Estimated Implementation Costs of DNA-informed Breeding in a Peach Breeding Program

Seth D. Wannemuehler[1], Chengyan Yue[1], William W. Shane[2], R. Karina Gallardo[3], and Vicki McCracken[4]

SUMMARY. Marker-assisted selection (MAS) use in breeding programs allows for examination of seedlings at an early stage before accumulation of high field costs. However, introducing MAS into a breeding program implies additional costs and uncertainties about effective incorporation. Previous simulations in apple (*Malus ×domestica*) have shown cost-effective applications of MAS. To further evaluate MAS cost-effectiveness in perennial crops, we conducted a cost-effectiveness analysis examining MAS in an upper midwestern U.S. peach (*Prunus persica*) breeding program. Breeding program procedures and associated costs were collected and used as input into spreadsheet-based simulations of the breeding program. Simulations compared a conventional breeding program to MAS with varying cull rates of low, medium, and high at multiple stages in the breeding cycle. Cost-effective MAS implementation was identified at the end of seedling trials with a break-even cull rate of 4%. These results inform breeders of cost-effectiveness of MAS use in a peach breeding program.

P each (*Prunus persica*) cultivation began in China as early as 1100 BCE (Bassi and Monet, 2008). Yet modern breeders continue striving for peach cultivars with improved disease resistance,

environmental adaptability, and fruit quality (Gallardo et al., 2015; Janick and Moore, 1996; Yue et al., 2012, 2014). Peach production is plagued by susceptibility to devastating diseases such as sharka (*Plum pox virus*), leaf curl (*Taphrina deformans*), and *Xanthomonas* species (Hancock et al., 2008). Additionally, changing climates create adverse growing environments with unstable temperatures for some peach breeders, forcing them to modify chilling requirements for better fruit production. Complex traits such as disease resistance or environmental adaptability are difficult to measure in the field using conventional breeding methods. However, breeding methods combining conventional practices with DNA marker–assisted technologies provide greater opportunities for improvement of these complex traits (Martínez-García et al., 2013). Breeders, including peach breeders, are seeking ways to increase the cost-effectiveness of their programs. Each breeding program is different, and thus the cost-effectiveness of DNA-informed breeding in apple (*Malus ×domestica*) and other crops does not mean it is cost-effective in peach. It is therefore worthwhile to explore whether it is cost-effective to apply in a peach breeding program. RosBREED included both large-scale

breeding programs that already apply DNA-informed breeding and relatively small-scale breeding programs that are exploring whether it is worthwhile to apply DNA-informed breeding. In this study, we use a decision support tool to examine the cost-effectiveness of implementing DNA-informed breeding in a small-scale peach breeding program exploring use of MAS.

DNA marker–assisted breeding consists of identifying a DNA marker, which is a fragment of DNA closely associated with the presence or absence of a target trait (Frey et al., 2004; Johnson, 2003). The association between the marker and the trait allows breeders to screen a population by testing for the presence or absence of the marker and select among offspring for individuals with the desired trait. This technique has been largely used in agronomic crops such as rice (*Oryza satvia*) and maize (*Zea mays*) (Alpuerto et al., 2009; Hoeck et al., 2003). In rice breeding, MAS has been used to increase crop yield (Hoeck et al., 2003), salinity tolerance (Alpuerto et al., 2009), and pyramid disease resistance [i.e., developing seedlings with multiple genetic regions associated with resistance to a disease (Suh et al., 2013)]. In peach, DNA markers have been associated with traits such as fruit quality, disease resistance, maturity date, and postharvest quality (Abbott et al., 2002; Hancock et al., 2008).

However, many traits are not controlled by a single large-effect gene (qualitative) but rather by multiple genes (quantitative) influencing the trait to varying degrees (Tartarini and Sansavini, 2002). Qualitative traits are more easily selected in conventional breeding with controlled crosses, but conventional breeding lacks screening methods to effectively select for quantitative traits (Lande and Thompson, 1990). For example, in disease-resistant plants, more than one resistance gene can elicit similar symptoms or responses and may be indistinguishable in the field by observation. Variations between resistance genes can be observed on the genetic level, allowing markers to select for multiple sources of resistance in a single plant, increasing the effectiveness of a breeding program (Chandler et al., 2012; Dreher et al., 2003; Whitaker, 2011).

