning to emerge from analyses of the RosBREED dataset for the storage disorders of internal browning, scald, and shrivel.

Md-PG1: The *Md-PG1* gene, putatively associated with fruit firmness, was previously given attention, together with the original *Md-ACS1* and *Md-ACO1* storability genes, during a 2008-2009 federally funded project with partial WTFRC funding and international collaborations. While *Md-PG1* remained promising for several years, recent analyses with a new DNA test (Md-PG1-SSR-10kd; Longhi et al. 2013a,b) screened on the RosBREED dataset confirmed that there is value for the WABP. Genotype outcomes for this DNA test account for more than 20% of observed variation for firmness, especially after storage, in many WABP families.

LG8A: Characterized by WSU RosBREED grad student Sujeet Verma (Verma 2013). This genomic region accounts for about a third of the observed variation for acidity in WABP germplasm. *LG8A* is highly predictive for fruit acidity differences among parents, seedlings, or selections, at harvest as well as after storage and especially in combination with DNA information at the *Ma* locus. Development and deployment in the WABP of a new DNA test for *LG8A* is recommended.

LG16C: Discovered and characterized by WSU RosBREED grad student Sujeet Verma (Verma 2013). This genomic region appears to explain a contrast in 'Honeycrisp' alleles in crispness after storage. However, this may the same effect already explained by the nearby Ma-indel DNA test. Further research is required to determine whether or not the effects are separate (and additive).

LG1Fru: Discovered and characterized by WSU RosBREED grad student Yingzhu Guan (Guan 2013). This genomic region accounts for about half or more of the observed variation among WABP seedlings for fruit fructose concentration and has almost as much explanatory power for glucose and sucrose concentrations. Development and deployment in the WABP of a new DNA test for *LG1Fru* is recommended.

Expected impact: The DNA tests for storability-related traits already available and soon to be developed for the WABP are valuable for use at various breeding stages. Their use in Phase 1 seedling selection, to eliminate thousands of seedlings predicted to have sub-par genetic potential, remains a powerful strategy for the WABP. The relatively large proportion of Phase 1 operational costs associated with rearing and evaluating seedlings after the greenhouse stage (Figure 1) highlights the value of this strategy. However, the biggest impact on the WABP from DNA tests is expected to continue to come from DNA-informed decisions – by helping avoid less efficient crosses and enabling more creative crosses to achieve target outcomes. (Yet this impact is difficult to quantify unless comparisons are made with hypothetical crosses that would have been made without DNA information.) With new families enriched for superior genetic potential, there is a reduced need for DNA testing of seedlings with the same tests. More opportunity is thereby afforded for selection among Phase 1 seedlings for other valuable attributes (including use of new DNA tests) or simply for identifying more one-in-a-million winners, which becomes more like one-in-a-hundred-thousand. Similarly, use of DNA tests in Phase 1 enriches the genetic potential of selections entering Phase 2.

Better individuals, and more of them, are therefore expected to result from Phase 2, even if operational components of Phase 2 trials themselves remain the same. Furthermore, as additional DNA tests become available, they can be used on Phase 2 selections and combined with performance data to inform advancement decisions. Or, given the proportionally large costs associated with Phase 2 fruit evaluation (Figure 2), new DNA tests can be used as soon as possible to identify Phase 2 selections to avoid evaluating phenotypically, or to chainsaw cull, in subsequent seasons.

Activity 2. Implement software (*Elite Advance*) for routine prediction of genetic potential

New software, called *Elite Advance*, for routine prediction of the genetic potential of candidates from Phase 2 trials was developed by Dr. Craig Hardner based on approaches developed in the 2011 and 2012 WTFRC projects, "Increasing decision confidence in cultivar development and adoption." Programming components and running of the new software are described in the box below.

Elite Advance
New software for routine prediction of genetic potential in WABP Phase 2 trials

Elite Advance utilizes the mixed model program ASReml implemented in R. R is a free software environment for statistical computing and graphics, although a little knowledge of programming in R is required for prediction of genetic potential to facilitate adoption by WABP personnel. The software RStudio is used as the interface for R. While R and RStudio are free, the software requires a valid license for ASReml. *Elite Advance* is run through a "Set_Parameters.R" file that defines:

- the paths for required data files and customized R code
- the traits that are in the data file for which predictions are required
- entries planted in the trials that will be used as standards to compare with Phase 2 selections
- the linear model to be used for the analysis
- subsets of the data to be used for the analysis
- directives to control the analysis.

Elite Advance is run by submitting the Set_Paramaters.R file. This file calls a function that creates separate analysis files for each trait listed in Set_Parameters.R. From this run, parameters are estimated for the linear model, and these parameters are used to predict the genetic potential for each candidate. Post-analysis processing includes testing the significance of the difference each Phase 2 selection and a specified standard. The last function of the single trait analysis is to collate results and output them into Excel files for easier investigation by the operator. Finally, after each trait is run the results are collated across traits.

