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LC-MS Analysis of Solanidane Glycoalkaloid Diversity among Tubers of Four Wild Potato Species and Three Cultivars (*Solanum tuberosum*)

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Secondary metabolites in potato tubers include both phytonutrients and plant defense compounds. The extent these small molecules vary among different potato genotypes is not well characterized. LC-MS analysis of tuber extracts from seven potato genotypes showed that one large source of small molecule variation is the glycoalkaloids. Glycoalkaloids are involved in the resistance of potatoes to pathogens and pests, but they also have implications for human health and nutrition. This study focused on glycoalkaloids with solanidane or solanidane-like aglycones, of which over 50 were tentatively identified, many of which appeared to be novel glycoalkaloids. Results suggested the variety of glycoalkaloids in potatoes is considerably greater than previously thought. Dissecting the role of these many glycoalkaloids in human health or pest and pathogen resistance will be a formidable undertaking.

KEYWORDS: Glycoalkaloids; potatoes; wild species; secondary metabolites;*Solanum tuberosum*; disease resistance

INTRODUCTION

Extensive genetic diversity exists among potato germplasm, which includes around 200 wild species found in extremely varied habitats throughout the Americas (*1*). However, only a small portion of this genetic diversity has been incorporated into modern cultivars, resulting in a narrow genetic pool (*2*). Consequently, wild species are a largely untapped resource that likely contain many novel genes useful for trait enhancement of domesticated potatoes.

Relatively little is known about the extent of metabolite diversity present among potato germplasm. Metabolomics holds promise toward understanding metabolite abundance and diversity in plants. GC-MS metabolic profiling of a few potato cultivars has been shown to be an effective tool (*3, 4*). Elegant metabolome analysis in *Arabidopsis* revealed a surprising amount of metabolite diversity among different accessions and was able to associate a majority of detected mass peaks with QTLs (*5*). As the potato metabolome becomes better defined, along with knowledge of the extent to which it varies among different genotypes, new opportunities for trait manipulation will become possible.

Preliminary LC-MS analysis of the seven genotypes in this study suggested glycoalkaloids were a large source of metabolite diversity. Glycoalkaloids are plant metabolites containing an oligosaccharide, a C_{27} steroid and a heterocyclic nitrogen component. New potato cultivars must contain less than 20 mg/100 g fresh weight (FW) of total glycoalkaloids (*6*). Solanine and chaconine are thought to comprise upward of 90% of the total glycoalkaloid complement of domesticated potatoes, with chaconine often more abundant than solanine (*7*).

The glycoalkaloid biosynthetic pathway is not fully delineated, even for the major potato glycoalkaloids, solanine and chaconine. Glycoalkaloids, derived from the mevalonate pathway via cholesterol (*8*), occur throughout the tuber but are primarily synthesized in the phelloderm (*9*). Surprisingly little has been elucidated about the genes and enzymology involved in conversion of cholesterol into the various glycoalkaloids. More is known about the glycosylation steps, and several glycosyltransferases have been characterized or cloned (*10–12*).

Identification of glycoalkaloid biosynthesis genes has enabled transgenic approaches to decrease potato glycoalkaloid content, because glycoalkaloids are typically regarded as antinutritive compounds capable of causing vomiting and other ill effects if ingested in high enough amounts (*13, 14*). Potatoes overexpressing a soybean sterol methyltransferase exhibited decreased amounts of both cholesterol and glycoalkaloids (*15*). Antisense expression of potato steroidal glycosyltransferases also was successful in reducing glycoalkaloid levels (*10, 16*).

Complicating assessment of the desirability of glycoalkaloids in potato is that they contribute to pest and pathogen resistance (*17*). Moreover, recent evidence suggests they have health-promoting effects in humans (*18*). Multiple studies show glycoalkaloids, including some found in potato, promote apo-

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ptosis and are effective against several different type of human cancer cells in in vitro assays (*19, 20*). Another study suggested glycoalkaloids boost innate immunity in mice (*21*).

Initial LC-MS screening suggested that among the hundreds of compounds detected in tubers, glycoalkaloid composition was particularly diverse. Potato glycoalkaloids can be divided into two general classes, those with solanidane or spirosolane aglycones (*22*), and this study focused on solanidine or solanidane-like glycoalkaloids. Therefore, LC-MS was used in this study for comparative small molecule profiling of four potato wild species and three commercial cultivars that represent a large percentage of the potato acreage planted in the United States.

MATERIALS AND METHODS

Materials. Solanine and demissidine were purchased from Sigma (St. Louis, MO). Chaconine was purchased from Fluka (St. Louis, MO). Tomatidine and solasodine were purchased from MP Biomedicals (Solon, OH). All standards were prepared as stock solutions at 10 mg/mL in methanol. Stock solutions were stored in darkness at −80 °C. Standard solutions remained stable over one year in these conditions. Tomato, eggplant, and leaf extracts of *S. kurtzianum* and *S. stoloniferum* were analyzed to validate the assignment of tomatine, dehydrotomatine, solamargine and solasodine. *S. chacoense* were used to assist in leptine and leptidine identification. *S. dulcamara* was used as a source of α- and β-solamarine.

Tubers from the *Solanum tuberosum* cultivars 'Shepody', 'Ranger Russet', and 'Russet Norkotah' and the wild species *Solanum spegazzinii* (PI205407), *S. stenotomum* (PI195204), *S. pinnatisectum* (PI184774), and *S. bulbocastanum* (PI243510) were harvested and washed prior to freeze-drying. Wild species were obtained from the U.S. Potato Genebank (Sturgeon Bay, WI). *S. pinnatisectum* and *S. bulbocastanum* are Mexican species with excellent resistance to several diseases and pests (*23–27*). *S. stenotomum* is a primitive Peruvian diploid and an Argentinian accession of *S. spegazzinii* was used that is a source of resistance to potato cyst nematode *Globodera pallida* (*28*). Cultivars were grown in the field and harvested at maturity. Wild species do not tuberize in the field, so were grown in the greenhouse.

Sample Preparation. Three slices with peel from each tuber were immediately frozen in liquid N_2 and then homogenized. Two tubers per replicate were used. These powdered samples were then freeze-dried and stored at −80 °C until analysis. Samples were extracted as previously described (*29*).

HPLC Analysis. An Agilent 1100 HPLC system equipped with a quaternary pump, refrigerated autosampler, column heater was used with DAD and MS detectors. Flow rate was 1 mL/min and injection volume was 5 μL. Column temperature was 35 °C. A 50 × 4.6 mm, 1.8 μm Zorbax Eclipse XDB C-18 (Agilent) was used at a flow rate of 1 mL/min with a gradient elution of 0−1.5 min, 0−4% B; 1.5−5 min, 10−25% B; 5−10 min, 35−65% B; 10−15 min, 70−90% B; 15−16 min, 95% B. (Buffer A: 10 mM formic acid pH 3.5 with NH_4OH. Buffer B: 100% methanol with 5 mM ammonium formate.) UV detection was at 210, 280, 320, and 360 nm.