Studies comparing MAS and conventional breeding methods in agronomic crops, such as maize and rice, show that incorporation of MAS into breeding schemes may reduce time requirements, costs, or both depending on implementation strategy (Dreher et al., 2003; Morris et al., 2003). A study by Slater et al. (2013) suggests a relationship between MAS cost-effectiveness and MAS application timing. They identified a cost-effective MAS breeding scheme in which MAS was used in the field before conventional disease resistance trials (Slater et al., 2013). These studies show that cost-effective incorporation of MAS in a breeding program depends on the conventional methods' costs relative to MAS costs, timing of marker application, and the efficiency of markers to identify elite seedlings. Most studies describing methods of cost-effective MAS focus on agronomic crops such as maize (Dreher et al., 2003, Morris et al., 2003; Stromberg et al., 1994), rice (Suh et al., 2013), and wheat [*Triticum aestivum* (Kuchel et al., 2005)]. Interest and incorporation of MAS in perennial crop breeding programs is growing due to prolonged maintenance costs as plants mature and the ability of MAS to remove inferior seedlings earlier in the program potentially reducing these costs (Collard and Mackill, 2008).

In perennial crops, the value of MAS is promising because DNA from young plants can be used to screen for the presence of traits expressed in mature plants. With conventional breeding methods, evaluation of traits related to fruit quality are delayed until after completion of the juvenile phase, which increases costs due to maintenance of inferior seedlings in addition to superior ones. Using marker-assisted seedling selection (MASS), a form of MAS, allows breeders to remove inferior seedlings earlier in their program before costs for seedling maintenance become significant (Collard and Mackill, 2008). Few studies have described MAS as a breeding tool and examined the costs associated with MAS incorporation for rosaceous crops [Rosaceae (Edge-Garza et al., 2009; Ru et al., 2015, 2016; Tartarini and Sansavini, 2002; Testolin, 2002)]. Luby and Shaw (2001) concluded that if conditions related to trait inheritance, trait expression timing, and testing costs

were met, then MAS had a higher probability of improving the efficiency of selection. Meeting each of these requirements ensures that the use of MAS is more cost-effective than conventional plant breeding, but not every condition is necessary to achieve cost-effectiveness.

A study by Edge-Garza et al. (2015) used trait inheritance, trait expression timing, and testing costs conditions to generate a decision support tool for MASS using apple, grape (*Vitis vinifera*), and strawberry (*Fragaria ×ananassa*) as case study crops. This study determined that application of MASS as early as possible was not necessary as long as MASS occurred before significant labor costs are incurred from seedling handling (e.g., planting in orchards and maintenance).

Using the decision support tool they developed, Wannemuehler et al. (2019) modeled a large-scale apple breeding program (here defined as >4000 seedlings per year) and showed that it is cost-effective to apply MAS in the program compared with conventional breeding. The tool can be adjusted to accommodate the unique characteristics of different breeding programs with varying breeding capacities. It provides an overview of a program's entire breeding process by taking into consideration the dynamic nature of breeding over multiple years and each program's unique procedures and costs. These characteristics of breeding programs are challenges to the development of a decision support tool capable of modeling multiple breeding programs. However, these challenges are overcome by incorporating detailed information from breeders and breeding program records, allowing the tool to capture the variability among programs. This decision support tool provides breeders with a tailored decision-aid tool to model and estimate comparative costs quickly for their breeding methods at any program stage.

However, whether the application of MAS is cost-effective for relatively smaller-scale rosaceous breeding programs remains unknown. Conducting a cost-effectiveness analysis of DNA markers use requires a program to maintain detailed costs for breeding practices, which is lacking for many breeding programs. Collecting and analyzing the cost data requires professionals who have interdisciplinary

knowledge: researchers who understand the detailed peach breeding process and the economic tools to analyze the data. The U.S. Department of Agriculture–funded RosBREED project brought researchers from different disciplines to work as a team, which overcomes the boundaries between different disciplines and makes this work achievable. We use the decision support tool developed by Wannemuehler et al. (2019) to assess the cost-effectiveness of a small-scale (defined here as generating <2000 seedlings per year) peach breeding program's adoption of MAS and explore the versatility of the decision support tool.

Materials and methods

A spreadsheet-based (Excel; Microsoft, Redmond, WA) simulation model based on a peach breeding program in the upper midwestern United States was developed to examine MAS costs as well as other costs associated with stages in the breeding program. The model inputs included itemized costs incurred by the peach breeding program during the 2016 field season including program costs for MAS. Additional detailed information regarding yearly operations and breeding practices were provided by the peach breeding program's breeder. Together the itemized costs, operational information, and breeding practices provided a unique timeline from parental crosses to grower trials and precommercialization for a program in the upper midwestern United States.

