‘*Candidatus Liberibacter solanacearum*’ Titers in and Infection Effects on Potato Tuber Chemistry of Promising Germplasm Exhibiting Tolerance to Zebra Chip Disease

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ABSTRACT

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Long-term sustainable management of zebra chip (ZC) disease of potato requires development of tolerant or resistant germplasm. To this end, 283 potato varieties and breeding clones were infected with the ZC putative causal agent ‘*Candidatus Liberibacter solanacearum*’ (Lso) by potato psyllid vector inoculations in 2010, 2011, 2012, and 2013. Potato germplasm was then examined for development of fresh and fried ZC symptoms. Over multiple years 29 breeding clones exhibited little to no symptoms in freshly cut tuber slices, and five exhibited little to no symptoms in fried slices. These five presumed tolerant breeding clones were chosen for further screening to determine whether the lack of physiological responses to Lso infection was the cause of observed tolerance.

To this end, tuber amino acid, sugar, and phenolic levels were compared between noninfected and Lso-infected plants. The five putative tolerant clones had less dramatic shifts in host physiology following Lso infection than the susceptible Atlantic cultivar. This suggested lack of host responses to Lso infection that result in major changes in tuber biochemistry is a potential mechanism of ZC resistance. However, the susceptible Atlantic cultivar did have consistently greater Lso titers compared with two of the tolerant entries, so for these reductions in Lso pathogen progression also might be a factor. Regardless, lack of host responses could still remain one trait that could be used to aid in selection of ZC-resistant potato varieties, as other tolerant lines had infection levels consistent with susceptible Atlantic cultivar. These results also suggest that germplasm derived from relatives of cultivated potato plants are viable sources of ZC disease resistance.

Additional keywords: carbohydrates, phenolics, plant defense responses, terpenoids, tomato–potato psyllid.

Zebra chip (ZC) disease, putatively caused by ‘*Candidatus Liberibacter solanacearum*’ (Lso) (syn. ‘*Candidatus Liberibacter solanacearum*’), is a serious threat to potato (*Solanum tuberosum* L.) production in Central America, Western North America, and New Zealand (Crosslin et al. 2012a, 2012b; Liefting et al. 2008; Munyaneza 2012; Munyaneza et al. 2007; Secor and Rivera-Varas 2004; Wen et al. 2009). Potato psyllids (sometimes called tomato–potato psyllids), *Bactericera cockerelli* (Sulc.) (Hemiptera: Trioizidae), vector Lso to potato and other solanaceous crops and are the primary source of ZC spread. As a result, current ZC management involves costly (~\$700/ha) chemical control programs for potato psyllids (Guenther et al. 2012, Greenway 2014). The continued use of insecticides for psyllid control could result in resistant insect populations that would render chemical-based control ineffective (Prager et al. 2013). Likewise, organic and other nonconventional growers would not be allowed to utilize pesticides. Therefore, long-term sustainable management for ZC disease requires exploiting natural host resistance to develop ZC-resistant or Lso infection-tolerant varieties (Munyaneza et al. 2011; Munyaneza 2012). These Lso-tolerant varieties could be potentially coupled with traits for

psyllid resistance to provide comprehensive protection from ZC (Butler et al. 2011; Cooper and Bamberg 2014; Diaz-Montano et al. 2014; Levy and Tamborindeguy 2014).

One of the biggest concerns with potatoes infected by ZC is symptoms that reduce the quality and overall marketability of fresh-market and processing tubers. Symptoms characteristic of ZC include increased oxidation (browning) and brown specking around the vascular tissues of freshly cut tubers and brown striping (‘zebra stripes’) when tuber slices are fried to make potato chips/crisps (Munyaneza 2012; Secor et al. 2009; Wen et al. 2009). In addition to symptoms in tubers, ZC causes above-ground portions of potato plants to undergo dwarfing, leaf scorch, chlorosis, and collapse (Munyaneza 2012; Secor et al. 2009; Wen et al. 2009).

Also characteristic of ZC is substantial changes in tuber physiology, with tubers from Lso-infected plants having greater levels of phenolic compounds (Alvarado et al. 2012; Navarre et al. 2009; Rashed et al. 2013; Wallis et al. 2012, 2014), reducing sugars (glucose and fructose) (Buchman et al. 2011a, 2012; Rashed et al. 2013; Wallis et al. 2014), defense-associated enzymes (Alvarado et al. 2012, Rashed et al. 2013, Wallis et al. 2012), amino acids (e.g., isoleucine, leucine, serine, tyrosine, and valine) (Navarre et al. 2009; Rashed et al. 2013, Wallis et al. 2012, 2014), and minerals (e.g., phosphorus, potassium, and calcium) (Miles et al. 2009) compared with noninfected tubers. Furthermore, changes in foliar and stem levels of amino acids, sugars, phenolics, and terpenoids may occur when potato hosts are infected with Lso, with potential implications on the expression of ZC symptoms (Wallis et al. 2015).

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Many of these increases in compound levels could be part of a coordinated defense response to Lso infection (Miles et al. 2010), especially increased phenolic compound levels, which are relatively consistent occurrences in potato tissues following various pathogen infections (Henriquez et al. 2012; Ngadze et al. 2012). It remains unlikely that these defenses are effective at halting Lso growth within susceptible potato hosts because otherwise ZC would not be a concern. However, some evidence does exist that Lso could be halted somewhat as lower titers might be observed where shifts in host physiology are greatest (Rashed et al. 2013; Wallis et al. 2012, 2014).

It is hypothesized that Lso itself likely does not cause many direct negative effects on its potato hosts, but rather host responses to bacterial presence result in ZC symptom formation (Rashed et al. 2013; Wallis et al. 2012, 2014). To be specific, greater phenolic (especially chlorogenic acid and its isomers) and polyphenol oxidase levels result in increased oxidative browning to occur upon exposure to air, as observed in freshly cut susceptible potato tubers infected with Lso (Mayer 2006). Greater levels of sugars and amino acids would provide more substrates for the Maillard reaction when potato slices are fried, with the result being increased formation of the brown-colored acrylamide (Friedman and Levin 2008; Stadler et al. 2002).

This study summarizes ongoing work to discover Lso infection-tolerant potato varieties. This study proceeded further by examining whether or not lack of host physiology shifts were responsible for putative Lso tolerance. This was done by comparing noninfected and Lso-infected tuber levels of amino acids, sugars, and phenolics of tolerant varieties with the same comparisons done for the susceptible Atlantic variety as a reference. If the lack of a host physiological response is consistent in putatively tolerant varieties, then this trait could be selected for in breeding programs aimed at developing germplasm that is tolerant of Lso infection. Such Lso-tolerant varieties will prove invaluable at countering the threat ZC poses to potato growers in areas where it is endemic.

MATERIALS AND METHODS

Germplasm screening. Over a 4-year period (2010 to 2013), 283 potato varieties and advanced breeding lines were planted in the field at the USDA-ARS research farm at Moxee, WA. These potatoes were contained in 1.83 m by 4.57 m cages, with each cage enclosing six to eight plants each, as described by Munyaneza et al. (2007) and Buchman et al. (2011a, b). Plants were inoculated with Lso by being exposed to Lso-positive potato psyllid adults (three insects/plant) at tuber initiation stage for 7 days, after which time the insects were eliminated through the use of pesticides and the plants were allowed to develop until harvest (Buchman et al. 2011a, b; Munyaneza et al. 2007). Upon harvest, the tubers were assessed for both fresh and fried ZC symptoms, as well as Lso infection confirmed by polymerase chain reaction (PCR) (Buchman et al. 2011a, b; Munyaneza et al. 2007).