MS Parameters. LC-MS analysis was conducted with an Agilent 1100 LC/MSD VL ion trap. Experiments were carried out with an ESI source in both positive and negative ion mode. The source was operated using 350 °C drying gas (N_2) at 12 L/min, 55 psi nebulizer gas (N_2), and the source voltage with a scan range of *m/z* 100−1300. Automated MS (*2*) analysis was conducted using SmartFrag software (Agilent) and ramped CID voltage of 1500−4500. Data analysis was performed using Agilent ChemStation software and ACD/MS Manager software (Advanced Chemistry Development Inc., Toronto, Canada).

RESULTS AND DISCUSSION

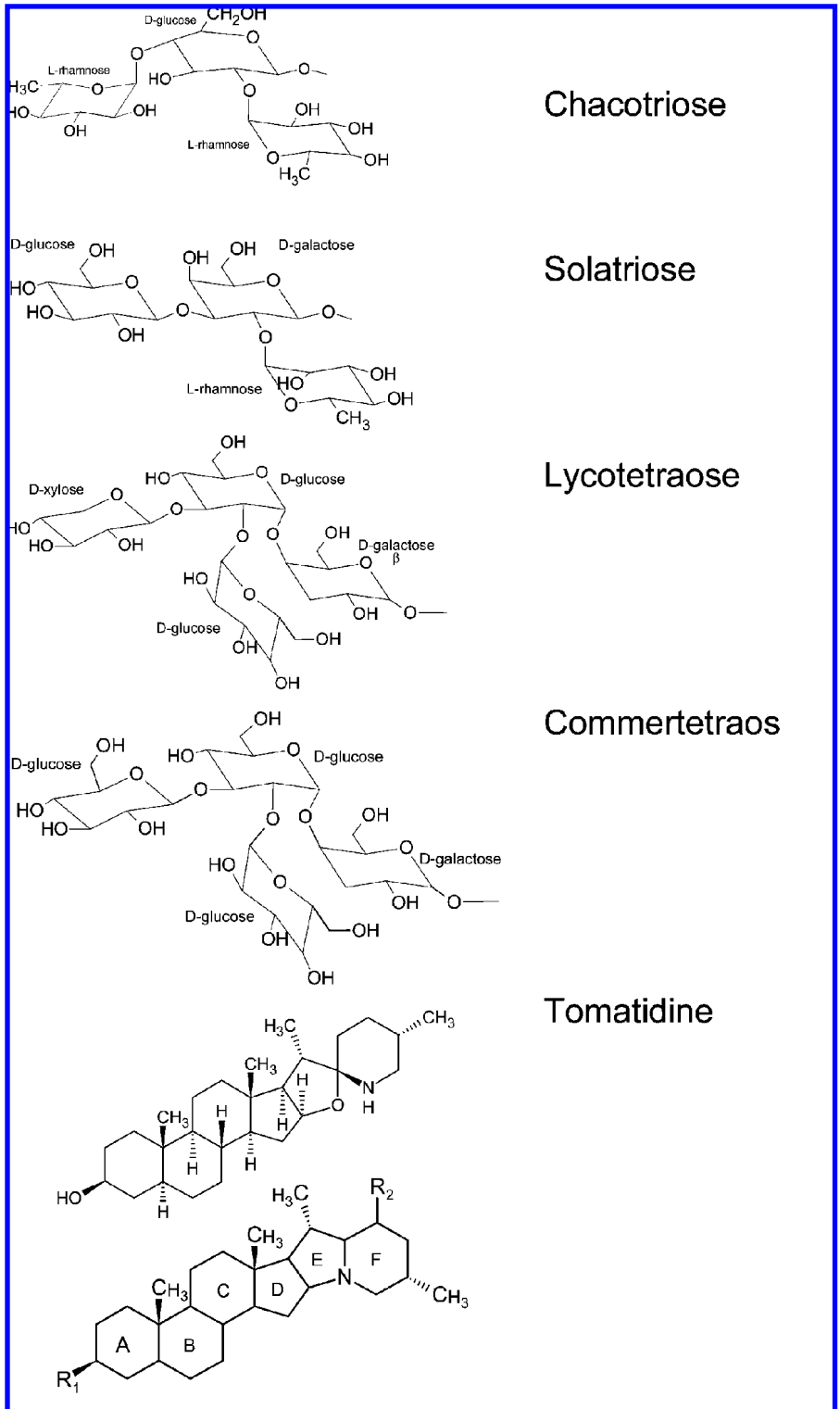
Genotypes Analyzed. Four wild species and three cultivars were used in this study. The three mainstream cultivars used, collectively make up over 50% of the total U.S. potato acreage. Initially, we were interested in characterizing the extent of diversity in secondary metabolites and particularly phenolics in these genotypes. However, LC-MS profiling quickly revealed that glycoalkaloids (**Figure 1**) were a large source of small molecule diversity. Total ion current chromatograms (**Figure 2**) of tuber extracts showed substantial differences between genotypes. Initial analysis suggested that many differences in these chromatograms were due to mass peaks that were glycoalkaloids. The glycoalkaloids were present in much greater numbers than anticipated, so we focused on those that were solanidanes or solanidane-like.

Glycoalkaloid Identification. Few glycoalkaloid standards are commercially available and most of those available are aglycones. Consequently, it is important to note that identifications reported in this study are tentative and LC-MS analysis alone cannot be definitive. Because of this limitation, we also used multiple reference plants known to contain particular glycoalkaloids.

The elution order for several key glycoalkaloids and aglycones was determined. Solanidine, demissidine, solasodine and tomatidine eluted at 15.2, 16.5, 17.3, and 17.5 min, respectively. The elution order for glycoalkaloids was leptinine I (8.6), commersonine (9.4), demissine (9.7), solanine (9.8), dehydrotomatine (10.3), tomatine (10.7), solasonine (10.8), and solarmargine (10.9). This information established that increasing the number of double bonds in a given glycoalkaloid causes it to elute earlier. Solanidanes eluted earlier than spirosolanes, and an aglycone with commertetraose or lycotetraose eluted before the same aglycone with solatriose or chacotriose.

Identifications were also based on the observation that all spirosolane glycoalkaloids examined had $(M + H - H_2O)^+$ as the base ion, whereas hydroxy-solanidane compounds may or may not preferentially lose water during fragmentation. MS^2 and MS3 analysis was used to help differentiate between spirosolanes and hydroxy-solanidanes. Previously it was reported that 273/255 and 271/253 product ions resulting from an E-ring rearrangement were major product ions of spirosolanes and dehydrospirosolanes, respectively (*30*). MS^2 analysis of solanidine and demissidine showed that these product ions were also present in the solanidanes. MS^2 analysis of solasodine, tomatidine, solanidine and demissidine or MS^3 analysis of these same aglycones from solasonine (884→414), tomatine (1034→416), and solanine (868→398) were used to look for differentiating fragmentation patterns. The five to seven most intense product ions for solasodine and tomatidine were highly consistent from run to run, whereas considerably more variability was found among solanidine and demissidine product ion relative intensity. An *m/z* 253 or 255 product ion was always detected in multiple analyses of solanine and demissidine and was usually one of the most abundant product ions, whereas the presence of *m/z* 271/273 was more variable in the solanidanes. A proposed fragmentation scheme for solanidine is shown in **Figure 3**. One of the more abundant product ions of solanidine was an *m/z* 327 ion that was not detected in demissidine, solasodine or tomatidine. Diagnostic ions that readily distinguish each class of aglycone were not detected with our instrumentation.