On the basis of data collected from the peach breeder, the breeding program included five stages: crossing, greenhouse growth, seedling trials, seedling clonal orchards, and grower trials and precommercialization orchards (Fig. 1). Costs for each stage were collected including labor costs and consumable costs for procedures occurring within each stage. Crossing stage procedures include germplasm maintenance, planning and selection of parents for crosses, pollination, and seed extraction. Greenhouse growth procedures consist mainly of seed germination and production of seedlings in containers in the greenhouse. Seedling trial procedures consist of planting of seedlings into the orchard. During the subsequent 6 years, evaluation and selection occur with desirable seedling
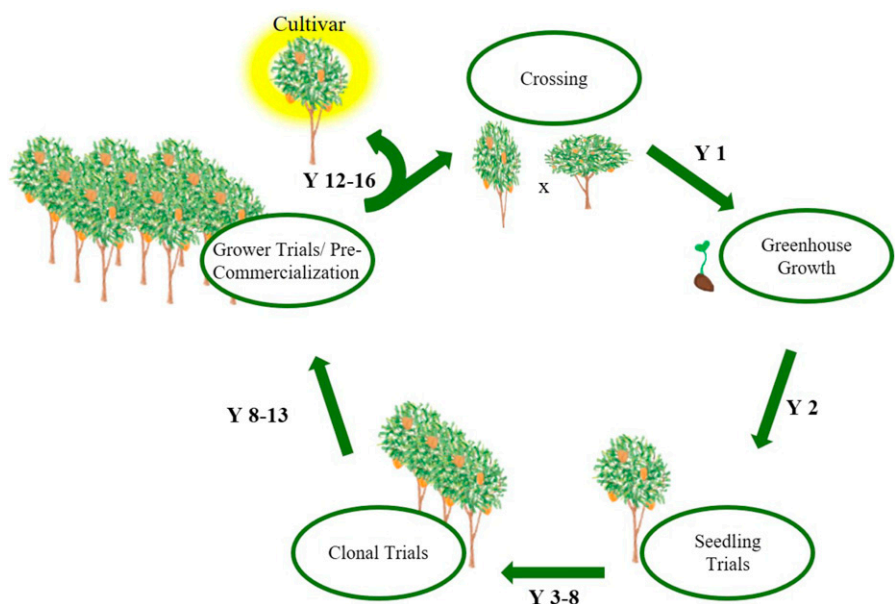
Fig. 1. Peach breeding program diagram following a single cohort of crosses to cultivar release between years (Y) 12 and 16. Crosses are made in spring of the first season with seed collection at the end of the same season. Seeds are germinated and then planted at the beginning of the second season. Seedlings remain in seedling trials for years 3 to 8. During this time, selections are made that advance to clonal trials. Selections remain here for years 8 to 13, during which time advanced selections occur. Advanced selections then move to grower trials and precommercialization, where they remain for years 12 to 16 in the program.

selections progressing to clonal trials. Clonal trials contain ≈12 asexual propagules of each selection onto rootstocks at a different location from seedling trials. After subsequent years of evaluation, a selection is made in clonal trials and progresses to grower trials and pre-commercialization. Grower trials are composed of ≈35 asexual propagules located in multiple test orchards. Costs for trials were estimated for establishment, maintenance, and fruit evaluations, while costs for crossing and greenhouse growth were based on specific activities described by the breeder.

After outlining breeding program operations and practice, we incorporated the following assumptions to make the models: 1) Environmental effects during the year of data collection are assumed average and consistent across years of the program. 2) Costs during the year of data collection are assumed to be typical of yearly expenditures. 3) Fixed costs (i.e., land rental, irrigation costs, machinery costs) are constant across years after accounting for inflation. 4) Reliable markers (i.e., markers with close linkage to a trait of interest and low failure rate due to technical issues) exist for

the purpose of selecting seedlings in a breeding program. 5) The cost of marker development is not included, but the potential for substantial marker development cost is recognized. 6) Incorporation of markers does not inherently reduce the number of selections made in a program. 7) The removal rate (i.e., the number of seedlings removed from a cohort of seedlings in the field trial) for a peach breeding program follows a normal distribution as observed in other rosaceous tree breeding programs (Wannemuehler et al., 2019).

In an average year, the modeled peach breeding program generates ≈1800 seedlings after greenhouse growth. To estimate the full cost of the breeding program, costs associated with crossing and greenhouse growth were estimated for 10 years. The repeated crosses result in overlapping activities as previous crosses begin to mature while new crosses are made. As time progresses, the overlap creates a dynamic structure of costs for the program as procedures from crossing through grower trials are conducted simultaneously each season.

Removal rate information was unavailable for this peach breeding

program. Removal rates have previously been used to estimate the distribution of juvenile phase in apple, a related species, and have indicated that variation for the length of juvenile phase in a breeding population follows a normal distribution. On the basis of the historical number of seedlings entering clonal trials yearly, ≈1.11% of seedlings in a crossing cohort progress to clonal trials. Assuming that the length of the juvenile phase follows a normal distribution, as is observed in apple, and using the historic number of seedlings selected for advancement from seedling trials, a distribution of selection from seedling trials was created to more accurately model the breeding program.

