Acrylamide-Forming Potential and Agronomic Properties of Elite US Potato Germplasm from the National Fry Processing Trial


ABSTRACT
Processed potato (Solanum tuberosum L.) products, such as chips and French fries, contribute to the dietary intake of acrylamide, a suspected human carcinogen. One of the most promising approaches for reducing its consumption is to develop and commercialize new potato varieties with low acrylamide-forming potential. To facilitate this effort, a National Fry Processing Trial (NFPT) was conducted from 2011 to 2013 in five states. More than 140 advanced breeding lines were evaluated for tuber agronomic traits and biochemical properties from harvest through 8 mo of storage. Thirty-eight and 29 entries had significantly less acrylamide in French fries than standard varieties Russet Burbank and Ranger Russet, with reductions in excess of 50%, after one and 8 mo of storage, respectively. As in previous studies, the glucose content of raw tubers was predictive of acrylamide in finished French fries ($R^2 = 0.64–0.77$). Despite its role in acrylamide formation, tuber free asparagine was not significant, potentially because it showed relatively little variation in the NFPT population. Even when glucose was included in the model as a covariate, genotype was highly significant ($p = 0.001$) for predicting acrylamide, indicating there may be yet-unidentified genetic loci to target in breeding. The NFPT has demonstrated that there exist many elite US breeding lines with low acrylamide-forming potential. Our ongoing challenge is to combine this trait with complex quality attributes required by the fry processing industry.

Potato is the most consumed vegetable in the United States, with raw product value of $3.7 billion in 2012 (National Potato Council, 2014). Fifty-seven percent of US potato consumption is in the form of processed products, such as French fries and chips (National Potato Council, 2014). Despite many decades of breeding effort, the most commonly planted variety in the United States remains Russet Burbank, a variety first grown in the 19th century (Bethke et al., 2014). Russet Burbank, which accounted for 37% of the 2014 potato production acreage in the top seven potato-producing states (USDA–NASS, 2014), is grown for both fresh and processing markets and is highly valued for its culinary quality and long-term storability. However, newer varieties, such as Ranger Russet (Pavek et al., 1992) and Umatilla Russet (Mosley et al., 2000), are slowly taking away market share for processing.
Acrylamide forms in carbohydrate-rich foods during high-temperature (>120°C) cooking as part of the Maillard reaction (Mottram et al., 2002). The acrylamide found in processed potato products is produced almost exclusively from asparagine (Asn) and glucose or fructose, all of which occur naturally in raw potato tubers (Bethke and Bussan, 2013). Asparagine is the most abundant free amino acid in potato tubers, accounting for roughly one-third of total free amino acids (Gerendas et al., 2007; Oruna–Concha et al., 2001). A condensation reaction between the amino group in Asn and the carbonyl of a reducing sugar (e.g., glucose or fructose) is the first step in the reaction sequence leading to acrylamide formation (Martins et al., 2001). Research with conventional potato varieties (Shepherd et al., 2010; Halford et al., 2012; Vinci et al., 2012; Bethke and Bussan, 2013; Muttucumaru et al., 2014) and with genetically modified lines (Rommens et al., 2008; Bhaskar et al., 2010; Chawla et al., 2012) has demonstrated that acrylamide content in finished products depends strongly on the concentration of acrylamide precursors in raw tubers.

Before it was detected in foods, acrylamide was known primarily as an industrial chemical. It was classified as a neurotoxin and “probable human carcinogen” (International Agency for Research on Cancer, 1994) based on toxicology studies conducted primarily with rats. At present, the effect of dietary acrylamide on human health at typical levels of consumption is uncertain and is an area of continuing investigation (Lawley et al., 2012). Although processed potato products are not the only dietary source of acrylamide, they tend to have higher amounts per serving than many other foods (Stadler and Scholz, 2004; Mills et al., 2008; DiNovi, 2006). According to a 2002–2004 survey by the USFDA of commercial products made from frozen processed potato (e.g., French fries, tater tots), the acrylamide content ranged from 109 to 1325 µg kg⁻¹, with a median of 330 µg kg⁻¹ (USFDA, 2013; Supplemental Fig. S1). In the past 2 yr, both the USFDA and its European counterpart have published information for industry and consumers to raise awareness about acrylamide in foods and have suggested strategies to reduce public exposure to acrylamide (USFDA, 2013; European Food Safety Authority, 2014). The use of potato varieties low in acrylamide precursors was recommended, and new potato breeding lines with reduced acrylamide-forming potential were viewed as a highly desirable component of an acrylamide mitigation portfolio (USFDA, 2013).

