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Evaluation of Glycoalkaloid Content in Potatoes Grown in Multiple Locations

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Background. Glycoalkaloids (GLKs) are of interest to the potato industry primarily because they can be toxic to humans if present in high concentrations. Eating a potato with high glycoalkaloids may first be noticed as a slight burning on the tongue or throat. At progressively higher concentrations, symptoms can include cramping, diarrhea, vomiting, rapid pulse, coma, and death. Documented cases of GLK poisoning are rare. Most often, if a potato has higher amounts of glycoalkaloids it is because the potato was exposed to light and turned green, or it is an old potato that is sprouting. Sprouts and foliage contain high amounts of GLKs.

On rare occasions, a cultivar that usually has normal amounts of GLKs may accumulate higher amounts in response to poorly understood environmental conditions. This occurred in Sweden in the 1980s with a popular cultivar, Magnus Bonum, in which GLK amounts inexplicably spiked high enough to cause gastrointestinal issues in some, but not all people. Similarly, a promising new chipping cultivar, Lenape, was developed in the 1960s but was later found to occasionally accumulate very high amounts of GLKs and so was withdrawn from commercial production.

GLKs have been scientifically studied for about a hundred years. Plants like potato, tomato, and eggplant synthesize them to help resist pests and pathogens. Numerous scientific studies published over the last two decades show health-promoting effects of glycoalkaloids, especially for efficacy against numerous types of cancer. Nevertheless, they are still perceived only as a “toxin” by most people, probably because this classification was established long before any of the health benefits of recent years were discovered.

What are some recent issues? While GLKs have been known for a long time and much studied, their impact on the industry has been increasing recently. This is largely because modern society has become more risk-averse, and with 24/7 global media coverage, any food safety incident can be widely publicized.

Two examples below highlight the increasing risk averseness. The Pacific Northwest has competitive advantages for trade with Asia, the fastest growing export market for PNW potatoes. The Pacific Rim market has an especially low tolerance for greening, which is associated with an increase in glycoalkaloids. A consumer in Taiwan reported on social media that “his tongue felt weird” after eating a green-tinted potato product at a quick service restaurant. This led to an investigation by the Taiwanese government, threats of fines to suppliers of green potatoes and for a while disrupted trade.

A second example is the recent recommendation of the "Department of Risk Assessment (BfR)" in Germany to lower the allowed maximum amount of GLKs in potatoes from 20 mg/100 gram fresh weight to 10 mg/100 g FW, cutting in half the amount that has been considered safe for decades. This new recommendation resulted from members of a family having stomach issues purportedly due to eating a potato salad made from potatoes later found to contain 24 mg/100 g FW.