After seedling selections enter clonal trials, the year at which a selection enters grower trials is uncertain within the program. Therefore, the year the cultivar is selected is randomized within the model with the probability of selection increasing as the number of seedlings increases. This probability is determined by the total number of seedlings within clonal trials with a 2-year lag, as seedlings enter the stage and are then grown to mature plants. As the number of seedlings increases the size of the selection pool, it is assumed that the likelihood of advancing a selection is increased. The peach breeding program is young relative to other rosaceous breeding programs in the nation, which prevented an estimation of how many seedlings are required to release a marketable cultivar. However, Wannemuehler et al. (2019) indicates that ≈1 selection may arise per 10,000 plants examined in seedling trials. For this reason, the models were conducted with 10 years of crossing to simulate a full breeding pipeline and achieve the estimated minimum number of screened seedling plants for cultivar production.

We developed a model to simulate the breeding program's application of MAS at the end of seedling trials. The number of seedlings being produced from greenhouse growth and the number entering and being evaluated in seedling trials were held constant. Three cull, or removal, intensities based on marker testing of 50 seedling selections at the end of seedling trials were used to estimate the effects of MAS at this stage. These cull rates were high (60% with 40

seedling selections removed), medium (40% with 20 seedling selections removed), and low (20% with 10 seedling selections removed). Overlapping evaluation periods in seedling trials, starting in year 7 and ending in year 17, were followed by entry into clonal trials after nursery propagation, which results in costs for stage 2 from year 9 to year 19.

An additional model was then constructed simulating application of MAS at the end of greenhouse growth. The upstream procedures of greenhouse growth and the number of seedlings entering greenhouse growth were held constant. Three cull, or removal, rates were chosen to estimate MAS impacts at high (50%), moderate (25%), and low (10%) removal rates. These values represent the percentage of seedlings out of 2500 that were removed after DNA marker testing with the assumption that every seedling underwent testing. The downstream procedures assumed proportional cost reduction associated with MAS.

SENSITIVITY ANALYSES. The cost of labor composes a significant portion of a breeding program costs. Additionally, MAS technology is improving in efficiency, which results in constantly decreasing costs associated with the technology. To examine capabilities of the model, we conducted sensitivity analyses. Costs for procedures before MAS application were assumed to be constant among analyses. Grower trials and precommercialization were held constant under the assumption that MAS does not negatively affect the likelihood of cultivar production. Effects of altering procedural costs were observed in greenhouse growth, seedling trials, and clonal trials.

The first sensitivity analysis explored decreasing costs for DNA marker tests. Marker technology is rapidly evolving and new technologies with lower costs are being developed quicker than ever. DNA marker test costs were estimated at 10%, 25%, and 50% cost reduction for laboratory costs (lower chemical costs, automation, etc.). These cost reductions were estimated for applications of MAS at both the greenhouse growth stage and seedling trials. The second sensitivity analysis examined cost reduction for evaluation of the disease bacterial spot caused by *Xanthomonas*

arboricola pv. *pruni* (Garita-Cambronero et al., 2018). Marker tests that accurately select for bacterial spot resistance will result in cost savings for field evaluations because time spent on phenotyping resistance will be reduced. Reduction in labor time and the corresponding reduction in cost for labor was examined by reducing estimated evaluation costs by 25%, 50%, and 75%.

Results
Conventional breeding

Conventional breeding costs are shown in Table 1. The conventional breeding model relies on an examination of physical characteristics for culling and selection, and MAS is not used in any stage of the program. Extensive evaluations result in increased labor costs for both seedling trials and clonal trials. Costs per seedling for crossing and greenhouse growth amount to about $2.80 with labor costs comprising 60% of the total cost. In seedling trials, costs are about $2.50 for planting in the first year and then approximately an

additional $0.70 per year for maintenance and $1.15 for each year of evaluation. Of the seedling trial costs, labor costs account for 71% of the total cost. Once selections enter clonal trials, the cost per selection for propagation and planting is $215.60, or $17.97 per tree. Each additional year a selection is grown requires $19.20 to maintain and an additional $19.20 for evaluation, if conducted. Clonal trials labor accounts for less than 26% of total costs. Overall the total cost for 10 years of crossing and subsequent evaluation of seedling cohorts through grower trials amounts to $584,690 (Table 1).