There has been a major focus in recent years on the identification of new potato clones with low acrylamide-forming potential. To facilitate this effort, a National Fry Processing Trial (NFPT) was initiated in 2011 in Idaho, North Dakota, and Washington and expanded to Maine and Wisconsin beginning in 2012. The NFPT is a unique public–private partnership involving breeders and other scientists from several land-grant universities and the USDA, experts in agronomy and food science from industry, potato growers and representatives from state grower associations, the US Potato Board, and the National Potato Council. We report here on results from the first 3 yr (2011–2013) of the NFPT. Our main objectives were to (i) evaluate the agronomic and biochemical properties of advanced breeding lines at harvest and during 8 mo of storage, (ii) determine the consistency of these traits across different production environments and storage regimes, and (iii) develop predictive models for acrylamide in French fries.

MATERIALS AND METHODS
Field Trials
Field trials were conducted at three locations in 2011: University of Idaho Aberdeen Research and Extension Center (Aberdeen, ID), Hoverson Farms (Larimore, ND), and the Washington State University Othello Research Station (Othello, WA). For 2012 and 2013, an additional two sites were used: University of Maine Aroostook Research Farm (Presque Isle, ME) and the University of Wisconsin Hancock Agricultural Research Station (Hancock, WI). These locations represent the range of production areas for fry processing potatoes in the northern tier of the United States. Planting in Idaho, Maine, Washington, and Wisconsin occurred between late April and mid-May. Planting in North Dakota occurred in mid-June. Field conditions of the trial locations are listed in Table 1. Seed spacing was either 25 or 31 cm, and row spacing was either 86 or 91 cm, depending on location. Tubers harvest at all locations occurred between mid-September and early October. Crop management strategies used in each season at each trial location were based on best management practices recommended by local extension personnel and were consistent across years.

A total of 149 genotypes were tested during field years 2011 through 2013, including the two check varieties Russet Burbank and Ranger Russet. The remaining 147 entries included advanced selections from potato breeding programs in Colorado, Idaho, Maine, Minnesota, North Dakota, and Wisconsin as well as a few recently released varieties (see Supplemental Table S1). The design of the trial was unbalanced with respect to year as entries with poor performance were dropped after 1 yr of testing, while entries with many favorable traits were included for all 3 yr. There were 81 entries in 2011, 88 in 2012, and 79 in 2013, with 30 common entries tested in all 3 yr (Supplemental Fig. S2). Approximately 25 fewer entries were planted in Maine each year because phytosanitary restrictions prevented the importation of seed tubers produced in some locations. In any year–location combination (i.e., one environment), the field layout was unreplicated, with one plot per entry.

Harvest and Postharvest Methods
After mechanical harvest at each location, tubers were culled for defects such as cracking, knobs, greening, and disease. Unculled tubers were divided into four weight categories: <113, 113 to 170, 170 to 283, and >283 g. Total yield was calculated from the total harvest weight, including culls. Marketable yield was calculated from the weight of unculled tubers >113...
Table 1. Characteristics of the National Fry Processing Trial (NFPT) locations, averaged over the 3 yr (2011–2013).

<table>
<thead>
<tr>
<th>Location</th>
<th>Idaho</th>
<th>Maine</th>
<th>North Dakota</th>
<th>Washington</th>
<th>Wisconsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latitude, longitude</td>
<td>42.95°N, 112.84°W</td>
<td>46.40°N, 68.01°W</td>
<td>47.8°N, 97.5°W</td>
<td>46.7°N, 119°W</td>
<td>44.08°N, 89.31°W</td>
</tr>
<tr>
<td>Trial duration (d)</td>
<td>140</td>
<td>141</td>
<td>123</td>
<td>147</td>
<td>157</td>
</tr>
<tr>
<td>Precipitation (cm)</td>
<td>4.2</td>
<td>85.0</td>
<td>41.1</td>
<td>8.0</td>
<td>39.6</td>
</tr>
<tr>
<td>Irrigation (cm)</td>
<td>50.8</td>
<td>0.0</td>
<td>41.7</td>
<td>57.6</td>
<td>58.7</td>
</tr>
<tr>
<td>Soil series and texture</td>
<td>Declo silt loam</td>
<td>Caribou loam</td>
<td>Sandy loam</td>
<td>Shano silt loam</td>
<td>Plainfield loamy sand</td>
</tr>
<tr>
<td>NFPT total yield (Mg ha⁻¹)</td>
<td>46a¹</td>
<td>46a</td>
<td>40a</td>
<td>84b</td>
<td>57a</td>
</tr>
</tbody>
</table>