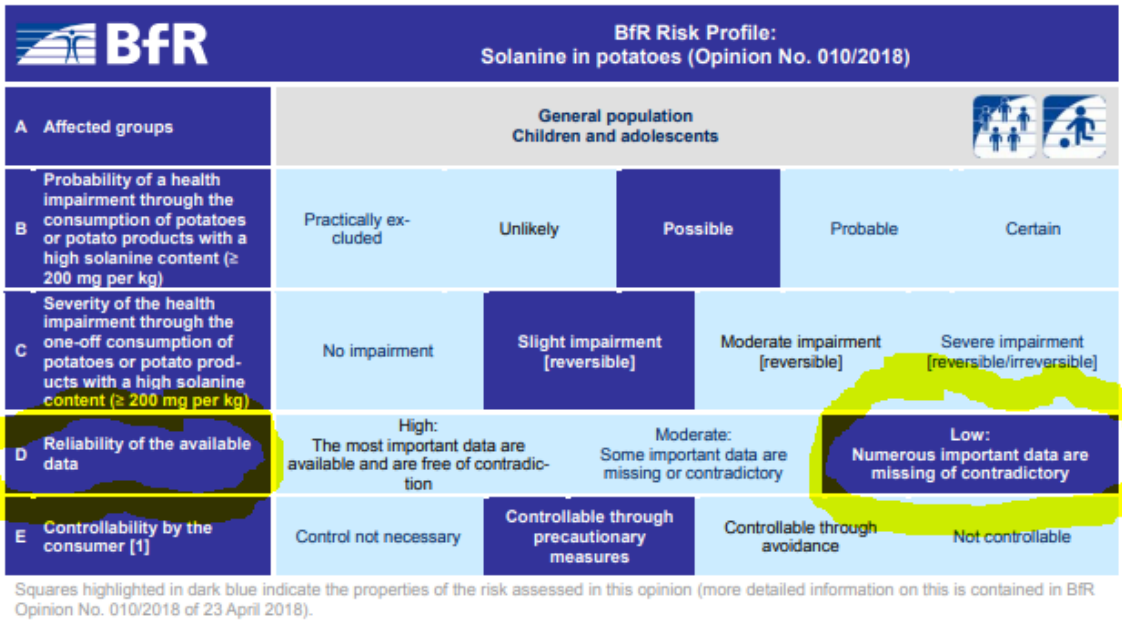

Figure 1.*Glycoalkaloid risk assessment by the German BfR.*

The rationale of the German BfR was that 24 mg/100 g FW was too close to the allowed limit of 20 mg. Note that the agency acknowledged that the data they based their provisional recommendation on is of low reliability and any effects were expected to be of "reversible slight impairment" (**Figure 1**). The extent to which this provisional recommendation will impact U.S. producers remains to be seen.

Relevance for Breeding. Potato breeders in the U.S. follow the voluntary guideline that any new cultivar must contain less than 20 mg GLKs/100 g fresh weight. One concern is that increasing risk averseness may cause breeding lines in the 10-20 mg/100g FW range (i.e. historically accepted as safe) to be eliminated as unsuitable. Pressure to do this could increase due to actions like that of the German BfR. For some, another concern about breeding lines or cultivars are worries that their GLKs *might* increase above 20 mg/100g FW in response to unpredictable environmental triggers. Therefore, breeding for as low an amount of GLKs as possible might be preferred, such as the low single digits. This worry exists because the environmental, physiological, and genetic regulation of GLKs in tubers is not well understood, and thus predictability is low.

However, there may be several negatives to an "as low as possible" approach.

1) If 10-20 mg is precluded as an acceptable range, this can slow new cultivar development and restrict the ability to use primitive germplasm as a source of highly desirable traits not found in the domesticated potato gene pool, because the progeny of crosses with primitive germplasm can have higher amounts of GLKs than the typical cultivar.

2) A line with outstanding performance could be rejected only because its GLKs fall in the 10-20 mg/100 g FW range, even though these amounts have been accepted for decades and such potatoes have been consumed for hundreds of years.

3) Unnecessarily low GLKs could decrease sustainability by lowering the plants ability to resist pests and pathogens, thereby necessitating greater pesticide use.

4) According to some studies, too low an amount of GLKs negatively affects flavor.

5) Given the numerous recent studies suggesting anti-cancer and other positive effects of GLKs, low

amounts may be desirable in the diet, although it should be noted that evidence for the health-promoting effects of GLKs comes largely from cell-culture and animal studies. Whether dietary amounts of GLKs have anti-cancer effects in humans has not been proven. While topical applications of GLKs have been effective in treatment of skin cancer in humans, testing the hypothesis that dietary GLKs have anti-cancer effects in human trials would be exceedingly difficult and expensive.

Objectives

One objective for this study was to examine the stability of GLK expression in Castle Russet. Castle is a promising new processing cultivar with many desirable characteristics, but analyses showed it has GLKs in the 10-20 mg/100 g FW range. This raised concerns about whether its GLKs might be prone to spiking higher; for example, if more widely grown.

Another goal of this work was to develop information that could be used to predict GLK amounts in potatoes. For example, the ability to predict whether a new advanced breeding line is prone to spikes in GLKs (or greening) would be very useful. However, there is no current biochemical or genetic test that can do this because the genetic and environmental factors that determine GLK concentrations or greening are not well understood. To study how GLKs are affected by environment, we grew 13 cultivars, each from the same seed lot, in six different states, Idaho, New York, North Dakota, Oregon, Texas, and Washington. The trials were repeated a second year. For each cultivar and location, four reps were prepared for analysis with each rep consisting of three tubers. GLK amounts were determined by LCMS using an Agilent triple quadrupole mass spectrometer in multiple reaction monitoring mode.