Based on findings from the first 2 years (2010 and 2011), a total of five promising breeding clones were selected for further evaluation in 2012 and 2013. The promising clones were: ARS1 (also known as 00-3115-2) and ARS2 (also known as 00-3115-11 or M5 [Jansky et al. 2011]) from the USDA-ARS, Madison, WI and Aberdeen, ID; BS2 (also known as T47022) from Bejo Seeds, Inc., Oceano, CA; and ZC73 and ZC74 from Frito-Lay, Inc., Rhinelander, WI. The ARS breeding clones were bred for resistance to cold-induced sweetening and have been characterized as having 25% of pedigree derived from wild potato species including *Solanum raphanifolium*, *S. tarijense*, and *S. chacoense* (Jansky et al. 2011). Each of these potato clones had six plants established in each of six cages. The susceptible cultivar Atlantic was established similarly for comparison. The promising clones and the susceptible Atlantic cultivar had half of the cages exposed to Lso-infective psyllids at tuber initiation stage, and the remaining cages were left as controls. At harvest

tubers were collected, ZC fresh and fried symptoms were assessed, and Lso infection confirmed by PCR as described by Munyaneza et al. (2007) and Buchman et al. (2011a, b). Subsets of noninfected and Lso-infected tubers for each entry were then shipped to USDA-ARS, Parlier, CA, for evaluation of tuber physiology as described below.

Biochemical analyses. For 2012, a total of nine Lso-infected and three noninfected tubers for each of the selected putatively tolerant breeding clones and susceptible Atlantic tubers were analyzed further, and for 2013 a total of five noninfected and five Lso-infected tubers were analyzed. Upon arrival to Parlier, CA, both noninfected and Lso-infected tubers from both 2012 and 2013 were sliced at the stolon end and examined for fresh ZC symptoms on a 0 to 3 scale (Rashed et al. 2013). Afterward, two additional small slices were made from each tuber. One slice per tuber was placed in a deep fryer containing corn oil for 3 min at 340°F (171°C), at which time the potato “chip” was removed and evaluated for fried ZC symptoms on a 0 (normal) to 3 (severe) scale. The other slice per tuber had the skin removed and was mashed using a mortar and pestle. The mashed tissue was then used to make two 0.10-g aliquots in labeled 1.5-ml microcentrifuge tubes, with the remaining mashed tissue saved for Lso titer analyses. These samples then were placed at –20°C until extractions were performed.

Lso titers were determined using DNA extracted using the DNeasy Plant mini kit (Qiagen, Valencia, CA) and the methods of Wallis et al. (2012) on both Lso-inoculated and noninoculated tubers. In brief, quantitative PCR to determine Lso titers involved 2 µl of extracted tissue DNA template, 12.5 µl of 2× iQ SYBR Green supermix (Bio-Rad, Hercules, CA), 0.5 µl of both 10 µM OA2 and 10 µM OI2c primers (Liefting et al. 2008), and 9.5 µl of water for a total reaction solution volume of 25 µl (Wallis et al. 2012). Ct values were calculated by the DNA Engine Opticon 2 Real-Time PCR Detection System (Bio-Rad) that was used to monitor the reactions, with control Lso template used to convert Ct values to nanogram template (Wallis et al. 2012).

To calculate amino acid, sugar, and phenolic levels, the methods of Rashed et al. (2013) and Wallis et al. (2008, 2012, 2014) were employed.

Briefly, one of the 0.10-g aliquots from each sample was twice-extracted overnight at 4°C in 0.5 ml of phosphate buffered saline (PBS) solution (pH 7.8) for use in amino acid and sugar analyses, for a total of 1 ml of extract. For amino acid analyses, 100 µl of this PBS buffer extract was used with the commercially available kit from Phenomenex (Torrance, CA) developed for analyzing amino acids by gas chromatography. Derivatized amino acid extracts from the kit were analyzed with a Shimadzu (Columbia, MD) GC-2010 gas chromatograph with a flame ionization detector and the kit-provided Zebron AAA column. Amino acid identifications and quantification were made using standard preparations from the Phenomenex amino acid kit and the Shimadzu Lab Solutions software.

Sugar analysis involved injecting 50 µl of the PBS buffer extract into a Shimadzu high-performance liquid chromatograph (HPLC) equipped with 300 mm by 7.8 mm Supelco C-611 carbohydrate column (Sigma-Aldrich, St. Louis, MO) and a refractive index detector (RID-10 from Shimadzu) (Rashed et al. 2013). Commercial standards of fructose and glucose were obtained from Sigma and used to identify peaks and convert peak areas to gram amounts.

To analyze phenolics, the remaining 0.10 g tuber tissue aliquot was twice-extracted overnight at 4°C in 0.5 ml of methanol (Sigma) each time, for a total of 1 ml of extract. A total of 50 µl of methanol extract per sample was injected into a Shimadzu HPLC system equipped with an XR-ODS C18 column (Shimadzu) and a Shimadzu photodiode array detector (set at 280 nm) to quantify phenolic compounds (Wallis et al. 2008, 2012). The binary solvent gradient used in the HPLC proceeded from 95% solvent A (water with 0.2%, vol/vol, acetic acid [Sigma]) to 100% solvent B (methanol with 0.2%, vol/vol, acetic acid) and back to reset for the following

injection over 40 min (Wallis et al. 2012). The majority of tuber phenolics were identified by Wallis et al. (2012) by matching retention times with commercial standards (from Sigma), matching UV/Vis spectra of peaks, or determining molecular weights by running representative samples on a Shimadzu LCMS-2020 liquid chromatograph-mass spectrometer. Area units of quantified peaks were converted to gram amounts by using standard curves to develop conversion factors for compounds within the same phenolic class and the same UV/Vis spectral profile (Rashed et al. 2013).

Statistical analyses. IBM-SPSS software version 22.0 (IBM, Armonk, NY) was used in all analyses with $\alpha = 0.05$. Normality was tested with the SPSS EXPLORE feature by examining residual plots. *t* tests were used to determine differences in symptoms and Lso titers between noninfected and infected tubers for each entry. Multivariate analyses of variance (MANOVA) of amino acids, sugars, or phenolics was utilized to determine significant differences between noninfected and infected plants for each of the tested entries for both years as well as each year separately. If the MANOVA Pillai's trace statistic was significant, differences in individual compounds were examined using follow-up analyses of variance (ANOVAs). ANOVAs were performed for all individual

compounds using data from both years (with year, year by infection status, year by entry, and the three-way interaction added to model with infection status, entry, and their interaction) as well as each year individually due to significant effects on compound levels due to year for almost every compound. ANOVAs followed by Tukey's honest significant difference (HSD) tests were performed for each individual compound using only Entry as an independent variable for each other treatment combination (noninfected or infected tubers for each year or both years). This allowed observations to be made regarding differences in chemistry between entries that might affect eventually Lso infection progression, as well as differences that developed when tubers were already infected with Lso.

In order to examine whether fold differences varied between entries, compound levels from five Lso-inoculated tubers were divided by the same compound levels from five noninoculated tubers for either year. The result was fold differences, with values greater than one representing increased levels in Lso-inoculated tubers, and levels less than one representing greater levels in noninoculated tubers. These values were subsequently analyzed by MANOVA with entry as the factor for each year separately for amino acids, sugars, or phenolics. Follow-up ANOVAs on individual compounds were performed, with Tukey's HSD tests utilized to determine pairwise differences between entries. In addition, a mean fold change for all amino acids or phenolics was calculated, and these values were

Fig. 1. Examples of fried tuber slices from noninfected and '*Candidatus Liberibacter solanacearum*' (Lso)-infected tubers of susceptible (Atlantic) and tolerant entries.

Fig. 2. Mean fried symptom ratings for tubers from each entry for both years, 2012, and 2013. Bars represent standard errors. Different letters represent means that differ significantly by Tukey's honest significant difference test.

Fig. 3. Mean '*Candidatus Liberibacter solanacearum*' titers from each entry for both years, 2012, and 2013. Bars represent standard errors. Different letters represent means that differ significantly by Tukey's honest significant difference test. * indicates that for 2012, the values represented on the graph are multiplied by 10 to allow for better observation.

analyzed by ANOVA followed by Tukey's HSD tests with entry as the factor to further compare and visualize fold differences.