¹ Means with the same letter are not significantly different at the 0.05 level (Tukey’s adjustment).

Table 2. Breeding targets for select traits.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Target</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity</td>
<td>1.084</td>
<td>1.080–1.095</td>
</tr>
<tr>
<td>Marketable yield &gt;170g</td>
<td>70%</td>
<td>68–74%</td>
</tr>
<tr>
<td>Marketable yield &gt;283g</td>
<td>32%</td>
<td>28–40%</td>
</tr>
<tr>
<td>Acrylamide level</td>
<td>75% reduction</td>
<td>&gt;50% reduction</td>
</tr>
</tbody>
</table>

g, and marketable yield percentage was defined as marketable yield divided by total yield. Two additional parameters of the size distribution were the percentage marketable tubers >170 g and percentage marketable tubers >283 g, calculated on a weight basis. Target ranges for the size distribution parameters and other traits are shown in Table 2.

After grading, 23 kg of tubers were randomly selected from each plot and shipped to the USDA Potato Research Worksite in East Grand Forks, MN. Upon arrival, tubers were put into climate-controlled storage lockers having 95% or more relative humidity, CO₂ concentration <2000 µL L⁻¹, and 12°C temperature. After 2 wk at 12°C for wound healing, storage temperature was ramped to 9°C where it was held for the rest of the storage season. Approximately 1 (early), 4 (mid), and 8 (late) mo after tubers went into storage, 3 kg of randomly selected tubers from each genotype—location combination (a storage-plot) were used to measure specific gravity, sucrose, and glucose. Specific gravity was calculated as (weight in air)/(weight in air − weight in water), and sucrose and glucose were measured using a YSI Analyzer, Model 27 (Sowokinos and Preston, 1988).

At each sampling time, an additional 5 kg of randomly selected tubers from each storage-plot were peeled using a high-pressure steam peeler, trimmed as needed with paring knives, and cut into strips 0.64 cm in width on each side with a Warner two-stage cutter. A 100-g subsample was frozen in liquid N₂ and stored at −80°C until shipped on dry ice to Covance Laboratories (Madison, WI) or University of Wisconsin–Madison (Madison, WI) for the measurement of free Asn by high-performance liquid chromatography. The remaining strips were blanched at 76°C for 8 min, par-fried in sunflower oil at 185°C for 90 s, and then frozen in a batch freezer at −10°C. Approximately 7 to 10 d later, 2.8 kg of frozen strips underwent a final fry at 182 to 185°C for 2 min and 50 s in a fryer that contains 132 L of sunflower oil. Finished French fries were frozen and shipped on dry ice to Covance Laboratories (Greenfield, IN) or UW–Madison for the measurement of acrylamide by liquid chromatography–tandem mass spectrometry or gas chromatography–mass spectrometry, respectively.

Data Analysis

Variance components for each trait were calculated using the following random effects model and the PROC MIXED function in SAS (version 9.3; SAS Institute, 2011):

\[
\gamma = \mu + \alpha_i + \beta_j + (\alpha \beta)_k + \gamma_l + (\gamma \alpha)_m + (\gamma \beta)_n + \varepsilon_{ijkl} \quad [1]
\]