Glycoalkaloids with Solanidine Aglycones. Solanine eluted at 9.8 min and was detected in all 7 genotypes. The most intense solanine mass peaks [*m/z* 868; $(M + H)^+$] were found in *S. stenotomum* and *S. spegazzini* and were a magnitude of order higher in intensity than in the cultivars (**Figure 4**). Very small amounts were present in *S. pinnatisectum*, which along with *S. bulbocastanum* had an additional *m/z* 868 peak, with an *m/z* 850 base peak that coeluted with solanine and appeared to be a spirosolane. Five of the genotypes that contained solanine,



Glycoalkaloid	[M+H] +	R_1	R_2	Double Bond
Chaconine	852	chacotriose	H	Δ5
Solanine	868	solatriose	H	Δ5
Demissine	1018	lycotetraose	H	-
Leptinine I	868	chacotriose	OH	Δ5
Leptine I	910	chacotriose	CH_3COO	Δ5
Dehydrocommersonine	1046	Commertetraose	H	Δ5

Figure 1. Structures of the four common glycoalkaloid sugar moieties found in potato, tomatidine, and the basic solanidane aglcyone.

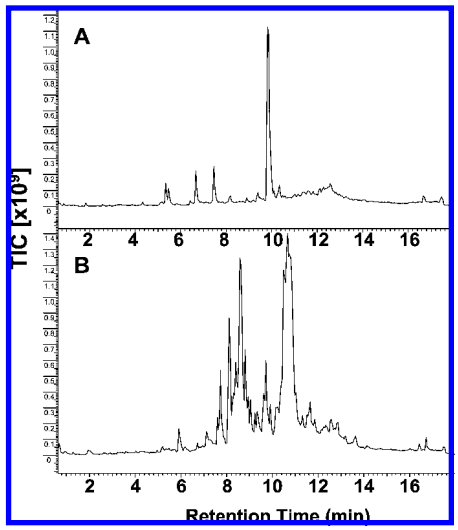


Figure 2. LC-MS chromatogram showing total ion current (positive mode) after injection of equal amounts of tuber extracts: (**A**) 'Shepody'; (**B**) *S. bulbocastanum*. Scale is the same.

also had a lower intensity *m/z* 868 peak elute at 10.3 min with similar MS^2 fragmentation, suggesting this is a solanine isomer (**Table 1**). This glycoalkaloid was more labile than solanine, because in the initial ionization, prior to MS^2, two product ions of *m/z* 398 $(M + H - \text{solatriose})^+$ and *m/z* 560 $(M + H - \text{rha} - \text{glu})^+$ were present in higher amounts than the *m/z* 868 parent ion.

Chaconine eluted at 9.9 min in all seven genotypes. The least intense chaconine mass peaks were in *S. bulbocastanum* and *S. pinnatisectum*, which were one to two magnitudes of order less than found in *S. spegazinni* and *S. stenotomum* (**Figure 4**). Two additional chaconine isomers were detected at 9.3 and 10.4 min in two of the wild species. *S. pinnatisectum* had by far the least amount of solanine/chaconine compounds.

A novel glycoalkaloid of *m/z* 1030 with an unusual carbohydrate moiety eluted at 9.6 min in *S. spegazzinii* and *S. stenotomum*. Product ions of *m/z* 884 $(M + H - \text{rha})^+$, 868 $(M + H - \text{glu})^+$, 706 $(M + H - \text{glu} - \text{glu})^+$, 560 $(M + H - \text{rha} - \text{glu} - \text{glu})^+$, and 398 $(M + H - \text{rha} - \text{glu} - \text{glu} - \text{glu})^+$ suggested the carbohydrate (*m/z* 632) contained one rhamnose and 3 hexoses conjugated to an aglycone with *m/z* 398. Such a glycoalkaloid sugar moiety does not appear to have been previously reported in potato. MS^3 analysis of the aglycone showed *m/z* 380 and 253 ions, consistent with that expected of solanidine.

Glycoalkaloids with Dehydrosolanidine Aglycones. A glycoalkaloid with *m/z* 850 eluted at 9.4 min in five genotypes and had an aglycone of *m/z* 396 $(M + H - \text{chacotriose})^+$ that is likely a solanidine derivative with an additional double bond, possibly at the C-3 position, as a solanida-3,5-diene has been reported in potato (*31*). Trace amounts of a dehydrochaconine with *m/z* 850 have been reported previously in potato (*32–34*). Additionally, a novel glycoalkaloid of *m/z* 850 eluted at 8.2 min in the same five genotypes, had similar product ions but was slightly less abundant than the 9.4 min compound. This strongly suggested the compound was an isomer of dehydrochaconine, however the fact that it elutes 1.7 min faster than chaconine, 1.2 min faster than dehydrochaconine and earlier than some of the solanidenols is somewhat surprising. The presence of multiple dehydrochaconines is consistent with our detection of two chaconine isomers described above and shows the saturation level of a given aglcyone is a mechanism through which potato is able to achieve glycoalkaloid diversity.

A glycoalkaloid with *m/z* 866 was detected at 9.4 min in the three cultivars but not in the wild species (**Table 1**). This glycoalkaloid was composed of solatriose and a *m/z* 396 aglycone, which is consistent with that of a solanida-diene-solatriose.

Two glycoalkaloids with *m/z* 1044 or 1016 were found only in in *S. spegazzinii*, eluted at 9.0 and 9.7 min and were likely a didehydrocommerosine and dehydrodemissine, respectively (*34*). Eluting at 9.4 min in *S. spegazzinii* was a glycoalkaloid of *m/z* 1046 composed of commertetraose and an aglycone of *m/z* 398 and identified as dehydrocommersonine (solanidene commertetraose).

Solanidane Aglycones with Two Oxygens. A hydroxysolanidine of *m/z* 868 eluting at 8.6 min was identified as leptinine I (**Table 1**). Leaf extracts from the reference plant *S. chacoense*, a well characterized leptinine source, was used to help establish leptinine identities (*35*). MS (*3*) data also supported this assignment (Table S1, Supporting Information). Leptinines and leptines are thought to be components of resistance to Colorado potato beetle, and efforts have been made to breed lines with elevated amounts (*36, 37*). Leptinines have been regarded as rare glycoalkaloids, found only in *S. chacoense* and only in foliage, not tubers (*36*). Leptinines may be more common than thought, given they were found in tubers from three of the four wild species we examined. Two additional leptinine-like glycoalkaloids with *m/z* 868 eluted at 9.0 min in *S. bulbocastanum* and 9.4 min in four genotypes.