Lastly, to analyze relationships between symptoms, Lso titers, and chemicals, Spearman's correlations were performed using only inoculated tubers and data for both years.

RESULTS

Field screening of potato cultivars for ZC resistance.

During the 4 years of the field screening trial, all the Atlantic control plants in the cages exposed to Lso-infective psyllids produced tubers with very severe fresh and fried ZC symptoms indicating that inoculation during each trial was successful. Furthermore, above ground plant symptoms in Atlantic were observed in all cages and plants generally died soon after ZC symptom development. Plant symptoms included leaf rolling of upper leaves, basal cupping of leaflets, foliar chlorosis, proliferation of axillary buds, shortened internodes, swollen nodes, and leaf scorching. Tuber symptoms from trial entries following Lso infection included collapsed stolons, brown discoloration of the vascular ring, necrotic flecking of internal tissues, and streaking of the medullary ray tissues. A total of 254 out of 283 potato entries had severe ZC tuber symptoms. The remaining 29 entries had ZC symptoms in fresh tubers ranging from light to almost none and this was consistent over multiple years. Lso

infection was confirmed in tubers collected from inoculated plants of all 283 lines and the Atlantic controls. Tuber samples of the 29 entries with no symptoms to light symptoms were processed into chips and fried as described by Munyaneza et al. (2007). Of the 29 with no symptoms to light fresh ZC symptoms, five breeding lines (ARS1, ARS2, BS2, ZC73, and ZC74) showed negligible to no ZC symptoms in fried potato slices. Therefore, tubers for each of the five promising clones, in addition to Atlantic for comparison, were sent to USDA-ARS Parlier in 2012 and 2013 for further symptom assessment and biochemical analyses.

Confirmation of fresh and fried tuber symptoms from susceptible and tolerant entries. Fresh tuber symptoms of putative tolerant clones were significantly less than Atlantic for both years (mean rating of 0.0 ± 0.0 SE for all tolerant clones except ARS1, which was 0.07 ± 0.07 , versus 1.43 ± 0.17 for Atlantic; $F = 48.992$; $P < 0.001$; $n = 84$ for entries; $F = 0.150$; $P = 0.700$; $n = 84$ for year; and $F = 0.282$; $P = 0.921$; $n = 84$ for the entry and year interaction), 2012 (mean rating of 0.0 ± 0.0 SE for all tolerant clones versus 1.44 ± 0.24 for Atlantic; $F = 35.579$; $P < 0.001$; $n = 54$), and 2013 (mean rating of 0.0 ± 0.0 SE for all tolerant clones except ARS1, which was 0.20 ± 0.20 , versus 1.44 ± 0.24 for Atlantic; $F = 18.880$; $P < 0.001$; $n = 30$).

When both years were analyzed together, Atlantic tubers had significantly greater ($F = 19.724$; $P < 0.001$; $n = 84$ for entries; $F = 0.849$;

TABLE 1. Mean ($\pm$ SE) amino acid levels (nmol/g FW) separated by entry for both years and each year separately^a