where \(\mu\) is the grand mean, \(\alpha, \beta, \gamma\) are random effects of location, year, and genotype, respectively. In the model, \(\alpha_i \sim N(0, \sigma^2_\alpha), \beta_j \sim N(0, \sigma^2_\beta), (\alpha \beta)_k \sim N(0, \sigma^2_{\alpha \beta}), (\alpha \gamma)_n \sim N(0, \sigma^2_{\alpha \gamma}), (\gamma \beta)_n \sim N(0, \sigma^2_{\gamma \beta}), (\gamma \alpha)_m \sim N(0, \sigma^2_{\gamma \alpha}), \) \(\sigma^2_\gamma\) are the variance components for location, year, location × year, genotype, genotype × location, genotype × year, and residual error, respectively. Data transformation was used when inspection of the residuals indicated the assumption of normality was strongly violated. All three yield traits expressed as percentages (marketable yield, marketable tubers >170 g, and marketable tubers >283 g) were transformed by arcsin(\(x\)). Specific gravity was transformed by arcsin(\(\sqrt{x}\)). Raw tuber glucose, sucrose, and free Asn, as well as acrylamide in finished French fries, were transformed by \(\log_{10}(x)\).

Broad-sense heritability (\(h^2\)) for the entries tested in all 3 yr of the NFPT was calculated as follows:

\[
h^2 = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_e/L + \sigma^2_g/Y + \sigma^2_e/LY} \quad [2]
\]

where the number of locations \(L\) was the mean of the 3 yr \((3 + 5 + 5)/3 = 4.3\) and \(Y = 3\) yr.

The multiyear, multilocation mean for each genotype was estimated based on Eq. [1], except that the genotype effect was fixed (i.e., best linear unbiased estimation using LSMEANS command in SAS). When testing for differences against a check variety (Ranger Russet or Russet Burbank), Dunnett’s method was used to adjust \(p\)-values for multiple comparisons. For ease of interpretation, in the figures, we show the genotype means on the original trait scale (using the appropriate inverse transformation). The complete set of genotype means is presented in Supplemental Table S1.

The genetic correlation between the early, mid-, and late measurements of the storage traits (specific gravity, sucrose, glucose, free Asn, and acrylamide) was estimated by multivariate mixed model analysis using a variation on Eq. [1]:

\[
y_{ijkl} = \mu + y_{i} + \log_{j} + (y_{i} \times \log_{j}) + t_{k} + (y_{i} \times \log_{j}) + (g \times \log_{j} \times \log_{k}) + \varepsilon_{ijkl} \quad [3]
\]
Table 3. Variance components and broad-sense heritability ($h^2$) of the entry means.

<table>
<thead>
<tr>
<th>Trait</th>
<th>$\sigma^2_g$†</th>
<th>$\sigma^2_{g\times l}/\sigma^2_g$†</th>
<th>$\sigma^2_{g\times y}/\sigma^2_g$ †</th>
<th>$\sigma^2_{g\times l\times e}/\sigma^2_g$ †</th>
<th>$h^2$‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total yield (Mg ha$^{-1}$)</td>
<td>68</td>
<td>0.65</td>
<td>0.04</td>
<td>1.92</td>
<td>0.76</td>
</tr>
<tr>
<td>Marketable yield percentage</td>
<td>0.017</td>
<td>0.14</td>
<td>0.00</td>
<td>0.91</td>
<td>0.91</td>
</tr>
<tr>
<td>Marketable yield &gt;170g (%)</td>
<td>0.026</td>
<td>0.10</td>
<td>0.00</td>
<td>0.79</td>
<td>0.92</td>
</tr>
<tr>
<td>Marketable yield &gt;283g (%)</td>
<td>0.016</td>
<td>0.25</td>
<td>0.04</td>
<td>1.04</td>
<td>0.87</td>
</tr>
<tr>
<td>Specific gravity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mo</td>
<td>$4.9 \times 10^{-5}$</td>
<td>0.16</td>
<td>0.05</td>
<td>0.71</td>
<td>0.90</td>
</tr>
<tr>
<td>4 mo</td>
<td>$4.2 \times 10^{-5}$</td>
<td>0.18</td>
<td>0.00</td>
<td>1.02</td>
<td>0.89</td>
</tr>
<tr>
<td>8 mo</td>
<td>$4.7 \times 10^{-5}$</td>
<td>0.05</td>
<td>0.04</td>
<td>1.06</td>
<td>0.90</td>
</tr>
<tr>
<td>Sucrose (mg g$^{-1}$ fresh wt.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mo</td>
<td>0.017</td>
<td>0.30</td>
<td>0.04</td>
<td>0.83</td>
<td>0.87</td>
</tr>
<tr>
<td>4 mo</td>
<td>0.020</td>
<td>0.18</td>
<td>0.19</td>
<td>1.36</td>
<td>0.83</td>
</tr>
<tr>
<td>8 mo</td>
<td>0.080</td>
<td>0.21</td>
<td>0.15</td>
<td>0.97</td>
<td>0.85</td>
</tr>
<tr>
<td>Glucose (mg g$^{-1}$ fresh wt.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mo</td>
<td>0.081</td>
<td>0.07</td>
<td>0.04</td>
<td>0.71</td>
<td>0.92</td>
</tr>
<tr>
<td>4 mo</td>
<td>0.101</td>
<td>0.00</td>
<td>0.09</td>
<td>0.76</td>
<td>0.92</td>
</tr>
<tr>
<td>8 mo</td>
<td>0.091</td>
<td>0.03</td>
<td>0.25</td>
<td>0.79</td>
<td>0.87</td>
</tr>
<tr>
<td>Asparagine (mg g$^{-1}$ fresh wt.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mo</td>
<td>0.0056</td>
<td>0.00</td>
<td>0.37</td>
<td>1.63</td>
<td>0.80</td>
</tr>
<tr>
<td>8 mo</td>
<td>0.0065</td>
<td>0.05</td>
<td>0.11</td>
<td>1.35</td>
<td>0.87</td>
</tr>
<tr>
<td>Acrylamide (µg kg$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mo</td>
<td>0.031</td>
<td>0.15</td>
<td>0.05</td>
<td>0.77</td>
<td>0.90</td>
</tr>
<tr>
<td>4 mo</td>
<td>0.039</td>
<td>0.12</td>
<td>0.12</td>
<td>0.78</td>
<td>0.89</td>
</tr>
<tr>
<td>8 mo</td>
<td>0.054</td>
<td>0.12</td>
<td>0.15</td>
<td>0.89</td>
<td>0.87</td>
</tr>
</tbody>
</table>