MAS application at the end of stage 1 seedling test

The MAS application model was built with applications of MAS at the end of seedling trials before clonal trial entry, and costs are shown in Table 2. All procedural costs and numbers of seedlings were held constant across simulations for crossing, greenhouse growth, and grower trials. Costs from conventional breeding

Table 1. Costs for conventional peach breeding program methods per procedure. Each cost represents costs for conducting procedures in a given year and repeating procedures over a number of seedling cohorts. No marker-assisted selection is used in this scenario.

Stage	Yr	Seedlings (no.)	Cost for conventional breeding methods ($)
Crossing	1	2,500	61,030
Greenhouse growth	2	2,500	12,630
Seedling trials	3	1,800	24,000
	4	1,800	12,000
	5	1,800	36,000
	6	1,800	36,000
	7	1,800	36,000
	8	1,800	42,400
Clonal trials	8	50	117,341
	9	50	19,191
	10	50	19,143
	11	50	19,143
	12	50	19,089
	13	50	45,795
Grower trials and precommercialization	12	10	43,930
	13	10	10,250
	14	10	10,250
	15	10	10,250
	16	10	10,250
Total			584,690

were used as the basis for procedural costs, and additional input of marker tests were used for MAS costs. Costs for tissue collection, DNA extraction, and MAS analysis were included in the final year of seedling trials for MAS utilization, unlike the Wannemuehler et al. (2019) analysis for apple. In conventional breeding, ≈50 seedlings were selected throughout seedling trials to continue on to clonal trials. The MAS model examined culling the 50 selections by 60% (high), 40% (medium), and 20% (low). These cull rates result in proportional clonal trial cost decreases, and the total costs were $446,911, $495,013, and $542,898, respectively, for the three cull rates (Table 2). Procedures for MAS incurred additional costs amounting to $6250 over a span of 10 years for evaluating 50 seedling selections per year. The simulation results showed MAS application was more cost-effective compared with conventional breeding methods. The break-even point, where the cost savings by removing seedling selections

from clonal trials was equivalent to the additional costs of MAS, was determined at an ≈4% cull rate or removal of two individuals (Table 2).

MAS application at the end of greenhouse growth stage

We also examined the impacts of MAS application at the end of the greenhouse growth stage before seedling planting in seedling trials by holding crossing and grower trials constant. The MAS models, shown in Table 3, examined the impacts of reduced seedling numbers in seedling trials at three cull rates assuming all seedlings were tested: 10% (low), 25% (medium), and 50% (high). Additional procedural costs for MAS were included in the model to capture the trade-offs associated with technology usage. The number of seedling selections entering clonal trials was held consistent at 50 seedlings across the models.

The 10% cull rate resulted in increased costs by 135% ($207,000) compared with conventional breeding methods. Medium and high cull

rates similarly resulted in increased costs by 130% ($180,000) and 123% ($135,000), respectively (Table 3). The MAS cost was $10.75 per individual, which was a relatively high cost for MAS, ≈$22,500 annually, and resulted in significant cost increases compared with conventional breeding that cost $8.00 per seedling over 6 years. Greenhouse growth MAS increased the stage cost by about nineteen times conventional greenhouse cost ($237,630) and only decreases seedling trials costs by half ($12,000). The break-even cull rate, or the cull rate at which using MAS became cost-effective, was estimated at 125.1%, indicating that more seedlings than those included in the program would have to be removed to compensate for the additional costs of MAS (Table 3). The 125.1% cull rate had a total cost of $584,692 compared with conventional breeding total of $584,690.

SENSITIVITY ANALYSIS 1. *MAS cost reduction*. This sensitivity analysis explored reductions in the laboratory

Table 2. Costs for seedling trials marker-assisted selection (MAS) application occurring at the end of seedling trials are shown per procedure under three levels of culling in the peach breeding program from the upper midwestern United States. Each cost represents costs for that procedure over multiple years. For MAS, the three levels of culling are 60% (20 selections out of 50), 40% (30 selections), and 20% (40 selections). The break-even point (BEP) is added to show at what cull rate MAS becomes cost-effective for this stage.