Compound	Year	Treatment	Atlantic	ARS1	ARS2	BS2	ZC73	ZC74	F		
									Between entry	Between year	Between treatment
Alanine	Both	Control	577 $\pm$ 134 c	525 $\pm$ 100 c	759 $\pm$ 161 abc	1110 $\pm$ 160 a	616 $\pm$ 110 bc	1040 $\pm$ 190 ab	4.708**	42.580***	12.477***
		Lso+	756 $\pm$ 71 a	1160 $\pm$ 230 a	1050 $\pm$ 260 a	1420 $\pm$ 210 a	775 $\pm$ 137 a	1410 $\pm$ 180 a	2.844*	0.071	
	2012	Control	799 $\pm$ 162 c	756 $\pm$ 105 c	914 $\pm$ 69 bc	1460 $\pm$ 180 ab	746 $\pm$ 108 c	1550 $\pm$ 110 a	8.319***		0.702
		Lso+	864 $\pm$ 75 b	904 $\pm$ 143 b	872 $\pm$ 104 b	1340 $\pm$ 210 ab	1050 $\pm$ 140 ab	1610 $\pm$ 180 a	4.271**		
	2013	Control	312 $\pm$ 162	247 $\pm$ 50	574 $\pm$ 345	692 $\pm$ 114	461 $\pm$ 193	430 $\pm$ 100	0.774		9.155**
		Lso+	559 $\pm$ 104	1620 $\pm$ 570	1360 $\pm$ 740	1550 $\pm$ 500	274 $\pm$ 86	1040 $\pm$ 350	1.42		
Asparagine	Both	Control	4160 $\pm$ 1440 b	10800 $\pm$ 4190 ab	10600 $\pm$ 4700 ab	13600 $\pm$ 4900 a	15200 $\pm$ 5700 a	13000 $\pm$ 4600 a	4.661**	230.266***	4.285*
		Lso+	6600 $\pm$ 2500 ab	11200 $\pm$ 4300 a	6630 $\pm$ 3130 ab	6860 $\pm$ 2650 ab	8220 $\pm$ 3200 ab	5420 $\pm$ 2050 b	4.685***	370.733***	
	2012	Control	154 $\pm$ 27 ab	94 $\pm$ 26 ab	72 $\pm$ 15 b	194 $\pm$ 51 a	85 $\pm$ 10 ab	134 $\pm$ 24 ab	2.725*		4.039*
		Lso+	356 $\pm$ 56 a	105 $\pm$ 14 b	78 $\pm$ 11 b	190 $\pm$ 33 b	93 $\pm$ 12 b	119 $\pm$ 14 b	13.434***		
	2013	Control	8970 $\pm$ 860 b	23700 $\pm$ 4500 ab	23200 $\pm$ 6900 ab	29800 $\pm$ 3600 a	33200 $\pm$ 5300 a	28500 $\pm$ 2100 a	3.804*		2.816
		Lso+	17900 $\pm$ 2500	31100 $\pm$ 4100	18400 $\pm$ 5900	18900 $\pm$ 2800	22800 $\pm$ 3100	15000 $\pm$ 1700	2.456		
Aspartic acid	Both	Control	5370 $\pm$ 1230	5050 $\pm$ 1340	7990 $\pm$ 2470	6990 $\pm$ 1660	5340 $\pm$ 1340	7220 $\pm$ 1980	1.011	80.734***	4.305*
		Lso+	13000 $\pm$ 1300 a	5090 $\pm$ 950 b	9350 $\pm$ 2370 ab	9160 $\pm$ 2280 ab	7060 $\pm$ 2140 ab	8890 $\pm$ 1850 ab	3.194*	28.919***	
	2012	Control	8520 $\pm$ 1060	8250 $\pm$ 1450	12600 $\pm$ 3600	11400 $\pm$ 1300	8350 $\pm$ 1600	11800 $\pm$ 2300	0.926		0.753
		Lso+	12600 $\pm$ 1940	6580 $\pm$ 1200	13100 $\pm$ 3000	13200 $\pm$ 2700	10000 $\pm$ 2900	12600 $\pm$ 1900	1.2		
	2013	Control	1580 $\pm$ 260	1210 $\pm$ 80	2450 $\pm$ 430	1750 $\pm$ 250	1730 $\pm$ 210	1740 $\pm$ 180	2.41		66.133***
		Lso+	13700 $\pm$ 1300 a	2400 $\pm$ 400 b	2640 $\pm$ 640 b	1940 $\pm$ 310 b	1820 $\pm$ 350 b	2170 $\pm$ 360 b	50.125***		
Glutamic acid	Both	Control	2340 $\pm$ 310	1770 $\pm$ 420	2100 $\pm$ 570	1800 $\pm$ 210	2140 $\pm$ 390	1750 $\pm$ 260	0.453	3.51	0.502
		Lso+	4900 $\pm$ 1210	1390 $\pm$ 330	2160 $\pm$ 590	2680 $\pm$ 730	1960 $\pm$ 700	2410 $\pm$ 570	2.182	26.183***	
	2012	Control	2330 $\pm$ 240	2370 $\pm$ 700	2450 $\pm$ 1030	2130 $\pm$ 310	2320 $\pm$ 630	1920 $\pm$ 470	0.098		6.241*
		Lso+	7060 $\pm$ 1440 a	1770 $\pm$ 470 b	3080 $\pm$ 750 ab	3680 $\pm$ 990 ab	2520 $\pm$ 1060 b	3280 $\pm$ 730 ab	3.681*		
	2013	Control	2360 $\pm$ 670	1060 $\pm$ 130	1680 $\pm$ 350	1410 $\pm$ 210	1930 $\pm$ 460	1540 $\pm$ 170	1.378		23.365***
		Lso+	1030 $\pm$ 250	717 $\pm$ 142	513 $\pm$ 155	869 $\pm$ 118	946 $\pm$ 163	838 $\pm$ 272	0.894		
Glutamine	Both	Control	2980 $\pm$ 1270	2780 $\pm$ 800	5660 $\pm$ 3310	4490 $\pm$ 1420	5150 $\pm$ 2000	4270 $\pm$ 1290	0.76	40.461***	16.553***
		Lso+	8550 $\pm$ 2980	8610 $\pm$ 4140	7520 $\pm$ 4040	8570 $\pm$ 3600	3840 $\pm$ 1490	4170 $\pm$ 1580	1.881	84.066***	
	2012	Control	746 $\pm$ 14	880 $\pm$ 134	740 $\pm$ 69	868 $\pm$ 52	640 $\pm$ 22	748 $\pm$ 26	1.815		0.063
		Lso+	870 $\pm$ 109 ab	838 $\pm$ 84 ab	1000 $\pm$ 120 a	762 $\pm$ 89 ab	608 $\pm$ 60 b	617 $\pm$ 73 b	2.785*		
	2013	Control	5660 $\pm$ 2360	5050 $\pm$ 1070	11600 $\pm$ 6700	8850 $\pm$ 1560	10600 $\pm$ 2900	8500 $\pm$ 960	0.643		10.664**
		Lso+	22400 $\pm$ 2600	22600 $\pm$ 8950	19200 $\pm$ 9800	22600 $\pm$ 6600	9650 $\pm$ 2600	10600 $\pm$ 2600	0.94		
Glycine	Both	Control	149 $\pm$ 35 b	139 $\pm$ 26 b	139 $\pm$ 20 b	229 $\pm$ 32 a	137 $\pm$ 21 b	221 $\pm$ 47 a	8.874***	231.410***	22.579***
		Lso+	391 $\pm$ 31 a	180 $\pm$ 20 c	148 $\pm$ 12 c	262 $\pm$ 36 b	178 $\pm$ 27 c	219 $\pm$ 41 bc	22.432***	59.090***	
	2012	Control	238 $\pm$ 32 bc	203 $\pm$ 24 c	189 $\pm$ 17 c	313 $\pm$ 21 ab	191 $\pm$ 16 c	352 $\pm$ 24 a	9.058***		3.376
		Lso+	371 $\pm$ 36 a	203 $\pm$ 27 c	169 $\pm$ 11 c	345 $\pm$ 30 ab	246 $\pm$ 14 bc	318 $\pm$ 28 bc	9.880***		
	2013	Control	42.2 $\pm$ 4.1 b	63.0 $\pm$ 9.8 b	78.8 $\pm$ 13.5 ab	129 $\pm$ 23 a	73.2 $\pm$ 6.5 b	63.0 $\pm$ 10.1 b	5.509		34.004***
		Lso+	426 $\pm$ 62 a	139 $\pm$ 16 bc	112 $\pm$ 16 bc	111 $\pm$ 7 b	56.6 $\pm$ 14.5 bc	40.6 $\pm$ 1.7 c	25.841***		
Histidine	Both	Control	168 $\pm$ 46	255 $\pm$ 92	365 $\pm$ 179	284 $\pm$ 35	346 $\pm$ 109	421 $\pm$ 207	0.438	1.319	12.403***
		Lso+	1550 $\pm$ 330 a	364 $\pm$ 125 b	671 $\pm$ 209 ab	841 $\pm$ 273 ab	332 $\pm$ 121 b	721 $\pm$ 201 ab	3.159*	1.219	
	2012	Control	204 $\pm$ 68	338 $\pm$ 164	406 $\pm$ 294	293 $\pm$ 49	319 $\pm$ 129	615 $\pm$ 374	0.426		6.443*
		Lso+	1680 $\pm$ 510	252 $\pm$ 135	754 $\pm$ 287	939 $\pm$ 411	386 $\pm$ 185	917 $\pm$ 291	2.365		
	2013	Control	125 $\pm$ 61	155 $\pm$ 33	315 $\pm$ 214	274 $\pm$ 54	378 $\pm$ 199	188 $\pm$ 34	0.623		25.841***
		Lso+	1300 $\pm$ 90 a	565 $\pm$ 246 ab	522 $\pm$ 304 ab	663 $\pm$ 237 ab	234 $\pm$ 80 b	368 $\pm$ 122 b	3.478*		
Isoleucine	Both	Control	325 $\pm$ 86 b	165 $\pm$ 19 b	159 $\pm$ 20 b	724 $\pm$ 106 a	304 $\pm$ 40 b	342 $\pm$ 45 b	12.712***	3.402	34.105***
		Lso+	997 $\pm$ 102 a	468 $\pm$ 90 b	334 $\pm$ 52 b	768 $\pm$ 114 a	243 $\pm$ 37 b	273 $\pm$ 28 b	30.684***	5.377*	
	2012	Control	463 $\pm$ 136 b	175 $\pm$ 25 b	120 $\pm$ 20 b	883 $\pm$ 161 a	236 $\pm$ 21 b	372 $\pm$ 57 b	9.553***		4.097*
		Lso+	779 $\pm$ 80 a	258 $\pm$ 31 b	239 $\pm$ 49 b	926 $\pm$ 153 a	312 $\pm$ 42 b	329 $\pm$ 31 b	14.973***		
	2013	Control	159 $\pm$ 13 b	153 $\pm$ 31 b	206 $\pm$ 24 b	534 $\pm$ 83 a	386 $\pm$ 70 ab	305 $\pm$ 74 ab	7.102***		59.662***
		Lso+	1390 $\pm$ 110 a	848 $\pm$ 120 b	506 $\pm$ 69 c	483 $\pm$ 43 cd	119 $\pm$ 19 e	173 $\pm$ 5 de	39.620***		
Leucine	Both	Control	278 $\pm$ 70 bc	172 $\pm$ 32 c	164 $\pm$ 13 c	453 $\pm$ 85 a	194 $\pm$ 22 c	366 $\pm$ 66 ab	9.156***	60.135***	26.920***
		Lso+	673 $\pm$ 47 a	333 $\pm$ 42 c	270 $\pm$ 20 c	548 $\pm$ 86 ab	250 $\pm$ 37 c	393 $\pm$ 70 bc	17.774***	56.546***	
	2012	Control	413 $\pm$ 100 ab	252 $\pm$ 30 b	177 $\pm$ 18 b	639 $\pm$ 102 a	249 $\pm$ 17 b	535 $\pm$ 51 a	8.192***		12.289***
		Lso+	692 $\pm$ 59 a	400 $\pm$ 52 bc	265 $\pm$ 25 c	733 $\pm$ 82 a	337 $\pm$ 30 bc	557 $\pm$ 53 ab	12.982***		
	2013	Control	115 $\pm$ 14 b	77.4 $\pm$ 9.7 b	149 $\pm$ 18 ab	231 $\pm$ 34 a	129 $\pm$ 13 ab	164 $\pm$ 36 ab	4.923**		35.239***
		Lso+	639 $\pm$ 83 a	213 $\pm$ 26 bc	279 $\pm$ 38 b	216 $\pm$ 20 bc	94.6 $\pm$ 14.2 c	96.6 $\pm$ 1.1 c	25.431***		

(continued on next page)

^a F statistics are provided. Different letters represent significant mean separations by Tukey's honest significant difference test. * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

$P = 0.360$; $n = 84$ for year; and $F = 10.130$; $P < 0.001$; $n = 84$ for the entry and year interaction) ZC fried symptoms than all other entries, and BS2 tubers had greater fried symptoms than ARS1 and ZC74 (Fig. 2A). In 2012, Atlantic tubers exhibited greater fried symptoms ($F = 19.904$; $P < 0.001$; $n = 54$) when infected with Lso than all of the putative tolerant breeding clones except BS2, with ARS1 and ZC74 exhibiting the least symptoms (Figs. 1 and 2). In 2013, Atlantic tubers exhibited greater fried symptoms ($F = 19.040$; $P < 0.001$; $n = 30$) when infected with Lso than all of the putative tolerant clones except ARS2, with ZC73 exhibiting the least symptoms (Fig. 2).