† Variance components: g, genotype as the main effect; g×l, genotype by location; g×y, genotype by year.
‡ For entries tested in all 3 yr. Entries tested for only 1 or 2 yr necessarily have lower entry-mean heritability.

The main effects of year (yr), location (loc), and storage time (t) were modeled as fixed, in addition to the two-way interactions involving year. The three-way interaction between genotype (g), location, and storage time was modeled as random with the following separable covariance structure:

$$\text{Var}[g \times l \times t] = I_{g \times l \times t} \otimes \Sigma_{\text{cons}}$$  \[4\]

with an unstructured covariance matrix for time:

$$\Sigma_{\text{time}} = \begin{bmatrix}
\sigma^2_{\text{early}} & \sigma_{\text{early}} \sigma_{\text{mid}} & \sigma_{\text{early}} \sigma_{\text{late}} \\
\sigma_{\text{early}} \sigma_{\text{mid}} & \sigma^2_{\text{mid}} & \sigma_{\text{mid}} \sigma_{\text{late}} \\
\sigma_{\text{early}} \sigma_{\text{late}} & \sigma_{\text{mid}} \sigma_{\text{late}} & \sigma^2_{\text{late}} 
\end{bmatrix}$$  \[5\]

Genetic correlations between time points are denoted by r(middle-late) in Eq. 5. This covariance modeling framework is analogous to the methods widely used for G×E modeling in plant breeding (Crossa et al., 2004; Smith et al., 2005). Because measurements over time were made on tubers from the same plot, we also allowed for correlated residuals with the following covariance structure:

$$\text{Var}[e] = I_{g \times l \times t} \otimes R_{\text{time}}$$  \[6\]

and using a compound-symmetric matrix for $R_{\text{time}}$:

$$R_{\text{time}} = \begin{bmatrix}
\sigma^2 & \rho \sigma^2 & \rho \sigma^2 \\
\rho \sigma^2 & \sigma^2 & \rho \sigma^2 \\
\rho \sigma^2 & \rho \sigma^2 & \sigma^2 
\end{bmatrix}$$  \[7\]

The above linear model was fit using PROC MIXED in SAS.