Two additional glycoalkaloids with *m/z* 868 were among the earliest eluting of all the glycoalkaloids, eluting at 7.7 and 7.9 min in *S. bulbocastanum* and *S. stenotomum*, respectively (**Figure 4**). These glycoalkaloids had a chacotriose and an aglycone of *m/z* 414. MS^3 of the aglycone included product ions of *m/z* 396, 343, and 157. The *m/z* 850 product ion $(M + H - H_2O)^+$ suggested the presence of a second oxygen on the aglycone. The fragmentation is consistent with that expected of a hydroxy-solanidane; however, the very early elution relative to some of the solanidane-ol, -diols and -triols might suggest the presence of a novel aglycone.

A solanidenol of *m/z* 884 eluting at 8.6 min with an aglycone of *m/z* 414 was identified as leptinine II and was detected in three of the four wild species (**Table 1**; **Figure 4**). Two other *m/z* 884 glycoalkaloids were found only in *S. bulbocastanum*, one of which eluted at at 7.6 min and was composed of an aglycone with *m/z* 414 and solatriose. The fragmentation was consistent with that expected from a solanidenol solatriose, although the very early elution, one minute faster than leptine II and faster than some solanidane-diols, was earlier than expected.

Three probable hydroxysolanidadiene glycoalkaloids with a molecular ion of *m/z* 866 $[M + H]^+$ and aglycone of *m/z* 412 eluted at 8.1, 8.3, and 8.8 min (**Table 1**). Only one such glycoalkaloid has previously been reported in potatoes. The 8.1 min eluting *m/z* 866 compound was one of the few glycoalkaloids found in all seven genotypes (**Figure 4**). The *m/z* 866 at 8.3 min was detected only in *S. bulbocastanum*, the genotype that contained the most hydroxysolanidine derivatives. In all of these *m/z* 866 molecular ions, the MS^2 base peak of *m/z* 720 $(M + H - \text{rha})^+$, and ions at 574 $(M + H - \text{rha} - \text{rha})^+$ and 412 $(M + H - \text{rha} - \text{rha} - \text{glu})^+$ indicated the presence of chacotriose and the *m/z* 848 $(M + H - H_2O)^+$ product ion indicated a water loss. A spirosolane glycoalkaloid could have an *m/z* 866 molecular ion, but the ion abundance would be different because spirosolanes preferentially lose a water molecule, nor would such a nonhydroxylated spirosolane be

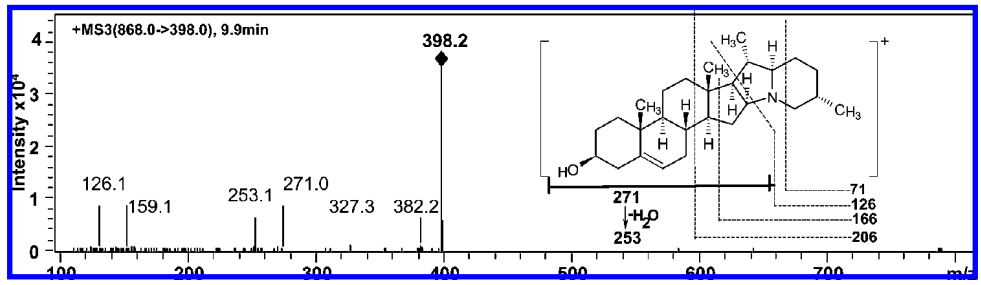


Figure 3. MS^3 mass spectrum (868→398) of solanine. Proposed fragmentation schemes of the *m*/*z* 398 aglycone are shown.

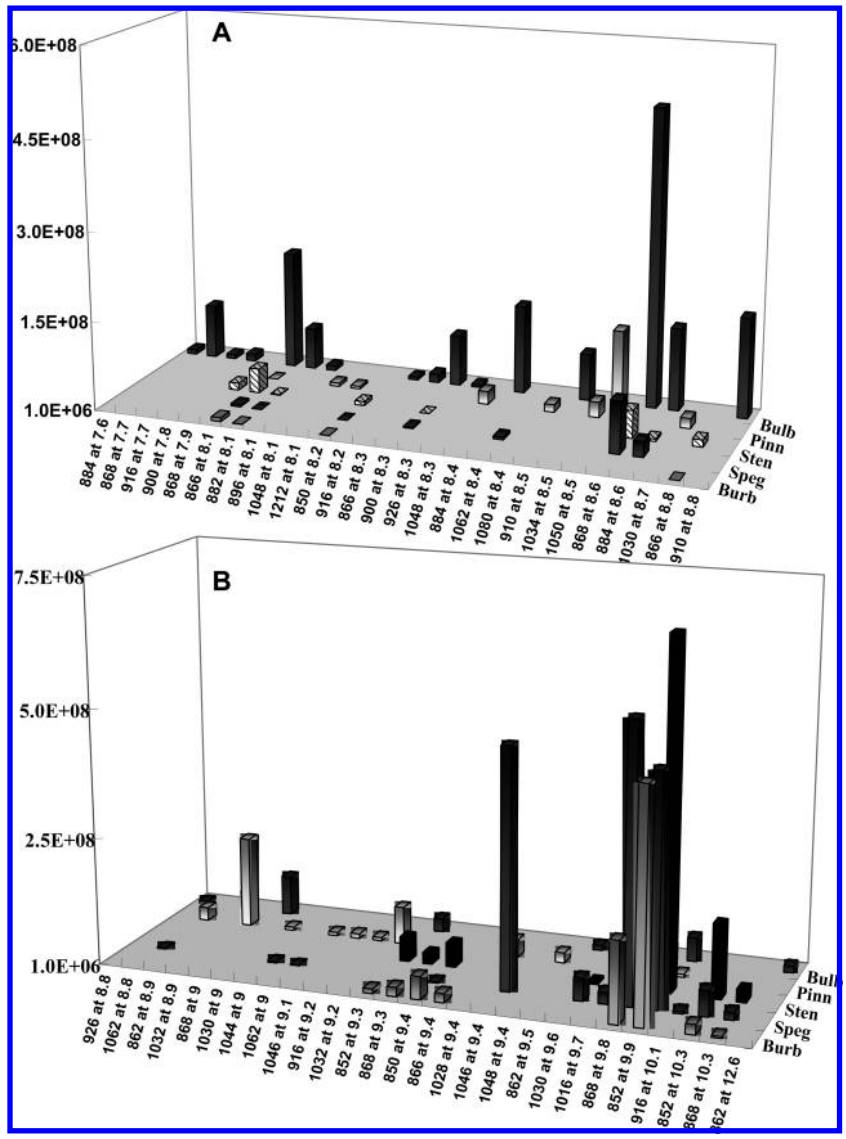


Figure 4. Glycoalkaloid occurrence and peak intensity: (**A**) glycoalkaloids eluting between 7.6 and 8.8 min; (**B**) glycoalkaloids eluting between 8.8 and 12.6 min. The $(M + H)^+$ ions, elution time (min), and intensity are shown in the four wild species and 'R. Burbank.' Ion intensity is shown in logarithmic scale. The cultivars had similar glycoalkaloid profiles, so only 'R. Burbank' is shown.

expected to elute this early. The best known hydroxylated solanine is commonly referred to as leptinine, so these hydroxysolanidadiene isomers with *m*/*z* 866 could be termed leptinidienes. An additional *m*/*z* 866 ion eluted at 10.6 min in *S. stenotomum* and *S. spegazzini* and was tentatively identified as solanidenone rha-rha-glu, possibly oxosolanidine.