All noninoculated tubers used in this study had Ct values from the qPCR analyses equivalent to negative controls and thus were deemed noninfected. Of Lso-inoculated plants, tubers from the Atlantic cultivar had greater Lso titers than ZC73 and ZC74 when both years were analyzed together ($F = 6.265$; $P < 0.001$; $n = 84$ for entry; $F = 37.720$; $P < 0.001$; $n = 84$ for year; and $F = 6.128$; $P < 0.001$; $n = 84$ for the entry and year interaction), and tubers from 2013 had greater titers than those from 2012 (Fig. 3). For 2012, Atlantic tubers had greater Lso titers than all other entries ($F = 9.367$; $P < 0.001$; $n = 54$) (Fig. 3). However, even though ANOVA suggested a significant difference in Lso titers due to entry in 2013 ($F = 3.213$; $P = 0.023$; $n = 30$), follow-up Tukey's HSD tests failed to separate means between entries, albeit Atlantic had marginally ($P < 0.06$) increased titers over ZC73 and ZC74 (Fig. 3).

Fresh and fried symptoms were positively correlated with each other ($\rho = 0.523$; $P < 0.001$; $n = 84$). Likewise, Lso titers were

positively correlated with both fresh ($\rho = 0.319$; $P = 0.003$; $n = 84$) and fried symptoms ($\rho = 0.494$; $P < 0.001$; $n = 84$).

Amino acid responses in susceptible and tolerant entries. A total of 18 amino acids (alanine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine) were analyzed in tubers in both 2012 and 2013.

Entry, infection status, and year significantly affected overall levels of amino acids (Pillai's trace $\Delta = 3.019$; $F = 8.986$; $P < 0.001$; $n = 150$ for entry; $\Delta = 0.827$; $F = 27.144$; $P < 0.001$; $n = 150$ for infection status; and $\Delta = 0.941$; $F = 90.657$; $P < 0.001$; $n = 150$ for year) and all interactions between variables were significant (Pillai's trace $\Delta = 2.099$; $F = 4.267$; $P < 0.001$; $n = 150$ for entry and infection status; $\Delta = 2.787$; $F = 7.423$; $P < 0.001$; $n = 150$ for entry and year; $\Delta = 0.852$; $F = 32.834$; $P < 0.001$; $n = 150$ for infection status and year; and $\Delta = 1.923$; $F = 3.684$; $P < 0.001$; $n = 150$ for entry, infection status and year).

Entry and infection status significantly affected overall levels of amino acids in both 2012 (Pillai's trace $\Delta = 3.200$; $F = 6.420$; $P < 0.001$; $n = 90$ for entry; $\Delta = 0.486$; $F = 3.200$; $P < 0.001$; $n = 90$ for infection status) and 2013 ($\Delta = 4.196$; $F = 10.143$; $P < 0.001$; $n = 60$ for entry; $\Delta = 0.984$; $F = 104.480$; $P < 0.001$; $n = 60$ for infection status). However, in both 2012 (Pillai's trace $\Delta = 1.541$; $F = 1.609$; $P = 0.001$; $n = 90$) and 2013 ($\Delta = 3.640$; $F = 5.206$; $P < 0.001$; $n = 60$), MANOVAs suggested significant differences due to the entry and infection interaction.

TABLE 1. (continued from preceding page)