To facilitate adoption of *Elite Advance* by the WABP, two deliverables were achieved. First, an instruction manual was prepared on the installation and running of the new software. Second, a workshop was presented on 17 July 2013 to Dr. Kate Evans, Lisa Brutcher, Bonnie Konishi, Dr. Cameron Peace, Yingzhu Guan, Sushan Ru, Julia Harshman, Paul Sandefur, and Jerry Tangren. As part of this workshop the alpha version of *Elite Advance* was transferred to the WABP team with a functional example. Further meetings on the following two days provided guidance and identified implementation issues and solutions. Since then, a beta version of *Elite Advance* was delivered and implemented by the WABP for prediction of genetic potential of Phase 2 selections using 2013 at-harvest fruit evaluation data. An updated version will be transferred to the team in Jan 2014, which is likely to be the final version of the software.

Lisa Brutcher (WABP operations team member) completed basic training in R script and has successfully implemented *Elite Advance* on the WABP Phase 2 dataset covering 2005 to 2012. Data output was particularly useful for identification of "outlier" data points which could then be checked

and corrected if erroneous. Outputs from *Elite Advance* of ranking of genetic potential among Phase 2 selections for the various traits were used to support decision-making for fall 2013 tree propagation for advancement to P3.

The WABP team is currently looking at different options in data output display to determine which is the easiest to interpret. The extended storage evaluations of samples from the 2013 season were not completed at the time of writing this report. Once the dataset is complete, data will be uploaded into *Elite Advance* in a timely manner to enable implementation of output analysis in the decision-making for the 2014 propagation season. The WABP operations team expects to use the system routinely from now on.

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EXECUTIVE SUMMARY

Genetic improvement underpins the long-term economic sustainability of the Washington apple industry Focusing on extended storage, our goal was to deliver routinely implementable methods to the Washington Apple Breeding Program (WABP) for revealing genetic potential for commercial performance. The two objectives of this project were to:

1. Optimize resource allocation in the WABP
2. Implement software for routine prediction of genetic potential

By fulfilling those objectives, we expect to improve the WABP's prospects for developing superior new cultivars that provide exceptional fruit quality like 'Honeycrisp' but without the storage flaws of that cultivar. 'WA 38' is an example of such a WABP output, mostly developed using breeding operations established at program's outset in 1994. We believe that we can increase the WABP's development of such superior cultivars – in number and/or performance levels for multiple valuable traits. We believe we can do so by streamlining WABP operations through the routine application of robust statistical genetics calculations and the routine application of predictive DNA tests. Both of these complementary strategies are designed to efficiently reveal genetic potential for superior performance across the spectrum of WABP germplasm and operations. Both strategies were advanced and successfully implemented in this project.

- Opportunities were identified to optimize resource allocation in the WABP.
- The WABP's Phase 1 and Phase 2 have been thoroughly dissected for their operational activities, costs of each activity, and traits evaluated at that phase. Complete datasets of historical WABP data for these two Phases have been compiled. Only with such dissection and dataset preparation can efficiencies be identified and alternatives objectively compared.
- The greatest proportion of costs for Phases 1 and 2 are in harvesting and fruit processing, especially labor, indicating that any reduction in fruit processing time while achieving the same or better genetic outcomes would substantially save costs.
- An experiment is underway to identify efficiencies that might be gained in Phase 1 fruit quality evaluations, to be complete by May 2014.
- In Phase 2 trials, efficiencies were identified in identifying selections superior in their genetic potential under extended storage: fruit quality evaluations after both normal storage (2 months) and extended storage (4 months) are unnecessary; conducting only one is sufficient. After a regular 2-month storage evaluation, a supplemental 4-month duration could be used just to reveal storage disorder fatal flaws.
- DNA testing capability was advanced for storability-related traits by refining the predictiveness and technical efficiency of previously available DNA tests, developing new DNA tests, and identifying new genomic regions to target.
- Deployment of DNA tests is enhancing efficiency, accuracy, and creativity in the WABP.
- New software was developed, delivered, and implemented in the WABP for routine prediction of genetic potential among Phase 2 selections.
- The first routine use of this software, *Elite Advance*, in 2013 by WABP staff supported decision-making by the breeder for advancement of certain selections from P2 to P3.
- *Elite Advance* was useful in identifying outlier data points.

In the remaining months of this project, once fruit of Phase 1 and Phase 2 trees are evaluated after extended storage, we will conduct final comparisons of alternative evaluation methods in early selection phases to improve the efficiency of identifying genetic potential for superior performance after extended storage.