S. bulbocastanum had an unusual group of three glycoalkaloids of *m*/*z* 862 $(M + H)^+$ composed of chacotriose and aglycone of *m*/*z* 408 eluting at 8.9, 9.5, and 12.6 min, which have not previously been reported in potato. The occurrence of low abundance *m*/*z* 844 $(M + H - H_2O)^+$ and 372 $(M + H - \text{chacotriose} - H_2O)^+$ product ions showed the presence of two oxygens on the aglycone. MS^3 analysis of the *m*/*z* 408 ion showed an *m*/*z* 390 ion as an abundant product ion. One possibility is that these compounds are solanidatetraenol isomers, which would account for an *m*/*z* 408 aglycone. Pentaene glycoalkaloids have previously been reported in potato (*38*). However, given the additional unsaturation, it would be expected such compounds would elute earlier than a solanidenol, which they do not. In particular it is hard to envision a solanidatetraenol eluting as late as 12.6 min. Moreover, the *m*/*z* 255 MS^3 fragment, while often found in

Table 1. LC-MS Analysis of Tuber Glycoalkaloids[a]

SN	compound	RT	sugar	occurrence	$(M + H)^+$	MS^2 ions
1	dehydrochaconine	8.2	cha	SS, SZ, RB, RN, SH	850	704, 558, 396, 378
2	dehydrochaconine	9.4	cha	SS, SZ, RB, RN, SH	850	704, 558, 396, 379, 378
3	chaconine isomer	9.3	cha	SB, SS, RB, RN, SH	852	706, 398, 380
4	α-chaconine	9.9	cha	SB, SP, SS, SZ, RB, RN, SH	852	706, 398, 560, 380
5	chaconine isomer	10.4	cha	SS, SZ, RB, RN, SH	852	706, 398, 560, 399, 380, 366
6	solanidatetraenol chacotriose	8.9	cha	SB	862	716, 408, 390, 570, 362, 380, 392, 846, 372, 844,
7	solanidatetraenol chacotriose	9.5	cha	SB	862	716, 408, 570,390, 378. 392. 846, 844
8	solanidatetraenol chacotriose	12.6	cha	SB	862	716, 408, 570, 390, 852, 844
9	solanidadienol chacotriose	8.1	cha	SB, SP, SS, SZ, RB, RN, SH	866	720, 848, 412, 702, 394, 574, 252, 253
10	solanidadienol chacotriose	8.3	cha	SB	866	720, 848, 574, 412, 394, 702
11	solanidadienol chacotriose	8.8	cha	SS, RB, RN, SH	866	720, 412, 574, 722, 394, 848
12	solanidenone chacotriose	10.6	cha	SS, SZ	866	720, 412, 574, 394, 380
13	solanidadiene solatriose	9.4	sol	RB, RN, SH	866	720, 396, 704, 558, 572, 848
14	solanidenol chacotriose	7.7	cha	SB	868	850, 704, 722, 253, 396, 378, 414, 271
15	solanidenol chacotriose	7.9	cha	SS	868	722, 576, 414, 850, 396, 704
16	leptinine I	8.6	cha	SB, SS, SZ	868	850, 704, 396, 253, 558, 378, 722, 414
17	leptinine isomer	9.0	cha	SB	868	850, 704, 251, 720, 396, 558, 414
18	solanidenol chacotriose	9.4	cha	SS, RB, RN, SH	868	722, 414, 866, 396, 704, 576, 850, 720, 378, 272
19	α-solanine	9.8	sol	SB, SP, SS, SZ, RB, RN, SH	868	398, 706, 722, 560, 380, 272
20	solanidadiene solatriose	10.3	sol	SS, SZ, RB, RN, SH	868	706, 722, 398, 560, 380
21	solanidadienol solatriose	8.1	sol	SB, SS, SZ, RB	882	736, 720, 864, 412, 540, 394, 850, 556, 382, 702
22	solanidenol solatriose	7.6	sol	SB	884	866, 396, 736, 414, 410, 720, 428, 704
23	solanidenediol chacotriose	8.4	cha	SB	884	866, 720, 738, 412, 848, 702, 253, 271, 574
24	leptinine II	8.6	sol	SB, SS, SZ	884	866, 396, 380, 378, 414, 704, 720, 253
25	methoxysolanidadiene solatriose	8.1	sol	SB	896	864, 866, 702, 394, 718, 734, 750, 704, 426, 720
26	solanidenetriol chacotriose	7.8	cha	SB	900	882, 864, 852, 880, 428, 846, 410, 446
27	solanidenediol solatriose	8.3	sol	SB, SS, SZ	900	882, 412, 394, 864, 271, 736, 253, 720, 754, 574
28	leptine I isomer	8.5	cha	SB	910	850, 396, 398, 704, 380
29	leptine I	8.8	cha	SB	910	850, 396, 832, 378, 704, 834
30	solanidenetriol solatriose	7.7	sol	SB	916	898, 880, 428, 410, 446, 590, 592, 253
31	solanidenetriol solatriose	8.2	sol	SB	916	898, 412, 394, 864, 880, 592, 574, 446, 428, 430
32	solanidenetriol solatriose	9.2	sol	SP	916	898, 880, 574, 736, 412, 754, 430, 592, 394
33	solanidenetriol solatriose	10.1	sol	SZ	916	898, 430, 868, 412, 592, 382, 754, 574, 394, 556
34	leptine II isomer	8.3	sol	SB	926	866, 848, 908, 378, 778, 396
35	leptine II	8.8	sol	SB	926	866, 720, 396, 704, 378, 844, 824, 840
36	leptine II isomer	9.0	sol	SB	926	866, 704, 378, 720, 674, 824, 396
37	dehydrodemissine	9.7	lyc	SZ	1016	854, 560, 884, 380, 398
38	solanidatrienol lycotetraose	9.4	lyc	SP	1028	866, 734, 572, 410, 392
39	solanidadienol lycotetraose	8.7	lyc	SP	1030	412, 898, 394, 574, 868, 1028, 736, 556, 376, 271, 253, 1012
40	solanidadienol lycotetraose	9.0	lyc	SP	1030	412, 898, 574, 1028, 868, 736, 1012
41	solanidene tetraose	9.6	U1	SS, SZ	1030	884, 398, 868, 706, 1028, 560
42	solanidenol lycotetraose	8.9	lyc	SP	1032	414, 900, 396, 576, 1014, 546, 720, 412, 738, 558
43	solanidenol lycotetraose	9.2	lyc	SP	1032	414, 396, 576, 900, 1014, 435, 801, 942, 558
44	hydroxydemissine	8.5	lyc	SP	1034	1016, 1032, 414, 902, 576, 416, 396
45	didehydrocommersonine	9.0	com	SZ	1044	882, 558, 720, 396
46	solanidadienediol lycotetraose	9.1	lyc	SP	1046	914, 1028, 428, 590, 410, 884, 752, 554, 734, 390
47	dehydrocommersonine	9.4	com	SZ	1046	884, 560, 380, 398, 722
48	solanidenediol lycotetraose	8.1	lyc	SP	1048	430, 592, 1030, 916, 412, 394, 574, 1016, 724, 898
49	solanidenediol lycotetraose	8.3	lyc	SP	1048	1030, 412, 916, 574, 430, 592, 394, 898, 724, 736
50	solanidenediol lycotetraose	9.4	lyc	SP	1048	1030, 916, 1012, 592, 430, 412, 574, 886, 898, 754
51	dihydroxydemissine	8.5	lyc	SP	1050	1032, 414, 432, 576, 918, 396, 540, 594, 900, 1014
52	solanideneol commertetraose	8.4	com	SZ	1062	1044, 1060, 396, 882, 412, 414, 394, 900, 559, 576
53	solanidenol commertetraose	8.8	com	SP, SZ	1062	414, 576, 396, 900, 1044, 412, 394, 1030
54	solanidenol commertetraose	9.0	com	SP, SZ	1062	414, 1030, 576, 900, 396, 938, 896, 1050, 1044
55	dihydroxycommersonine	8.4	com	SP	1080	1062, 414, 432, 576, 594, 396, 918, 255, 273
56	dihydroxydemissine pentaose	8.1	U2	SP	1212	432, 1050, 594, 1048, 1210, 1192, 592, 1202, 1030, 574, 414, 412, 1080