Compound	Year	Treatment	Atlantic	ARS1	ARS2	BS2	ZC73	ZC74	F		
									Between entry	Between year	Between treatment
Lysine	Both	Control	308 ± 75	395 ± 125	612 ± 271	660 ± 58	613 ± 183	745 ± 317	0.65	1.302	18.732***
		Lso+	3440 ± 720 a	890 ± 294 b	1090 ± 337 b	1880 ± 560 ab	539 ± 208 b	1650 ± 430 ab	4.045**	0.24	
		Control	306 ± 57	518 ± 222	643 ± 434	633 ± 59	740 ± 300	1010 ± 580	0.489		8.113**
	2012	Lso+	3740 ± 1130 a	486 ± 268 b	949 ± 355 ab	2070 ± 840 ab	687 ± 316 b	1990 ± 622 ab	3.324**		
		Control	312 ± 162	247 ± 50	574 ± 345	692 ± 114	461 ± 193	430 ± 100	0.774		24.417***
		Lso+	2920 ± 157 a	1620 ± 570 ab	1360 ± 740 ab	1550 ± 500 ab	274 ± 86 b	1040 ± 350 ab	3.501*		
Methionine	Both	Control	104 ± 12	145 ± 30	213 ± 70	194 ± 54	202 ± 36	181 ± 37	1.299	9.887**	33.010***
		Lso+	615 ± 141 a	198 ± 59 b	193 ± 49 b	238 ± 50 b	196 ± 52 b	208 ± 32 b	25.106***	72.873***	
		Control	90.5 ± 15.5	140 ± 48	121 ± 64	57.7 ± 7.3	180 ± 45	177 ± 67	1.047		24.417***
	2012	Lso+	256 ± 65	64.3 ± 23.1	119 ± 44	148 ± 50	196 ± 81	216 ± 49	1.612		
		Control	120 ± 16	151 ± 37	324 ± 124	357 ± 59	229 ± 61	187 ± 32	2.156		43.899***
		Lso+	1260 ± 47 a	439 ± 86 b	327 ± 88 b	400 ± 59 b	196 ± 34 b	194 ± 24 b	42.866***		
Phenylalanine	Both	Control	225 ± 49 a	150 ± 31 c	160 ± 34 c	468 ± 52 b	302 ± 39 c	315 ± 82 c	24.640***	26.192***	68.789***
		Lso+	1390 ± 240 a	279 ± 52 c	418 ± 105 bc	721 ± 123 b	238 ± 52 c	365 ± 54 c	35.309***	41.739***	
		Control	277 ± 83 a	116 ± 36 a	114 ± 51 a	458 ± 77 a	305 ± 60 a	378 ± 139 a	2.904*		4.547*
	2012	Lso+	812 ± 159 a	187 ± 51 b	217 ± 41 b	587 ± 165 ab	268 ± 79 b	351 ± 78 b	5.272***		
		Control	164 ± 31 b	191 ± 52 b	215 ± 33 b	480 ± 77 a	297 ± 54 ab	240 ± 72 ab	4.195**		106.885***
		Lso+	2420 ± 170 a	445 ± 70 bcd	779 ± 210 bc	961 ± 134 b	185 ± 38 d	389 ± 72 cd	38.821***		
Proline	Both	Control	636 ± 228 c	2110 ± 640 ab	1020 ± 280 bc	3380 ± 900 a	718 ± 135 bc	1740 ± 450 bc	7.738***	44.371***	5.735*
		Lso+	4360 ± 430 a	1650 ± 310 bc	1270 ± 270 bc	2510 ± 560 b	1150 ± 250 c	1250 ± 170 bc	15.558***	1.046	
		Control	925 ± 391 c	3630 ± 690 ab	1110 ± 500 bc	5450 ± 1030 a	943 ± 203 c	2720 ± 570 bc	8.797***		1.099
	2012	Lso+	3860 ± 580 a	1700 ± 490 bc	849 ± 262 c	3260 ± 770 ab	1620 ± 270 bc	1520 ± 210 bc	5.939***		
		Control	291 ± 33 a	282 ± 69 a	910 ± 203 a	901 ± 267 a	448 ± 71 a	562 ± 137 a	3.382*		107.638***
		Lso+	5280 ± 422 a	1570 ± 130 bc	2030 ± 450 b	1170 ± 70 bcd	297 ± 36 d	769 ± 34 cd	46.549***		
Serine	Both	Control	1650 ± 200 c	3000 ± 530 ab	3270 ± 490 ab	3590 ± 480 a	2630 ± 360 abc	2410 ± 300 bc	6.890***	49.665***	24.682***
		Lso+	5310 ± 790 a	2990 ± 460 bc	2860 ± 360 c	3940 ± 400 b	2020 ± 180 c	2760 ± 200 c	30.940***	94.464***	
		Control	1900 ± 320	1610 ± 270	2370 ± 320	2480 ± 150	1780 ± 170	1980 ± 150	1.974		10.932**
	2012	Lso+	3280 ± 320 a	1870 ± 210 b	2350 ± 280 ab	3190 ± 380 a	2150 ± 270 ab	2640 ± 270 ab	3.672**		
		Control	1360 ± 130 b	4670 ± 410 a	4350 ± 800 a	4910 ± 650 a	3650 ± 460 ab	2920 ± 600 ab	5.904**		11.707**
		Lso+	8960 ± 380 a	5010 ± 440 b	3790 ± 750 bc	5290 ± 480 b	1780 ± 110 d	2970 ± 290 cd	30.553***		
Threonine	Both	Control	210 ± 33 b	432 ± 57 ab	514 ± 102 a	502 ± 48 a	323 ± 32 ab	281 ± 39 ab	5.093***	0.075	28.129***
		Lso+	950 ± 104 a	610 ± 113 b	607 ± 80 b	614 ± 49 b	286 ± 52 c	311 ± 43 c	16.788***	2.993	
		Control	282 ± 40	360 ± 42	496 ± 128	451 ± 32	296 ± 35	340 ± 54	1.776		11.535**
	2012	Lso+	820 ± 122 a	363 ± 58 b	610 ± 102 ab	605 ± 72 ab	386 ± 57 b	385 ± 52 b	5.011***		
		Control	124 ± 13 b	517 ± 110 ab	534 ± 180 ab	562 ± 96 a	355 ± 58 ab	209 ± 38 ab	3.487*		15.585***
		Lso+	1180 ± 150 a	1050 ± 170 ab	600 ± 144 bc	629 ± 55 bc	106 ± 14 d	179 ± 17 cd	15.295***		
Tryptophan	Both	Control	76.5 ± 17.2	64.4 ± 15.2	140 ± 64	165 ± 36	95.0 ± 38.8	84.5 ± 16.2	1.771	14.148***	33.798***
		Lso+	333 ± 95 a	282 ± 88 ab	163 ± 69 abc	243 ± 80 abc	63.1 ± 8.2 c	134 ± 35 bc	7.553***	78.518***	
		Control	74.3 ± 7.5 a	47.2 ± 5.6 a	63.8 ± 14.8 a	76.5 ± 15.0 a	34.3 ± 8.0 a	62.0 ± 7.7 a	2.554*		1.123
	2012	Lso+	88.3 ± 12.3	149 ± 84	48.2 ± 15.9	73.8 ± 6.0	61.2 ± 9.9	53.4 ± 9.9	1.08		
		Control	79.0 ± 39.3	85.0 ± 32.0	230 ± 135	271 ± 40	170 ± 75	112 ± 32	1.308		23.972***
		Lso+	774 ± 73 a	520 ± 154 ab	370 ± 160 ab	547 ± 147 ab	66.4 ± 16.0 b	278 ± 49 ab	4.541**		
Tyrosine	Both	Control	48.5 ± 14.4	53.0 ± 23.8	35.9 ± 12.0	46.8 ± 12.2	56.9 ± 20.0	69.1 ± 30.3	0.318	17.268***	7.032**
		Lso+	231 ± 60 a	42.9 ± 12.0 b	57.5 ± 14.1 b	113 ± 37 ab	82.7 ± 36.1 b	157 ± 50 ab	2.525*	21.367***	
		Control	57.7 ± 22.5	91.5 ± 37.7	53.5 ± 19.4	74.7 ± 13.3	80.0 ± 34.3	118 ± 48	0.571		8.611**
	2012	Lso+	336 ± 72 a	56.2 ± 17.3 b	77.0 ± 18.3 b	160 ± 52 ab	112 ± 55 b	229 ± 67 ab	4.192**		
		Control	37.6 ± 18.1	6.80 ± 1.56	14.8 ± 5.3	13.4 ± 6.0	29.2 ± 9.0	10.0 ± 4.2	1.778		4.215*
		Lso+	40.6 ± 6.4	19.0 ± 3.9	22.4 ± 11.0	29.8 ± 7.0	30.2 ± 5.1	27.8 ± 7.4	1.09		
Valine	Both	Control	743 ± 186 b	465 ± 64 b	380 ± 33 b	2150 ± 310 a	633 ± 64 b	864 ± 121 b	22.262***	16.232***	22.712***
		Lso+	1720 ± 143 b	1280 ± 270 bc	759 ± 90 c	2400 ± 340 a	806 ± 145 c	769 ± 80 c	15.442**	1.438	
		Control	1120 ± 250 b	558 ± 86 b	352 ± 28 b	2720 ± 429 a	619 ± 48 b	1070 ± 134 b	16.079***		4.362*
	2012	Lso+	1920 ± 190 b	619 ± 68 c	602 ± 86 c	2900 ± 450 a	1100 ± 150 bc	925 ± 86 c	17.316***		
		Control	294 ± 15 b	355 ± 78 b	413 ± 67 b	1470 ± 230 a	650 ± 137 b	621 ± 164 b	10.160***		43.469***
		Lso+	1360 ± 110 b	2480 ± 310 a	1040 ± 130 bc	1500 ± 140 b	269 ± 20 d	489 ± 21 cd	25.809***		

Due to all interactions being significant, subsequent ANOVAs were conducted with treatment combinations separated out. ANOVAs suggested that infection status significantly affected most amino acids with the exception of alanine, aspartic acid, glutamine, glycine, proline, and tryptophan in 2012, and most amino acids with the exception of asparagine in 2013, and most amino acids in with the exception of glutamic acid when both years were analyzed together (Table 1). ANOVAs also suggested differences in most amino acid levels due to year, with the exception of noninfected levels of glutamic acid, histidine, isoleucine, lysine, and threonine; and Lso-infected levels of alanine, histidine, lysine, proline, threonine, and valine (Table 1).

Regarding differences of amino acid levels between entries, it was clear that interactions between infection status and entry

existed. BS2 often had greater amino acid levels than other entries when tubers were noninfected, such as for alanine, glycine, isoleucine, leucine, proline, serine, threonine, and valine (Table 1). However, Atlantic had greater amino acid levels than other entries when tubers were Lso-infected, such as for glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, and tryptophan (Table 1). Dependent on year and infection status, other patterns existed between entries for different amino acids, as shown in Table 1.