Analysis of covariance was used to compare the influence of genotype, specific gravity, tuber sucrose, glucose, and free Asn on the acrylamide content of finished French fries. Genotype was modeled as a fixed effect, and the other storage traits were treated as numeric covariates. F-test $p$-values were computed by PROC MIXED in SAS at both the early (1 mo) and late (8 mo) storage times.

RESULTS

Variance component estimates are presented in Table 3. Of the three genotypic components of variance—genotype as the main effect (g), genotype by location (g×l), and genotype by year (g×y)—the main effect was the largest. Total yield in megagrams per hectare had the largest genotype variance relative to the main effect at $\sigma^2_g / \sigma^2_g = 0.76$. For marketable yield percentage that is expressed as a percentage of total yield, the magnitude of the interaction variance was much smaller: $\sigma^2_{g \times y} / \sigma^2_g = 0.14$. For all other traits, the g×l and g×y variance estimates were less than 30% of the main effect. The accuracy of the genotype means is reflected in the estimated broad-sense heritabilities ($h^2$), with the lowest value being that for total yield ($h^2 = 0.76$) because of its larger g×l and g×y variances. The $h^2$ results in Table 3 are for the 30 genotypes tested in all 3 yr; for the genotypes tested in only one or 2 yr, the $h^2$ values were necessarily somewhat lower (data not shown).

Many National Fry Processing Trial Genotypes made Low Acrylamide French Fries

Figure 1 shows the distribution of acrylamide concentration in French fries made at the early-, mid-, and late-storage times. Acrylamide tended to increase with time in

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storage, from a trial median of 495 µg kg\(^{-1}\) after 1 mo to 700 µg kg\(^{-1}\) after 8 mo. By comparison, the check variety Ranger Russet (Fig. 1, black arrows) produced French fries at 723 and 1227 µg kg\(^{-1}\) after 1 and 8 mo, respectively, and French fries from Russet Burbank (Fig. 1, grey arrow) increased from 1010 to 1350 µg kg\(^{-1}\) during the same time period. Compared with Ranger Russet (the more stringent standard), there were 38, 34, and 29 genotypes with significantly lower acrylamide (p < 0.05) after 1, 4, and 8 mo of storage, respectively. From the estimated means, the magnitude of the acrylamide reduction was greater than 50% for all these genotypes. Of the entries with sufficient overall merit (e.g., size, shape, and yield) to remain in the NFPT for all 3 yr, a handful were distinguished for having 75% less acrylamide than Ranger Russet (i.e., <25% of the Ranger Russet estimate) after 8 mo of storage: A0012-5, A02507-2LB (Payette Russet), A03921-2, AF3001-6 (Easton), Dakota Trailblazer, and W8152-1rus. From this group, only W8152-1rus exceeded the 75% acrylamide reduction target at every sampling time (1, 4, and 8 mo).

### Yield and Size Distribution

High total yield and high marketable yield in megagrams per hectare and as a percentage of total yield are desirable traits. The genotype estimates for total yield of Russet Burbank and Ranger Russet were 62.9 and 62.6 Mg ha\(^{-1}\), respectively, with standard errors (SE) of 9 Mg ha\(^{-1}\). Although 34 genotypes had higher estimated means than Ranger Russet (the trial maximum was 84.6 Mg ha\(^{-1}\)), these differences were not significant, even at the 0.1 level, because of the large SE. Similar statistical results were observed for the genotype means of marketable yield (Supplemental Table S1).

The distribution of marketable yield as a percentage of total yield is shown in Fig. 2. Most NFPT entries had values of 70 to 90%, compared with 72 and 81% for the standards Russet Burbank and Ranger Russet, respectively (SE 6%). A large number of entries (75) had estimated means higher than Ranger Russet (maximum 92%), but only three were significant at the 0.1 level: AF4124-7, AF4320-7, and W9433-1rus (all >90%).

Tuber size targets (percentage marketable tubers >170 and >283 g) have been established to produce French fries with the proper size distribution (Table 2). Figure 3 shows how well the NFPT entries met these targets, which correspond to the region inside the gray square. The genotype estimates for Russet Burbank were within the desired range for both parameters, while Ranger Russet may have been slightly above target for percentage marketable tubers >170 g (error bars show ±1 SE of the mean). Of the remaining 147 entries, 64 had genotype estimates within the target range for at least one size parameter, and 14 were on-target for both size parameters (Supplemental Table S1).