Stages	Yr	MAS 60% cull rate		MAS 40% cull rate		MAS 20% cull rate		BEP 4% cull	
		Seedlings (no.)	Costs ($)	Seedlings (no.)	Costs ($)	Seedlings (no.)	Costs ($)	Seedlings (no.)	Costs ($)
Crossing	1	2,500	61,030	2,500	61,030	2,500	61,030	2,500	61,030
Greenhouse growth	2	2,500	12,630	2,500	12,630	2,500	12,630	2,500	12,630
Seedling trials	3	1,800	24,000	1,800	24,000	1,800	24,000	1,800	24,000
	4	1,800	12,000	1,800	12,000	1,800	12,000	1,800	12,000
	5	1,800	36,000	1,800	36,000	1,800	36,000	1,800	36,000
	6	1,800	36,000	1,800	36,000	1,800	36,000	1,800	36,000
	7	1,800	36,000	1,800	36,000	1,800	36,000	1,800	36,000
	8	1,800	48,650	1,800	48,650	1,800	48,650	1,800	48,650
Clonal trials	8	20	46,725	30	70,440	40	93,920	48	112,704
	9	20	7,661	30	11,501	40	15,322	48	18,432
	10	20	7,680	30	11,482	40	15,283	48	18,317
	11	20	7,680	30	11,482	40	15,283	48	18,317
	12	20	7,642	30	11,443	40	15,245	48	18,317
	13	20	18,283	30	27,425	40	36,605	48	43,949
Grower trials and precommercialization	12	10	43,930	10	43,930	10	43,930	10	43,930
	13	10	10,250	10	10,250	10	10,250	10	10,250
	14	10	10,250	10	10,250	10	10,250	10	10,250
	15	10	10,250	10	10,250	10	10,250	10	10,250
	16	10	10,250	10	10,250	10	10,250	10	10,250
Total			446,911		495,013		542,898		581,275

Table 3. Costs for marker-assisted selection (MAS) application occurring at the end of greenhouse growth with three levels of culling in the peach breeding program from the upper midwestern United States. One thousand seedlings from 1800 are theoretically screened, and cull rates are estimated at 10%, 25%, and 50%. Each cost represents costs for that procedure of a period of multiple years. The break-even point (BEP) cull rate is included to represent at what level MAS becomes cost-effective.

Stages	Yr	MAS 10% cull rate Seedlings (no.)	Costs ($)	MAS 25% cull rate Seedlings (no.)	Costs ($)	MAS 50% cull rate Seedlings (no.)	Costs ($)	BEP 125.1% cull Seedlings (no.)	Costs ($)
Crossing	1	2,500	61,030	2,500	61,030	2,500	61,030	2,500	61,030
Greenhouse growth	2	2,500	12,630	2,500	12,630	2,500	12,630	2,500	12,630
Seedling trials	3	1,620	21,600	1,350	18,000	900	12,000	(452)	(6,000)
	4	1,620	10,800	1,350	9,000	900	6,000	(452)	(3,000)
	5	1,620	32,400	1,350	27,000	900	18,000	(452)	(9,000)
	6	1,620	32,400	1,350	27,000	900	18,000	(452)	(9,000)
	7	1,620	32,400	1,350	27,000	900	18,000	(452)	(9,000)
	8	1,620	38,800	1,350	33,400	900	24,400	(452)	(2,600)
Clonal trials	8	50	117,341	50	117,341	50	117,341	50	117,341
	9	50	19,191	50	19,191	50	19,191	50	19,191
	10	50	19,143	50	19,143	50	19,143	50	19,143
	11	50	19,143	50	19,143	50	19,143	50	19,143
	12	50	19,089	50	19,089	50	19,089	50	19,089
	13	50	45,795	50	45,795	50	45,795	50	45,795
Grower trials and precommercialization	12	10	43,930	10	43,930	10	43,930	10	43,930
	13	10	10,250	10	10,250	10	10,250	10	10,250
	14	10	10,250	10	10,250	10	10,250	10	10,250
	15	10	10,250	10	10,250	10	10,250	10	10,250
	16	10	10,250	10	10,250	10	10,250	10	10,250
Total			791,690		764,690		719,692		584,692

costs of DNA testing for MAS by 10% (low), 25% (medium), and 50% (high) to examine whether cost-effectiveness improved. As shown in Table 4, the reduction in MAS costs was analyzed for both the application of MAS at the greenhouse growth stage and that at the end of seedling trials. The baseline models used for the two applications were the models with the greatest culling rates from Tables 2 and 3. The baseline model for the greenhouse growth MAS application had a 50% cull rate with a total cost of $719,692, whereas the baseline model for MAS application at the end of seedling trials had a 60% cull rate with a total cost of $446,911. MAS at the end of seedling trials was cost-effective compared with the conventional breeding model for each modeled cost decrease with totals of $446,3160, $445,603, and $444,416 for low, medium, and high MAS cost reduction, respectively. The cost savings for these models were 0.13% (low), 0.29% (medium), and 0.56% (high) total cost

reductions relative to the baseline model for MAS application at the end of seedling trials. Greenhouse growth MAS application models had a greater cost-savings but were not cost-effective compared with the conventional breeding model. These models had totals of $702,590, $676,940, and $634,190, resulting in 2.38%, 5.94%, and 11.88% total cost reductions, respectively, relative to the baseline model for MAS application at the end of greenhouse growth (Table 4).