Regarding differences in fold changes due to entries, MANOVA suggested that overall amino acid levels significantly differed between entries for both years ($\Delta = 3.199$; $F = 3.085$; $P < 0.001$; $n = 59$ for entry; $\Delta = 0.953$; $F = 30.949$; $P < 0.001$; $n = 59$ for year; and

TABLE 2. Mean ($\pm$ SE) fold differences of '*Candidatus Liberibacter solanacearum*' (Lso)-infected tubers over control levels of each individual amino acid by entry for both years and each year separately^z

Compound	Year	Atlantic	ARS1	ARS2	BS2	ZC73	ZC74	F	
								Between entry	Between year
Alanine	Both	2.19 $\pm$ 0.64	4.36 $\pm$ 2.05	1.40 $\pm$ 0.35	1.92 $\pm$ 0.81	1.35 $\pm$ 0.28	1.84 $\pm$ 0.58	1.59	10.133**
	2012	1.05 $\pm$ 0.22 ab	0.87 $\pm$ 0.15 ab	0.87 $\pm$ 0.13 ab	0.78 $\pm$ 0.10 b	1.69 $\pm$ 0.32 a	0.97 $\pm$ 0.19 ab	2.745*	
	2013	3.34 $\pm$ 1.08	7.85 $\pm$ 3.57	2.06 $\pm$ 0.67	3.05 $\pm$ 1.51	1.01 $\pm$ 0.44	2.70 $\pm$ 1.04	1.787	
Asparagine	Both	2.14 $\pm$ 0.37 a	1.27 $\pm$ 0.19 ab	0.79 $\pm$ 0.14 b	0.83 $\pm$ 0.16 b	1.02 $\pm$ 0.17 b	0.66 $\pm$ 0.12 b	6.314***	0.858
	2012	2.20 $\pm$ 0.69	1.11 $\pm$ 0.32	0.93 $\pm$ 0.22	0.93 $\pm$ 0.26	1.24 $\pm$ 0.26	0.76 $\pm$ 0.21	2.003	
	2013	2.08 $\pm$ 0.38 a	1.42 $\pm$ 0.23 ab	0.61 $\pm$ 0.15 b	0.73 $\pm$ 0.21 b	0.79 $\pm$ 0.18 b	0.55 $\pm$ 0.10 b	6.903***	
Aspartic acid	Both	5.72 $\pm$ 1.75 a	1.41 $\pm$ 0.29 b	0.76 $\pm$ 0.12 b	1.00 $\pm$ 0.25 b	1.10 $\pm$ 0.18 b	1.06 $\pm$ 0.19 b	13.980***	18.862***
	2012	1.60 $\pm$ 0.24 a	0.78 $\pm$ 0.17 ab	0.70 $\pm$ 0.17 b	0.67 $\pm$ 0.18 b	1.06 $\pm$ 0.24 ab	0.78 $\pm$ 0.11 b	3.570*	
	2013	9.84 $\pm$ 2.31 a	2.04 $\pm$ 0.38 b	0.84 $\pm$ 0.18 b	1.32 $\pm$ 0.45 b	1.15 $\pm$ 0.29 b	1.34 $\pm$ 0.33 b	11.785***	
Glutamic acid	Both	1.84 $\pm$ 0.56 a	0.76 $\pm$ 0.12 b	0.56 $\pm$ 0.15 b	0.84 $\pm$ 0.16 b	0.74 $\pm$ 0.16 b	0.82 $\pm$ 0.15 b	5.751***	22.223***
	2012	3.20 $\pm$ 0.69 a	0.79 $\pm$ 0.19 b	0.82 $\pm$ 0.18 b	0.98 $\pm$ 0.29 b	0.90 $\pm$ 0.28 b	1.05 $\pm$ 0.15 b	7.275***	
	2013	0.47 $\pm$ 0.08	0.73 $\pm$ 0.17	0.22 $\pm$ 0.04	0.70 $\pm$ 0.16	0.57 $\pm$ 0.15	0.59 $\pm$ 0.23	1.273	
Glutamine	Both	3.65 $\pm$ 1.12 a	2.74 $\pm$ 0.77 ab	1.51 $\pm$ 0.27 ab	2.13 $\pm$ 0.79 ab	1.11 $\pm$ 0.16 b	1.14 $\pm$ 0.20 b	3.618**	18.144***
	2012	1.19 $\pm$ 0.18	1.03 $\pm$ 0.04	1.51 $\pm$ 0.35	0.96 $\pm$ 0.05	1.08 $\pm$ 0.07	0.99 $\pm$ 0.17	1.292	
	2013	6.12 $\pm$ 1.59 a	4.45 $\pm$ 1.11 ab	1.51 $\pm$ 0.48 ab	3.31 $\pm$ 1.46 ab	1.15 $\pm$ 0.34 b	1.30 $\pm$ 0.38 b	3.644*	
Glycine	Both	5.90 $\pm$ 1.72 a	1.52 $\pm$ 0.29 b	1.01 $\pm$ 0.13 b	1.02 $\pm$ 0.12 b	1.10 $\pm$ 0.14 b	0.77 $\pm$ 0.09 b	26.648***	28.979***
	2012	1.41 $\pm$ 0.15 a	0.74 $\pm$ 0.08 b	0.82 $\pm$ 0.08 b	1.03 $\pm$ 0.11 ab	1.42 $\pm$ 0.12 a	0.82 $\pm$ 0.12 b	7.061***	
	2013	10.39 $\pm$ 1.78 a	2.30 $\pm$ 0.24 b	1.24 $\pm$ 0.23 b	1.02 $\pm$ 0.24 b	0.77 $\pm$ 0.16 b	0.72 $\pm$ 0.13 b	24.427***	
Histidine	Both	12.22 $\pm$ 2.90 a	2.56 $\pm$ 1.11 b	1.62 $\pm$ 0.39 b	2.25 $\pm$ 1.11 b	1.29 $\pm$ 0.37 b	1.42 $\pm$ 0.37 b	13.172***	10.426***
	2012	6.58 $\pm$ 2.10 a	0.92 $\pm$ 0.28 b	1.58 $\pm$ 0.59 b	0.88 $\pm$ 0.24 b	1.18 $\pm$ 0.36 b	0.84 $\pm$ 0.14 b	6.080***	
	2013	17.85 $\pm$ 4.19 a	4.20 $\pm$ 2.03 b	1.68 $\pm$ 0.58 b	3.61 $\pm$ 2.13 b	1.40 $\pm$ 0.68 b	2.01 $\pm$ 0.64 b	8.343***	
Isoleucine	Both	5.28 $\pm$ 1.30 a	3.62 $\pm$ 0.95 b	1.83 $\pm$ 0.25 c	0.93 $\pm$ 0.18 c	0.90 $\pm$ 0.21 c	0.76 $\pm$ 0.13 c	32.539***	56.505***
	2012	1.61 $\pm$ 0.44	1.18 $\pm$ 0.20	1.59 $\pm$ 0.31	0.79 $\pm$ 0.25	1.42 $\pm$ 0.20	0.77 $\pm$ 0.18	1.863	
	2013	8.94 $\pm$ 0.82 a	6.06 $\pm$ 1.03 b	2.14 $\pm$ 0.40 c	1.07 $\pm$ 0.27 c	0.38 $\pm$ 0.14 c	0.75 $\pm$ 0.22 c	34.977***	
Leucine	Both	3.81 $\pm$ 0.93 a	2.05 $\pm$ 0.36 b	1.40 $\pm$ 0.15 b	0.99 $\pm$ 0.15 b	1.13 $\pm$ 0.15 b	0.81 $\pm$ 0.13 b	14.573***	16.761***
	2012	1.63 $\pm$ 0.42	1.21 $\pm$ 0.21	1.20 $\pm$ 0.04	0.90 $\pm$ 0.19	1.46 $\pm$ 0.15	0.82 $\pm$ 0.09	2.016	
	2013	5.99 $\pm$ 1.15 a	2.89 $\pm$ 0.45 b	1.65 $\pm$ 0.30 b	1.07 $\pm$ 0.24 b	0.79 $\pm$ 0.16 b	0.79 $\pm$ 0.25 b	13.483***	