[a] Glycoalkaloids are listed in order of increasing mass. MS^2 product ions are listed in order of abundance. Sugars: cha, chacotriose; sol, solatriose; com, commertetraose; lyc, lycotetraose; U1, a rha-hex-hex-hex; U2, a pentaose. Occurrence lists the genotypes in which the glycoalkaloid was detected, where SB is *S. bulbocastanum*, SP is *S. pinnatisectum*, SS is *S. stenotomum*, SZ is *S. spegazzinni*, RB is 'R. Burbank', SH is 'Shepody', and RR is 'Ranger Russet'.

glycoalkaloids, would not necessarily be expected from a tetraene aglycone, unless none of the double bonds were in the A−D rings. Thus, it is possible this is a novel aglycone ring structure as opposed to a known ring structure with decreased saturation.

Additional hydroxylated solanidines were found in *S. pinnatisectum*. One (*m/z* 1028) eluted at 9.4 min and yielded product ions that indicated the presence of a lycotetraose moiety and *m/z* 410 aglycone (**Table 1**). MS^3 fragmentation of an *m/z* 572 product ion $(M + H)^+$ included *m/z* 410 $(M + H - glu)^+$ and 392 $(M + H - H_2O - glu)^+$ product ions, which would be consistent with the compound being solanidatrienol lycotetraose. However, unlike the other hydroxy-solanidanes, this glycoalkaloid was unique in that it did not as readily lose a water molecule during ionization, suggesting the oxygen modification of this aglycone may occur as an aldehyde instead of a hydroxyl group. Also found only in *S. pinnatisectum* was an *m/z* 1030 glycoalkaloid eluting at 8.7 min identified as solanidadienol lycotetraose. MS^2 analysis of the *m/z* 574 product ion (1030→574) showed *m/z* 253 and 271 as the two most abundant ions, with *m/z* 556 $(M + H - xyl - glu - glu - H_2O)^+$ and 376 $(M + H - lycotetraose - H_2O - H_2O)^+$ ions

also detected. A second, less intense *m/z* 1030 ion eluted slightly later at 9.0 min in *S. pinnatisectum* with very similar fragmentation, suggesting it was an isomer of the earlier *m/z* 1030 glycoalkaloid.

Four genotypes had a glycoalkaloid eluting at 8.1 min with a molecular ion of *m/z* 882 $(M + H)^+$ that consisted of solatriose plus an aglycone with *m/z* 412. Traces of this may have also been present in 'Russet Norkotah' and 'Shepody'. An *m/z* 850 ion resulting from a loss of 32 amu was indicative of a methanol neutral loss. This *m/z* 850 ion was consistently one of the most abundant ions in repeated runs of these samples. A solanidadienol with a hydroxy group attached to a methyl group at the C-18, 19 or 21 positions would give an *m/z* 412 aglycone and could allow losses of either water or methanol to produce *m/z* 864 or 850 product ions.

A novel glycoalkaloid of *m/z* 896 was only found in *S. bulbocastanum*, eluted at 8.1 min, and was composed of solatriose and an *m/z* 426 aglycone. MS^2 analysis showed distinctive product ions at *m/z* 750 $(M + H - rha)^+$ and 588 $(M + H - rha - glu)^+$ and corresponding ions with loss of 32 amu at *m/z* 718 $(M + H - MeOH - rha)^+$ and 556 $(M + H - MeOH - rha - glu)^+$ suggesting the loss of a methanol. A glycoalkaloid with a methanol group could show a loss of 18 amu due to loss of water. However, no product ions attributable to loss of water, such as an *m/z* 878 $(M + H - H_2O)^+$ or 732 $(M + H - H_2O - rha)^+$ were found and the relatively abundant *m/z* 394 ion suggested the loss of 32 amu could be due to a neutral loss of a methoxy group, in which case water loss would not be observed, unlike with a methanol group. Thus, the *m/z* 896 glycoalkaloid was tentatively identified as a methoxysolanidadiene solatriose.

Three glycoalkaloids of *m/z* 1062 composed of commertetraose and an *m/z* 414 aglyone were detected that appeared to be solanidenol commertetraose isomers. Each of these glycoalkaloids showed product ions resulting from loss of two hydrogens, which was characteristic of relatively few glycoalkaloids in this study. The two glycoalkaloids of *m/z* 1062 eluting at 8.8 and 9.0 min had an *m/z* 1030 product ion that suggested the loss of methanol $(M + H - MeOH)^+$ (**Table 1**).

An *m/z* 1032 peak was detected at 8.9 min in *S. pinnatisectum* composed of lycotetraose and an aglycone of *m/z* 414 that was also the base peak, plus product ions of *m/z* 720 $(M + H - H_2O - xyl - glu)^+$ and 1014 $(M + H - H_2O)^+$ suggesting the presence of a hydroxy group. It may be a solanidenol lycotetraose; however, this aglycone (solanidenol) with a lycotetraose would be expected to elute earlier than the same aglycone with solatriose or chacotriose, which it does not. A spirosolane of *m/z* 414 could also show this type of fragmentation, but would not be expected to elute so early. One possible explanation is that the position of the hydroxylation can substantially influence a glycoalkaloid's retention characteristics. Another peak of *m/z* 1032 eluted at 9.2 and had an almost identical fragmentation pattern. A glycoalkaloid of *m/z* 1034 eluting at 8.5 min in *S. pinnatisectum* was composed of lycotetraose and an *m/z* 416 aglycone and the product ions were consistent with that predicted from hydroxydemissine. This compound was unusual in its tendency to lose two hydrogens, as seen in the product ions of *m/z* 1032, 414, and 396.