### Changes during Storage

Table 4 shows the genetic correlation between the early-, mid-, and late-storage time points for several tuber traits. Specific gravity was very consistent, with a correlation exceeding 0.98 across the entire 8 mo duration. There were 82, 82, and 77 entries with specific gravity in the target range.

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**Figure 1.** Distribution of genotype means (2011–2013) for acrylamide content in finished French fries after (a) 1, (b) 4, and (c) 8 mo of storage. The values for the check varieties Russet Burbank and Ranger Russet are indicated with arrows.
range (1.080–1.095) after 1, 4, and 8 mo of storage, respectively (Supplemental Table S1). The early and late measurements for free Asn and sucrose were also strongly correlated ($r = 0.93$ for both; Table 4). For glucose and acrylamide, the genetic correlation between the early and mid-time points was high ($r > 0.9$), but the early and late time points were more modestly correlated ($r = 0.59$ and 0.72, respectively).

**Relationships between Acrylamide Precursors and Acrylamide Content in French Fries**

It was noted earlier that acrylamide levels in the NFPT population increased with storage time (Fig. 1), and a similar trend was observed for tuber glucose. After 1 mo of storage, the median glucose for the population was 0.50 mg g$^{-1}$ fresh wt., compared with 0.77 mg g$^{-1}$ fresh wt. for Ranger Russet and 1.16 mg g$^{-1}$ fresh wt. for Russet Burbank. By 8 mo the population median increased to 0.77 mg g$^{-1}$ fresh wt., while Ranger and Burbank were 1.57 and 1.12 mg g$^{-1}$ fresh wt., respectively.

Figure 4 shows that glucose was predictive of acrylamide across all three storage times, with $R^2$ values in the range 0.64 to 0.77. The slope of the regression line was fairly consistent, with an increase of approximately 550 µg kg$^{-1}$ acrylamide per mg g$^{-1}$ fresh wt. glucose. In contrast, there was no relationship between tuber free Asn and acrylamide in finished French fries ($R^2 < 0.01$). To determine whether there was residual genetic variance for acrylamide after accounting for the role of glucose, an analysis of covariance was performed. The results in Table 5 show that genotype was highly significant ($p < 0.001$) in affecting acrylamide levels in the finished French fries at both the early and late storage times.

**DISCUSSION**

Minimizing potential health concerns related to dietary acrylamide is a priority for the potato industry. One of the key goals of the NFPT was to identify varieties that produce French fries with less acrylamide than current varieties, such as Russet Burbank and Ranger Russet. Depending on the storage period, 29 to 38 genotypes met this objective, including six that exceeded the 75% reduction target after 8 mo of storage. These results indicate that breeding for reduced acrylamide-forming potential is quite feasible.

The real challenge is to combine low acrylamide with the complex agronomic and food quality traits that are required of new varieties. High marketable yield and an appropriate tuber size distribution are examples of key agronomic traits. Fry color and texture are important aspects of product quality, which are influenced by traits such as dry matter content (specific gravity 1.080–1.095) and the concentration of reducing sugars (which drives the Maillard reaction, e.g., nonenzymatic browning). The NFPT has generated multiple years of data for these traits for the best fry processing germplasm in the United States. Comparable datasets have been generated from regional trials, but the NFPT is the first national variety trial of this scope for the fry processing industry. By providing five site-years of data in one calendar year, from all the major regions where russet potatoes are grown for processing, the NFPT has enabled a higher level of selection efficiency for US potato breeding. The value of the data for selection is reflected in the generally high estimates of broad-sense heritability (Table 3), which measures the squared correlation between the true and estimated genotypic values.

Because of the large number of genotypes in the NFPT, field and storage management conditions could not be optimized for each entry. Optimal commercial management for fry processing varieties seeks to maximize marketable yield while keeping reducing sugars in a range that produces acceptable product (Sowokinos and Preston, 1988). It is expected that many NFPT genotypes, if produced under conditions optimized for their unique characteristics, would have lower reducing sugar content and produce French fries with lower acrylamide than the values reported here.