Results

The average amount of GLKs in Castle Russet in each State is shown in **Figure 2**. As seen, the average GLK amount in each location was well under 20 mg/100g FW. The two-year average of all locations was 11.0 mg/100 g FW. The largest year to year differences were seen in Oregon where the average was 7.7 in year 1 and 17.1 in year 2, and in North Dakota where the average was 16.1 in year 1 and 10.2 in year 2.

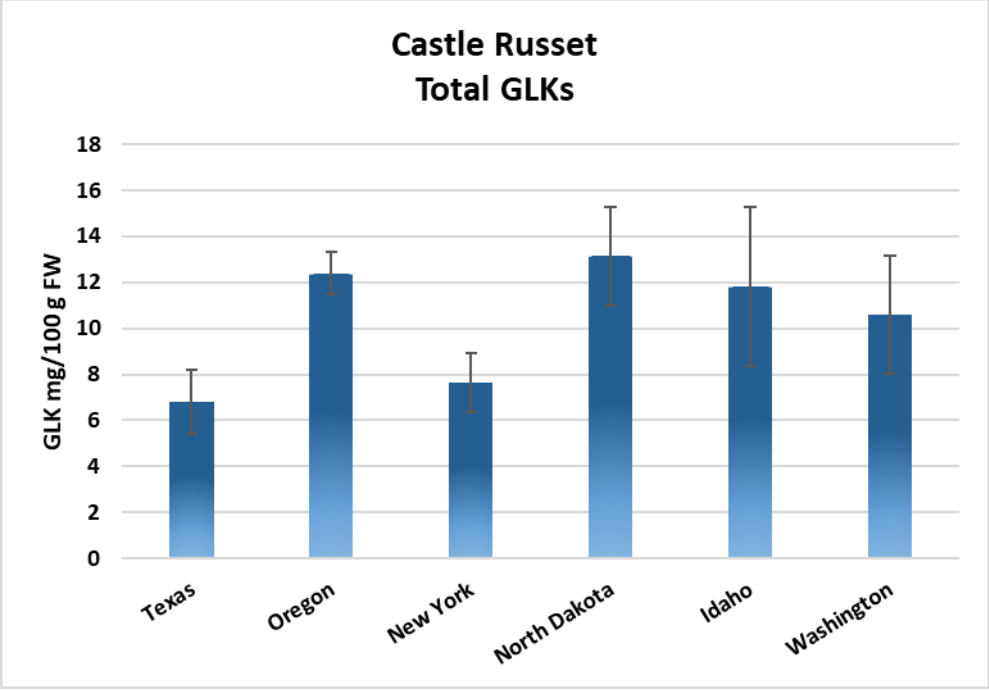

Figure 2. *GLK concentrations in Castle Russet grown in six states over two years. Solanine and chaconine amounts were determined and total combined amounts are shown here. Standard deviation is shown.*

Average amounts of total GLKs in the 13 varieties over two years and across all locations are shown in **Figure 3.** All 13 averaged well below 20 mg/100 g FW. OR09256-2 and Castle Russet had the highest amounts, whereas Classic Russet had the lowest. Snowden was included in this study because it is known to occasionally accumulate higher levels of GLKs, but in this study it had relatively low amounts, which might indicate the trials during these two years did not encounter conditions that promoted GLK accumulation.