SENSITIVITY ANALYSIS 2. *Labor cost reduction.* The model used itemized costs for 10 seedling cohorts with procedures from crossing to grower trials modeled for multiple years. In the model, it was assumed that MAS was applied to all seedlings at the greenhouse stage with an estimated cull rate of 50%. MAS application decreased the number of seedlings entering seedling trials, and seedlings entering the stage had an increased likelihood of disease resistance based on DNA tests. Under these assumptions, a sensitivity

analysis was conducted by decreasing the labor cost for disease resistance evaluations by 25%, 50%, and 75% (Table 5). Bacterial black spot resistance screening was estimated to account for 20% of total evaluation labor, which results in estimated decreases of 5%, 10%, and 15% for evaluation labor costs. After labor cost reductions, the totals decreased to $717,292, $714,892, and $712,492 for the 5%, 10%, and 15% cost reductions, respectively. Compared with the base model, these costs are 0.33%, 0.67%, and 1.00% reductions of the total program cost.

Discussion and conclusions

Plant breeders are constantly searching and evaluating for more accurate and cost-effective procedures to deliver improved crops. One method breeders have begun adopting is the use of DNA markers at various stages in their programs (Xie and Shizhong, 1998). Basic assumptions for DNA marker use include an increased likelihood of

Table 4. Sensitivity analysis 1 exploring the reduction in marker test costs. Early and late marker-assisted selection (MAS) application effects on total program costs of MAS cost reduction for early application (during greenhouse growth) and later application (at the end of seedling trials) are shown for the peach breeding program from the upper midwestern United States. Greenhouse growth MAS is held constant at a 50% cull rate, and Stage 1 MAS is held constant with a 60% cull rate (20 selections out of 50). In both scenarios, marker test costs are reduced by 10%, 25%, and 50%. Costs for each procedure reflect multiple years of operation.

Stage	Yr	Greenhouse growth MAS cost reduction, at 50% cull rate ($)	MAS cost reduction ($) 10%	25%	50%	Stage 1 MAS cost reduction, at 60% cull rate ($)	MAS cost reduction ($) 10%	25%	50%
Crossing	1	61,030	61,030	61,030	61,030	61,030	61,030	61,030	61,030
Greenhouse growth	2	237,630	220,530	194,880	152,130	12,630	12,630	12,630	12,630
Seedling trials	3	12,000	12,000	12,000	12,000	24,000	24,000	24,000	24,000
	4	6,000	6,000	6,000	6,000	12,000	12,000	12,000	12,000
	5	18,000	18,000	18,000	18,000	36,000	36,000	36,000	36,000
	6	18,000	18,000	18,000	18,000	36,000	36,000	36,000	36,000
	7	18,000	18,000	18,000	18,000	36,000	36,000	36,000	36,000
	8	24,400	24,400	24,400	24,400	48,650	48,175	47,463	46,275
Clonal trials	8	117,341	117,341	117,341	117,341	46,666	46,666	46,666	46,666
	9	19,191	19,191	19,191	19,191	7,647	7,647	7,647	7,647
	10	19,143	19,143	19,143	19,143	7,661	7,661	7,661	7,661
	11	19,143	19,143	19,143	19,143	7,661	7,661	7,661	7,661
	12	19,089	19,089	19,089	19,089	7,623	7,623	7,623	7,623
	13	45,795	45,795	45,795	45,795	18,293	18,293	18,293	18,293
Grower trials and precommercialization	12	43,930	43,930	43,930	43,930	43,930	43,930	43,930	43,930
	13	10,250	10,250	10,250	10,250	10,250	10,250	10,250	10,250
	14	10,250	10,250	10,250	10,250	10,250	10,250	10,250	10,250
	15	10,250	10,250	10,250	10,250	10,250	10,250	10,250	10,250
	16	10,250	10,250	10,250	10,250	10,250	10,250	10,250	10,250
Total		719,692	702,590	676,940	634,190	446,790	446,316	445,604	444,416

genetic gains while not incurring substantial additional costs. Cost savings may be achieved in perennial breeding programs by offsetting the MAS use costs with reductions in the management costs of inferior seedlings at various stages. Previous studies indicate that efficient MAS incorporation may be achieved with MAS applications at early stages of breeding programs before maintenance costs (Edge-Garza et al., 2015: Luby and Shaw, 2001). However, implementation of MAS early in a breeding program assumes that the cost of MAS is less than the cost of maintenance and evaluation. A study conducted by Slater et al. (2013) examining MAS incorporation in potato (*Solanum tuberosum*) breeding concluded that MAS was more efficient if applied at a later stage in the breeding program and not at the greenhouse stage. The Slater et al. (2013) study found that cost-effectiveness for application of

MAS depended on MAS costs vs. maintenance costs, as well as marker efficiency. Luby and Shaw (2001) indicated that traits requiring high added costs for conventional screening impact the cost-effectiveness of MAS incorporation.