Beyond its value for assessing the commercial potential of new varieties, the NFPT has also contributed to our understanding of acrylamide formation in fry processing genotypes. In most previous studies, the relationships between acrylamide, reducing sugar, and free Asn have been explored using only a few varieties from potentially several market classes (Wicklund et al., 2006; De Meulen et al., 2008; Viklund et al., 2008), while the present study involved 149 fry processing varieties. Using 17 varieties, Amrein et al. (2003) also found that acrylamide was strongly predicted by reducing sugar content ($R^2 = 0.88$) but not by free Asn ($R^2 < 0.01$), although they noted a slight increase in goodness of fit ($R^2 = 0.02$) when a
bilinear regression term involving reducing sugar and acrylamide was added to the sugar-only model.

Amrein et al. (2003) rationalized the lack of influence of free Asn by noting that, on a molar basis, the average concentration of free Asn was 3.7 times that of glucose in their population, and, hence, it would not be expected to be rate limiting; there was also less variation for free Asn than glucose and fructose. Both trends were also observed in the NFPT dataset. The molar ratio between free Asn and glucose was 11.0 after 1 mo of storage and 5.6 after 8 mo (Supplemental Table S1). The genetic standard deviation for glucose was 50% of the population mean after 1 mo of storage and declined to 30% by 8 mo (Table 3; Supplemental Table S1). By contrast, the genetic standard deviation for free Asn was only 2% of its mean. Although large reductions in tuber free Asn by genetic engineering have been shown to decrease acrylamide content in French fries (Rommens et al., 2008; Chawla et al., 2012), the narrow range of Asn observed in the NFPT germplasm may explain the lack of relationship between free Asn and acrylamide in this study.

**Future Research**
To make the most efficient use of resources in variety development, it is important to identify which environments provide nonredundant information. This is one of the main goals of genotype × environment analysis, which is most often applied in the context of different locations (e.g., Cullis et al., 2010). However, the genetic correlation between storage environments is also important to consider, particularly for fruit and vegetable crops (Rak et al., 2013).

For future years of the NFPT, a key resource allocation question is how many times should glucose, Asn, and acrylamide be measured during storage? Because of the high correlation between the early and late times for free Asn, at most, one sampling time is probably needed, but since Asn was not predictive of acrylamide in 2011 through 2013, one might question whether it should continue to be measured at all. The value of such an effort would be the potential identification of new breeding lines with exceptionally low free Asn and to facilitate breeding for that trait. For both glucose and acrylamide, our results indicate it should be possible to eliminate either the measurement after 1 mo or after 4 mo of storage without much loss of information because the data at these two sampling periods were highly correlated ($r > 0.9$; Table 4). The correlation between the early (1 mo) and late (8 mo) storage environments was low enough for glucose ($r = 0.59$) and acrylamide ($r = 0.72$) that for these traits there is value in continuing to sample at both time points if the 1-mo sample is retained.

Of particular interest for future research was the finding that genotype had a strong effect on acrylamide content, even after controlling for the effect of tuber glucose. This finding was in agreement with models presented by Mutucumaru et al. (2014). Additional research will be needed to uncover the compositional differences and genetic loci that contribute to this result. To facilitate this effort, we are in the process of genotyping the NFPT entries with a set of genome-wide markers (Hamilton et al., 2011). As
Figure 4. Relationship between tuber glucose and acrylamide in finished French fries after (a) 1, (b) 4, and (c) 8 mo of storage. Each circle corresponds to a National Fry Processing Trial (NFPT) genotype, while the check varieties Russet Burbank and Ranger Russet are denoted by the square and triangle symbols, respectively. The slope of the regression line was fairly consistent throughout storage, with an increase of approximately 550 µg kg\(^{-1}\) acrylamide mg\(^{-1}\) glucose g\(^{-1}\) fresh wt.
the cumulative size of the NFPT population continues to grow, we are hopeful that the marker and phenotype data can be combined to identify new loci associated with acrylamide and other traits (Yu et al., 2006).

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References


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Table 5. Analysis of covariance p-values for predicting acrylamide in finished French fries.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Early (1 mo)</th>
<th>Late (8 mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>8 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
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<tr>
<td>Glucose</td>
<td>&lt;10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.078</td>
<td>0.159</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.667</td>
<td>0.659</td>
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<tr>
<td>Specific gravity</td>
<td>0.056</td>
<td>0.159</td>
</tr>
</tbody>
</table>