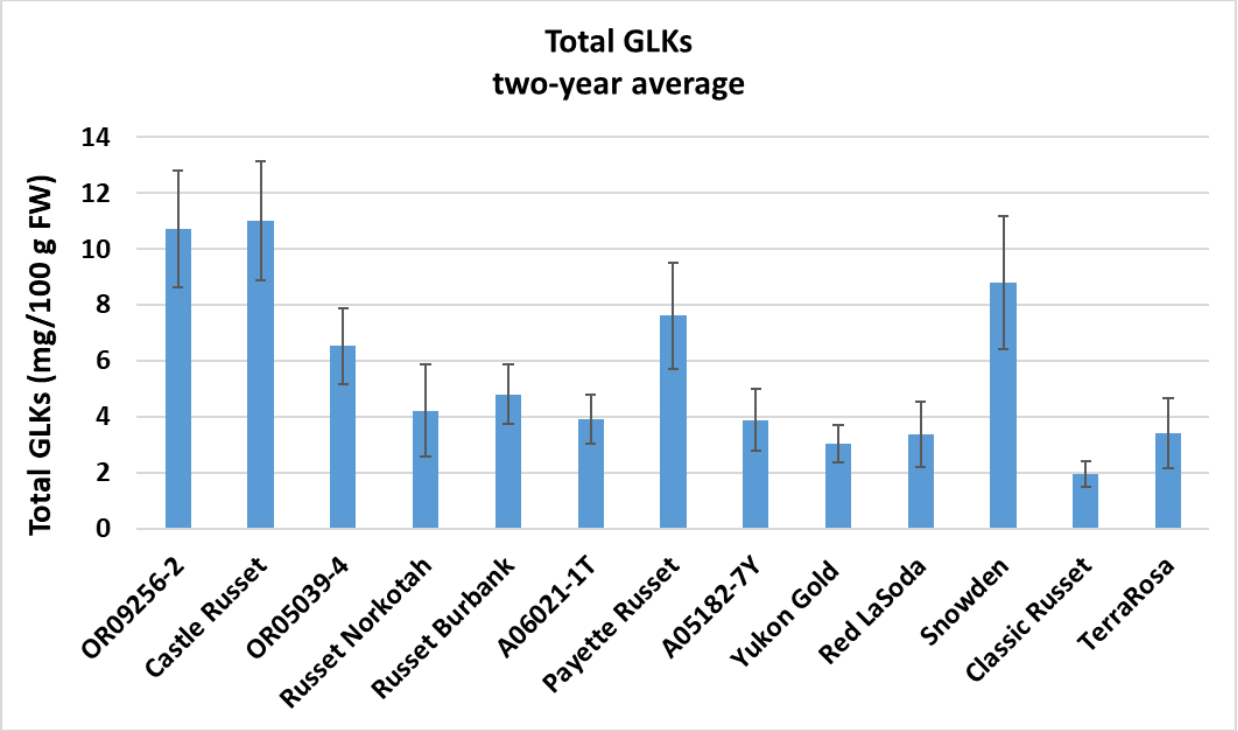

Figure 3. *Average amounts of total glycoalkaloids measured in 13 genotypes grown in Idaho, New York, North Dakota, Oregon, Texas and Washington over two years. Standard deviation is shown.*

So, while the averages were all well below 20 mg/100 g FW, it is also of interest to know how much variation occurred in the GLK content of each line among the locations. As seen in **Figure 4**, considerable differences in GLK amounts occurred within the same genotype over the course of the study. For example, amounts in Norkotah were quite a bit higher in North Dakota in year one (13.6 mg/100 g FW) than they were in the other locations or in North Dakota in year 2 (Figure 4, Table 1). Similarly, Payette grown in Oregon in year 2 of the trial was markedly higher (19 mg/100 g FW ±2.0 mg) than the other locations in year two, all of which were below 7 mg/100 g FW. The error bars in Figure 4 give an indication of how much variability occurred in GLK amounts in each cultivar by state. The Payette tubers with high amounts showed no obvious greening. The variability in GLK amounts highlights the fact that GLKs are inducible and that we do not understand all genetic x environment factors responsible for occasional increases in GLKs. Yukon Gold and Red LaSoda had consistent amounts among different locations, as did Classic Russet, which had the lowest amount of GLKs among the studied lines.

An important question for breeding programs is whether lines with higher average GLKs are prone to larger changes in GLK amounts than potatoes with lower average amounts. In other words, would a cultivar that has average GLK amounts of 5 mg/100 g FW be less prone to spikes than one with 10 mg? Or would one with 10 mg be less prone than one with 15 mg? This relates to the question of whether cultivars or breeding lines with intermediate amounts of GLKs might be more prone to spikes than potatoes with lower amounts. OR09256-3 had among the highest average GLK amounts (10.7 mg/100 g FW) and also had the highest amount of GLKs (23.9 in one sample comprised of 3 tubers) observed among any of the samples. This high value was 13.2 mg above the average for OR09256-3. This was the highest increase above average observed for any cultivar. However, Castle Russet, which averaged 11 mg/100 g FW, had a high reading only 6.1 mg/100 g FW above average, which is lower than the increase seen for Russet Norkotah and Russet Burbank

(9.4 and 8.2 mg respectively, Table 1). Both Norkotah and Burbank averaged less than 5 mg/100 g FW. Similarly, Payette (average 7.6 mg/100 g FW) had a maximum amount of 19.0 mg, which is greater than the highest amount for Castle Russet, 17.1 mg/100 g FW.