Solanidane Aglycones with Three or More Oxygens. A glycoalkaloid with *m/z* 884 found only in *S. bulbocastanum*, eluted at 8.4 min and was composed of chacotriose with an *m/z* 430 aglycone. Among the more abundant product ions were *m/z* 866 $(M + H - H_2O)^+$, 848 $(M + H - H_2O - H_2O)^+$, 702 $(M + H - H_2O - H_2O - rha)^+$, and 412 $(M + H -$ chacotriose $- H_2O)^+$ that suggested the glycoalkaloid was a solanidenediol chacotriose.

Leptines or leptine-like compounds were present in *S. bulbocastanum*, including two (acetyl-leptidine-rha-rha-glu or leptine I) glycoalkaloids of *m/z* 910 eluting at 8.5 and 8.8 min. These compounds were composed of chacotriose and an *m/z* 456 aglycone. MS^2 analysis showed a base peak of *m/z* 850 $(M + H -$ acetoxy$)^+$ derived from a neutral loss of the acetoxy group. Similarly, an *m/z* 396 ion resulted from loss of the acetoxy group from the aglycone. The glycoalkaloid eluting at 8.8 was identified as leptine I based on use of leaf extracts from the reference plant *S. chacoense*. Three similar compounds of *m/z* 926 with solatriose instead of chacotriose (acetyl-leptidine-rha-glu-gal or leptine II) and an aglycone with *m/z* 456, which eluted at 8.3, 8.8, and 9.0 min were also detected only in *S. bulbocastanum*. No similar aglycones with lycotetraose or commertetraose moieties were detected.

S. bulbocastanum had a compound with *m/z* 900 eluting at 7.8 min consisting of chacotriose and an *m/z* 446 aglcyone that appeared to be a solanidenetriol chacotriose. Product ions of *m/z* 882 $(M + H - H_2O)^+$, 864 $(M + H - H_2O - H_2O)^+$, and 846 $(M + H - H_2O - H_2O - H_2O)^+$ corresponded to sequential loss of the hydroxy groups. The early elution is also consistent with what would be expected from a glycoalkaloid with multiple hydroxy groups. Another compound with *m/z* 900 was detected at 8.3 min in all of the wild species except *S. pinnatisectum* and tentatively identified as a solanid-en-diol (**Table 1**).

Four glycoalkaloids with *m/z* 916 were detected, eluting at 7.7 and 8.2 min in *S. bulbocastanum*, 9.2 min in *S. pinnatisectum*, and 10.1 min in *S. spegazzinii*. These all contained solatriose and an *m/z* 446 aglycone and had similar MS^2 product ions. Product ions of *m/z* 898 and 880 indicated progressive loss of water molecules. For the most part, the fragmentation was consistent with that expected of a solanidenetriol solatriose. Any solanidane triol would be expected to show ions corresponding to loss of each of the three hydroxy groups. However, these four *m/z* 916 glycoalkaloids consistently had some product ions that were 2 amu larger than expected. For example *m/z* 592 instead of *m/z* 590 $(M+ H - rha - glu - H_2O)^+$, *m/z* 574 instead of 572 $(M + H - rha - glu - H_2O - H_2O)^+$ or *m/z* 394 instead of 392 $(M + H - rha - glu - gal - H_2O - H_2O - H_2O)^+$, which clearly showed the loss of 16 amu instead of one of the expected 18 losses. Loss of an *m/z* 16 ion is relatively rare and typically occurs due to loss of an amide nitrogen (which can easily be excluded in this instance) or from an N−O bond. The compounds with *m/z* 916 clearly have one nitrogen and this nitrogen could not be so modified in a solanidane. Thus, these four glycoalkaloids may contain a novel aglycone with an N−O bond. Alternatively, the presence and position of 3 hydroxy groups may result in a rearrangement during fragmentation that results in one of the oxygens being lost as an *m/z* 16 ion.

Whereas *S. bulbocastanum* was distinctive among these seven genotypes in the number of hydroyxsolanidines it contained, *S. pinnatisectum* was unique in the numerous glycoalkaloids it contained with lycotetraose or commertetraose sugars. Three glycoalkaloids of *m/z* 1048 with an *m/z* 430 aglycone were present in *S. pinnatisectum*, eluting at 8.1, 8.3, and 9.4 min. MS^2 analysis showed these glycoalkaloids contained lycotetraose with a solanidenediol aglycone (**Figure 5**).

Also in *S. pinnatisectum* was a novel *m/z* 1050 glycoalkaloid eluting at 8.5 min, composed of lycotetraose and an *m/z* 432

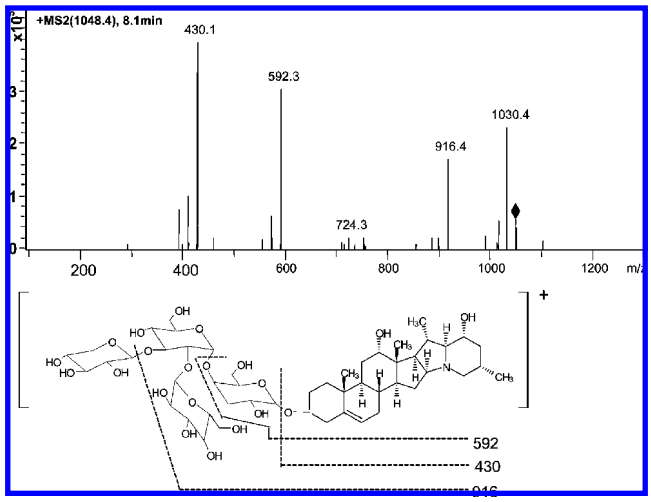


Figure 5. MS^2 analysis of solanidenediol lycotetraose. *Y*-axis represents ion intensity. Product ions resulting from cleavage of the lycotetraose moiety are shown.

aglycone. This compound was likely dihydroxydemissine (solanidediol lycotetraose) and represented one of the relatively few demissidine aglycones detected in these seven genotypes, as the large majority of solanidane glycoalkaloids were desaturated. A tomatidine-ol could give similar product ions, but would not be expected to elute this early, over 2 min earlier than tomatidine. Additionally, there were later eluting glycoalkaloids of *m*/*z* 1050 detected that were likely hydroxyspirosolanes.