In the conventional peach breeding program, greenhouse growth and seedling trials require minimal cost input compared with clonal trials (Table 1). Lower maintenance and evaluation costs for seedling trials prevent cost-effective MAS application at the end of the greenhouse growth stage. This study shows that costs associated with MAS are greater than individual seedling costs accumulated during the entirety of seedling trials (Table 3). A break-even point of 125.1% culling rate indicates that MAS would require greater culling than the number of individuals generated on a yearly basis at the end of the greenhouse growth stage to make up for

potential costs of MAS implementation, which is not feasible. Previous studies indicated that costs of marker technologies prevented adoption (Dreher et al., 2003; Luby and Shaw, 2001; Ru et al., 2015), but with a modeled discount of 50% for MAS associated costs, MAS remains cost-ineffective with costs increasing $49,754 compared with conventional breeding.

Of the models examined, MAS achieves the most cost-effectiveness compared with conventional breeding with application at the conclusion of seedling trials with a break-even cull rate of 4% (Table 2). The minimum cull rate modeled for seedling trials was a 20% reduction of advanced selection seedlings and indicates that even at the lowest modeled cull rate, MAS applied at the end of seedling trials is cost-effective. The sensitivity analysis exploring MAS cost reductions (Table 4) confirms that MAS

Table 5. Sensitivity analysis 2 examining the effects of cost reduction in bacterial spot evaluation labor costs are shown for the peach breeding program from the upper midwestern United States. Marker-assisted selection (MAS) was assumed to occur at the end of greenhouse growth to reduce normal labor costs for seedling trials bacterial spot evaluations comprising 20% of total evaluations. Procedures are repeated for multiple years and the costs reflect the dynamic nature of the breeding program.

Stage	Yr	Greenhouse growth MAS, at 50% cost reduction ($)	Bacterial spot evaluation cost reduction at different cull rates ($)		
			5%	10%	15%
Crossing	1	61,030	61,030	61,030	61,030
Greenhouse growth	2	237,630	237,630	237,630	237,630
Seedling trials	3	12,000	12,000	12,000	12,000
	4	6,000	6,000	6,000	6,000
	5	18,000	17,400	16,800	16,200
	6	18,000	17,400	16,800	16,200
	7	18,000	17,400	16,800	16,200
	8	24,400	23,800	23,200	22,600
Clonal trials	8	117,341	117,341	117,341	117,341
	9	19,191	19,191	19,191	19,191
	10	19,143	19,143	19,143	19,143
	11	19,143	19,143	19,143	19,143
	12	19,089	19,089	19,089	19,089
	13	45,795	45,795	45,795	45,795
Grower trials and precommercialization	12	43,930	43,930	43,930	43,930
	13	10,250	10,250	10,250	10,250
	14	10,250	10,250	10,250	10,250
	15	10,250	10,250	10,250	10,250
	16	10,250	10,250	10,250	10,250
Total		719,692	717,292	714,892	712,492

cost-effectiveness at the end of seedling trials will increase as marker technology costs decrease (Edge-Garza et al., 2015). This study suggests that program costs and MAS costs relative to each other influence MAS cost-effectiveness and indicates that MAS is capable of reducing costs. The tool we developed informs breeders of program specific cost-effective MAS strategies and indicates that MAS application in the later stages of peach breeding can be cost-effective.

Additionally, this study explores the cost sensitivity to program changes beyond MAS. The tool encompasses the entire breeding program cost structure and can be used to examine alterations to evaluation time and costs, independently of MAS use. Assuming MAS application at the greenhouse stage before disease screening, there is potential disease evaluation cost reductions (Table 5). Although the maximum savings ($7238.40) is relatively low compared with total cost, this shows the ability of the tool to capture any changes in total operational costs. The detailed inputs allow modeling flexibility, which enables breeders to estimate effects of new practices beyond MAS, such as changes in management practices or phenotyping methods. This study shows that the tool used for estimating financial decisions is versatile enough to handle perennial fruit crops such as peach and apple.

The results reported are applicable only to the modeled peach breeding program. Because breeding practices and associated cost structures are different between programs, a limitation of our reported results is that they are not representative of all peach breeding programs. However, this study shows that using the spreadsheet tool developed by Wannemuehler et al. (2019), a breeder can adjust

inputs to reflect their unique breeding program characteristics and identify cost-effective strategies.

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