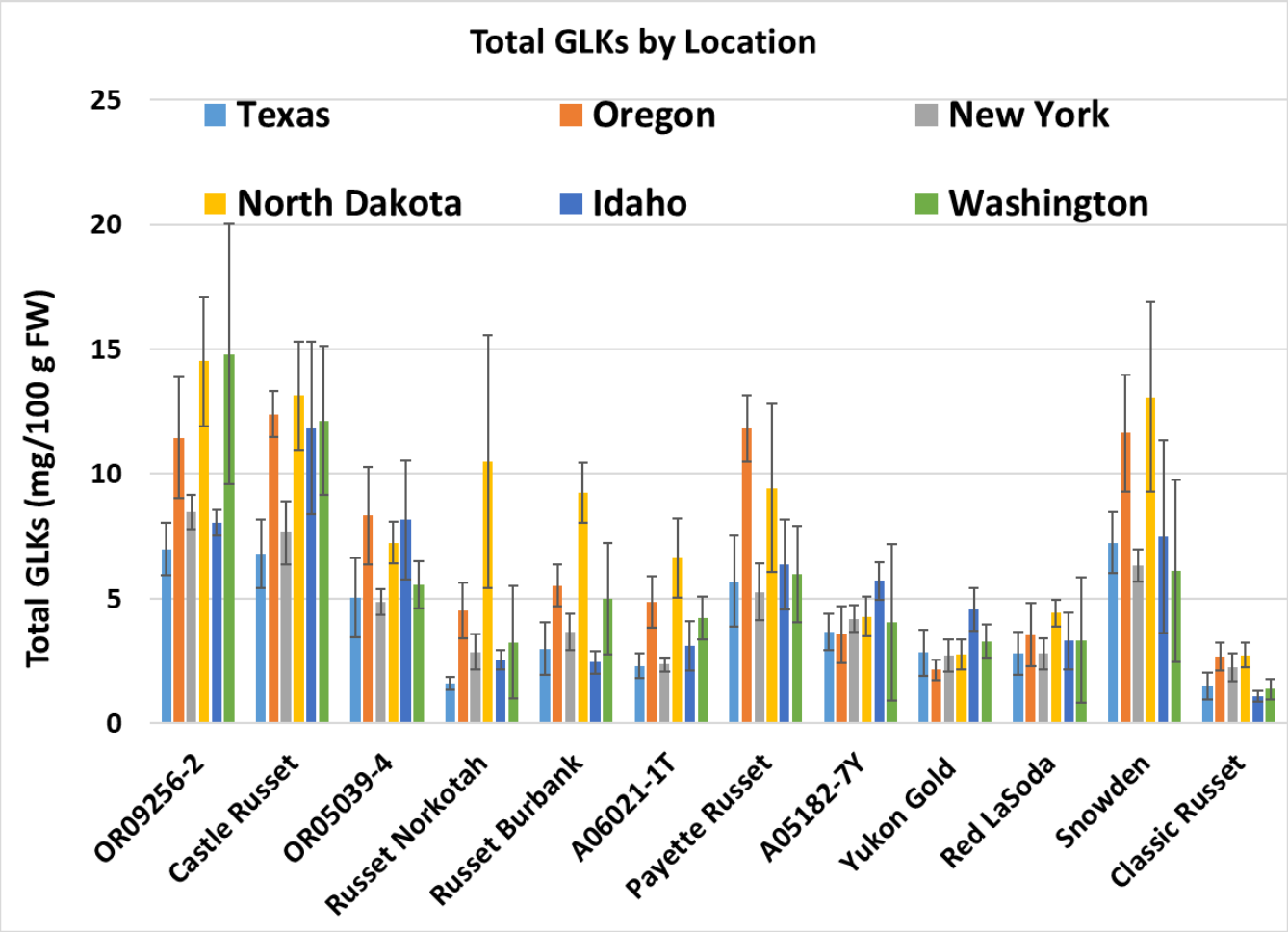

Figure 4. *Each bar represents the two-year average of total GLKs in each line grown in Idaho, New York, North Dakota, Oregon, Texas and New York for two years. Standard deviation is shown.*

Therefore, this study does not support the hypothesis that lines that have higher average amounts of GLKs, will *always* be more susceptible to large spikes. However, an even larger study would be needed to definitively answer this question, and would require measuring GLK amounts annually over many years and locations using diverse genotypes.