A glycoalkaloid of *m*/*z* 1046 eluted at 9.1 min in *S. pinnatisectum* that contained lycotetraose and an *m*/*z* 428 aglycone that showed loss of three water molecules and was tentatively identified as a solanida-diene-diol-lycotetraose. A glycoalkaloid of *m*/*z* 1080 with a commertetraose side chain eluted at 8.4 min in *S. pinnatisectum* extracts with an aglycone of *m*/*z* 432. Product ions representing each of three water losses were detected, suggesting the compound is a commersonine-diol. Another novel *S. pinnatisectum* glycoalkaloid of *m*/*z* 1212 eluted at 8.1 min with an aglycone of *m*/*z* 432 and a novel carbohydrate side chain with *m*/*z* 780 that contained xylose and 4 hexoses. MS^3 analysis of the *m*/*z* 432 ion was consistent with that of dihydroxydemissine.

Glycoalkaloid Analysis. Numerous points are important in interpreting these glycoalkaloid data. As mentioned previously, these glycoalkaloid assignments are tentative identifications due to the unavailability of standards and the many possible glycoalkaloid isomers that would have similar mass spectra. Confidence is high that these are indeed glycoalkaloids, including because of the characteristic conjugated sugars, the presence of nitrogen in the aglycone, aglycone product ions typical of glycoalkaloids, the proportions of M + 1 and M + 2 peaks and the time range in which all eluted.

Many instances were observed of glycoalkaloids of the same mass and similar product ions eluting at different times. Explanations for this may include attachment of the functional groups to different carbons, altered placement of double bonds, and stereoisomers. Carbohydrate moieties with the appropriate masses were assumed to be chacotriose, solatriose, lycotetraose or commertetratose, but the possibility that isomers of these occur cannot be excluded. Solasodine and tomatidenol, and tomatidine and soladulcidine are examples of glycoalkaloid isomers that elute separately and have similar product ions. Furthermore, another tomatine isomer had highly similar fragmentation and was thought to be a C-22 stereoisomer (*30*). Additional glycoalkaloids with similar mass spectra and thought to be isomers have been reported (*34*). Finally, some of the glycoalkaloids with the same mass, similar product ions but different retention times are only found in some genotypes. For example, the glycoalkaloid with *m*/*z* 916 eluting at 8.2 min is found only in S. bulbocastanum and has similar product ions to the *m*/*z* 916 peak eluting at 10.1 min only in *S. spegazzinii*. Thus, it is probable that such compounds are indeed unique compounds as opposed to the same compound eluting in multiple peaks.

For the tentatively identified glycoalkaloids, confidence is high that the correct mass of the sugar and aglycone was identified. Another assumption used for identification was that if a glycoalkaloid did not preferentially lose water, then it was unlikely to be a spirosolane and this, along with retention time, was used to help distinguish between solanidanes and spirosolanes. In tentatively identifying a glycoalkaloid, the greatest difficulty was in distinguishing between a solanidane with two or more oxygen in the aglycone and a spirosolane with three or more oxygen. This was because the earlier elution time of the solanidanes was the most useful characteristic in distinguishing them from the spirosolanes, but hydroxylated spirosolanes will elute earlier and have the potential to overlap with some hydroxysolanidanes.

Glycoalkaloid Diversity in Potato. Collectively, this data shows that tuber glycoalkaloid content is diverse and included numerous previously unidentified glycoalkaloids in just the few wild species we profiled. Furthermore, this study did not examine spirosolane glycoalkaloids, which our preliminary results suggest are present in equal numbers to the solanidanes. In addition to the glycoalkaloids mentioned, numerous additional glycoalkaloid-like compounds were detected but were too low in abundance to give reliable data. **Figure 4** shows a comparison of the occurrence and peak intensity of the glycoalkaloids in the four wild species and 'Russet Burbank'. This was not a quantitative study, but the peak intensity of each glycoalkaloid is shown for comparative purposes. No glycoalkaloids with peak intensity below 1×10 (*6*) were included in this study.

When the LC-MS total ion current of an entire extract is used to calculate the top ten ions detected using positive mode, clear differences are evident among the genotypes (Table S2, Supporting Information). For example, the *S. pinnatisectum* profile instantly suggested this genotype had numerous glycoalkaloids with four-sugar sidechains and had abundant spirosolanes. The individual glycoalkaloid profiles of these genotypes were distinctive, with the cultivars relatively similar to each other, but each of the wild species clearly differentiated.

Very few glycoalkaloids were detected in all seven genotypes (Figure S1, Supporting Information). A majority of the glycoalkaloids were detected only in one genotype. Of the solanidane or solanidane-like glycoalkaloids detected, *S. bulbocastanum* contained the largest number of glycoalkaloids (Figure S1, Supporting Information). The cultivars had the fewest, which is not unexpected given that glycoalkaloids have been selected against during the domestication of potato and that modern cultivars incorporate only a fraction of the genetic diversity available. Chacotriose was the most common carbohydrate component of these glycoalkaloids and commertetraose the least (Figure S1, Supporting Information). *S. pinnatisectum* contained the most glycoalkaloids with four-sugar carbohydrates side chains, whereas no such sugars were detected in the cultivars, *S. bulbocastanum* or *S. stenotomum*.

The ability to monitor large numbers of potato glycoalkaloids increases the possibilities for assessing the role of glycoalkaloids in pest and pathogen resistance, and also in human health.

Friedman et al. (*39*) have suggested that replacing solanine and chaconine in potatoes with less toxic glycoalkaloids such as tomatine might be beneficial. More research is needed to determine whether dietary glycoalkaloid intake can provide health benefits. The ideal glycoalkaloid concentration in potato might be one that is low enough to avoid negative effects, but not so low as to lose any potential positive health benefits or reduce glycoalkaloid contribution to potato disease and pest resistance.

The amount of glycoalkaloid diversity in only seven genotypes is unexpected, and all the more so given only tubers were examined. Tubers are well-known to have markedly lower glycoalkaloid concentrations than leaves, fruits or sprouts. Some of the wild species we used have been examined for glycoalkaloids before, but only a fraction of the glycoalkaloids described herein were found. This probably reflects the much greater selectivity and sensitivity of MS relative to methods typically used in older literature. Additionally, unlike most other older glycoalkaloid analytical methods, we did not utilize any precipitation, precolumn cleanup or other purification steps that decrease glycoalkaloid recovery. Our preliminary results indicate that these genotypes have a roughly equal number of spirosolanes. Given there are around 200 potato wild species, it is likely that potato contains many more glycoalkaloids than previously realized.

Dissecting the role of these many glycoalkaloids in pest and pathogen resistance, or in human health will be a formidable undertaking. Until such questions can be better answered, it will not be possible to determine what would constitute an ideal glycoalkaloid profile in a potato. Lacking such guidance, potato development programs will likely continue to focus on keeping the total glycoalkaloid concentrations below the 20 mg/100 g FW guidelines.

Supporting Information Available: Table S1 containing glycoalkaloid MS^3 data, Table S2 showing the 10 most intense ions in each genotype, and Figure S1 showing the distribution of glycoalkaloids among the genotypes, the number of glycoalkaloids detected in each genotype, and the distribution of sugars. This material is available free of charge via the Internet at http://pubs.acs.org.

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