It is interesting that the three cultivars with the lowest average amounts (Yukon Gold, Red LaSoda, and Classic Russet; Table 1) never increased above 5 mg/100 g FW and had high values that were only 1.7, 1.5 and 2.3 mg respectively above their average. This suggests that potatoes with average GLK amounts in the very low end of the accepted range of 0-20 mg/100 g FW, may be less prone to significant increases in GLKs in response to environment.

This study supports a role for genetics in determining how variable GLK content will be and to what extent a cultivar will be prone to spikes. However, the molecular mechanisms that regulate a potato's GLK content remain unknown, as does why two genotypes might respond differently to the same environmental stimuli in terms of effect on GLK synthesis. In the absence of robust predictors that forecast a genotype's propensity to spike to unacceptable levels, the current more laborious method of measuring GLKs in each breeding line grown in multiple locations can give the needed information. Based on these results, Castle Russet did not show a greater tendency to spike relative to other potatoes. Thus, one cannot assume that all breeding lines with intermediate levels of GLKs will be more prone to spiking than current commercial potatoes. Studies are underway in our program examining GLK-related gene expression, and also greening.

Table 1. Average, minimum, and maximum amounts of GLKs measured in each line over all locations and years. "Fold change" was calculated by dividing max by min value. "Amount of highest value above average" was calculated by taking the highest average amount from any individual location and subtracting the average amount across all locations and years.

	Average GLKs	Minimum Amount	Maximum amount	Fold Change	Difference between high and low amounts (mg/100 g FW)	Amount (mg) of highest value above average
	Total GLKs (mg/100 g FW)					
OR09256-3	10.7	5.3	23.9	4.5	18.7	13.2
Castle Russet	11.0	5.7	17.1	3.0	11.4	6.1
OR05039-4	6.5	4.0	12.0	3.0	8.0	5.5
Russet Norkotah	4.2	1.1	13.6	12.2	12.5	9.4
Russet Burbank	4.8	1.1	13.0	11.7	11.9	8.2
A06021-1T	3.9	1.7	9.2	5.4	7.5	5.3
Payette Russet	7.6	4.6	19.0	4.1	14.4	11.4
A05182-7Y	3.9	2.4	6.6	2.8	4.3	2.7
Yukon Gold	3.0	1.1	4.7	4.5	3.7	1.7
Red LaSoda	3.4	1.7	4.9	2.8	3.1	1.5
Snowden	8.8	4.1	16.9	4.2	12.9	8.1
Classic Russet	1.9	0.7	4.3	6.3	3.6	2.3

GLKs in foods like potatoes, tomatoes, eggplants, and peppers are a nuanced topic. On the one hand, society is much more risk averse today than in previous generations and any food safety incident can generate negative publicity. On the other hand, consumers are more interested in the health-promoting aspects of food and sustainability of production than previous generations. Generation Z is reported to be the most health-conscious yet. However, it seems that the negative aspects of GLKs are more widely known currently than the positive aspects.

Supporting the current voluntary standard that new breeding lines must have less than 20 mg/100 g FW is that this has been a successful standard for decades, during which time billions of pounds of potatoes have been consumed. A point worth considering about the GLK content of breeding lines is that some studies have shown that commercial processing involving peeling, blanching, and cooking can decrease GLKs over 70% in the finished product from the amount present in raw potatoes. Thus, processed products from cultivars grown for French fries or chips would be expected to contain considerably lower amounts of GLKs than raw potatoes, whereas cultivars grown as table stock and that are consumed with the skin and minimal processing would be expected to have similar amounts as in the raw form.

In conclusion, this two-year study covering six States gives an indication of how much GLKs vary within a genotype in different years and environments. The results support a role for both genetics and environment. If planted on a larger scale over a longer period it's likely even greater variability would be observed. This study suggests that some genotypes like ORO9256-3 may be more prone to spikes, whereas other genotypes, such as Classic Russet and Yukon Gold, may be less prone to variation and have consistently low GLK amounts.