Lysine	Both	13.79 $\pm$ 2.69 a	4.41 $\pm$ 2.04 b	1.50 $\pm$ 0.38 b	2.06 $\pm$ 0.79 b	0.98 $\pm$ 0.26 b	1.85 $\pm$ 0.57 b	12.105***	5.960*
	2012	11.14 $\pm$ 4.12 a	0.97 $\pm$ 0.21 b	1.06 $\pm$ 0.38 b	1.08 $\pm$ 0.18 b	0.96 $\pm$ 0.33 b	1.00 $\pm$ 0.09 b	5.924**	
	2013	16.44 $\pm$ 3.49 a	7.85 $\pm$ 3.57 ab	2.06 $\pm$ 0.67 b	3.05 $\pm$ 1.51 b	1.01 $\pm$ 0.44 b	2.70 $\pm$ 1.04 b	6.743***	
Methionine	Both	7.02 $\pm$ 1.49 a	1.90 $\pm$ 0.62 b	0.77 $\pm$ 0.16 b	1.43 $\pm$ 0.26 b	0.92 $\pm$ 0.17 b	0.97 $\pm$ 0.17 b	41.444***	40.259***
	2012	3.03 $\pm$ 0.99 a	0.47 $\pm$ 0.14 b	0.63 $\pm$ 0.19 b	1.50 $\pm$ 0.39 ab	0.77 $\pm$ 0.23 b	0.77 $\pm$ 0.15 b	4.464**	
	2013	11.02 $\pm$ 1.00 a	3.32 $\pm$ 0.82 b	0.95 $\pm$ 0.26 b	1.35 $\pm$ 0.39 b	1.07 $\pm$ 0.26 b	1.17 $\pm$ 0.28 b	44.082***	
Phenylalanine	Both	9.35 $\pm$ 2.43 a	2.16 $\pm$ 0.44 b	2.13 $\pm$ 0.40 b	1.53 $\pm$ 0.49 b	0.70 $\pm$ 0.09 b	1.34 $\pm$ 0.35 b	40.137***	57.847***
	2012	2.63 $\pm$ 0.46 a	1.53 $\pm$ 0.39 ab	1.60 $\pm$ 0.44 ab	0.61 $\pm$ 0.18 b	0.69 $\pm$ 0.11 b	0.65 $\pm$ 0.21 b	5.895**	
	2013	16.07 $\pm$ 1.94 a	2.79 $\pm$ 0.71 b	2.80 $\pm$ 0.62 b	2.44 $\pm$ 0.80 b	0.70 $\pm$ 0.16 b	2.03 $\pm$ 0.51 b	34.800***	
Proline	Both	12.86 $\pm$ 2.71 a	3.52 $\pm$ 1.23 b	2.72 $\pm$ 1.04 b	1.35 $\pm$ 0.50 b	1.77 $\pm$ 0.48 b	1.17 $\pm$ 0.32 b	22.602***	14.997***
	2012	6.66 $\pm$ 2.89 a	0.33 $\pm$ 0.16 b	3.42 $\pm$ 1.88 ab	0.53 $\pm$ 0.17 ab	2.77 $\pm$ 0.71 ab	0.55 $\pm$ 0.19 ab	2.931*	
	2013	19.05 $\pm$ 2.35 a	6.72 $\pm$ 1.31 b	1.83 $\pm$ 0.42 bc	2.17 $\pm$ 0.88 bc	0.77 $\pm$ 0.18 c	1.78 $\pm$ 0.47 bc	33.461***	
Serine	Both	4.51 $\pm$ 1.01 a	1.09 $\pm$ 0.13 b	0.71 $\pm$ 0.07 b	1.13 $\pm$ 0.18 b	0.86 $\pm$ 0.15 b	1.16 $\pm$ 0.16 b	26.952***	11.391**
	2012	1.98 $\pm$ 0.48 a	1.08 $\pm$ 0.24 ab	0.74 $\pm$ 0.08 b	1.04 $\pm$ 0.21 ab	1.21 $\pm$ 0.20 ab	1.09 $\pm$ 0.11 ab	2.675*	
	2013	7.04 $\pm$ 1.10 a	1.10 $\pm$ 0.12 b	0.67 $\pm$ 0.12 b	1.23 $\pm$ 0.31 b	0.51 $\pm$ 0.05 b	1.23 $\pm$ 0.31 b	25.072***	
Threonine	Both	6.35 $\pm$ 1.51 a	1.50 $\pm$ 0.29 b	0.95 $\pm$ 0.16 b	1.25 $\pm$ 0.26 b	0.82 $\pm$ 0.19 b	0.89 $\pm$ 0.10 b	28.947***	18.840***
	2012	2.59 $\pm$ 0.42 a	0.78 $\pm$ 0.11 b	0.89 $\pm$ 0.21 b	1.07 $\pm$ 0.21 b	1.30 $\pm$ 0.20 b	0.81 $\pm$ 0.10 b	8.868***	
	2013	10.11 $\pm$ 1.74 a	2.22 $\pm$ 0.34 b	1.02 $\pm$ 0.29 b	1.42 $\pm$ 0.49 b	0.33 $\pm$ 0.06 b	0.98 $\pm$ 0.19 b	22.428***	
Tryptophan	Both	10.71 $\pm$ 4.46 a	4.59 $\pm$ 1.79 ab	1.19 $\pm$ 0.56 b	1.83 $\pm$ 0.53 b	1.57 $\pm$ 0.33 b	1.89 $\pm$ 0.48 b	5.825***	16.351***
	2012	1.11 $\pm$ 0.14 b	0.88 $\pm$ 0.19 b	0.44 $\pm$ 0.15 b	1.18 $\pm$ 0.35 ab	2.32 $\pm$ 0.35 a	0.81 $\pm$ 0.34 b	5.676**	
	2013	20.32 $\pm$ 6.60 a	8.30 $\pm$ 2.75 ab	2.11 $\pm$ 1.14 b	2.48 $\pm$ 0.98 b	0.81 $\pm$ 0.31 b	2.97 $\pm$ 0.60 b	5.842**	
Tyrosine	Both	4.98 $\pm$ 1.34	1.82 $\pm$ 0.58	0.99 $\pm$ 0.16	2.48 $\pm$ 0.87	2.05 $\pm$ 0.75	3.45 $\pm$ 1.66	2.176	1.862
	2012	7.15 $\pm$ 1.75 a	0.54 $\pm$ 0.11 b	0.98 $\pm$ 0.22 b	1.12 $\pm$ 0.26 b	1.87 $\pm$ 0.84 b	0.92 $\pm$ 0.08 b	9.697***	
	2013	2.80 $\pm$ 1.61	3.10 $\pm$ 0.82	0.99 $\pm$ 0.27	3.84 $\pm$ 1.56	2.22 $\pm$ 1.35	5.98 $\pm$ 3.04	0.901	
Valine	Both	3.10 $\pm$ 0.58 a	4.28 $\pm$ 1.23 a	1.79 $\pm$ 0.25 b	1.04 $\pm$ 0.19 b	1.18 $\pm$ 0.26 b	0.90 $\pm$ 0.15 b	22.878***	50.955***
	2012	1.53 $\pm$ 0.32 ab	0.91 $\pm$ 0.13 b	1.42 $\pm$ 0.12 ab	0.87 $\pm$ 0.24 b	1.86 $\pm$ 0.24 a	0.74 $\pm$ 0.09 b	4.655**	
	2013	4.67 $\pm$ 0.44 b	7.64 $\pm$ 1.06 a	2.26 $\pm$ 0.46 c	1.21 $\pm$ 0.30 c	0.51 $\pm$ 0.13 c	1.06 $\pm$ 0.29 c	26.667***	

^z Values over 1 represent greater levels in Lso-infected tubers, whereas values under 1 represent levels greater in noninfected control. F statistics are provided. Different letters represent significant mean separations by Tukey's honest significant difference test. * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

$\Delta = 3.106$; $F = 2.849$; $P < 0.001$; $n = 59$ for the entry and year interaction), 2012 ($\Delta = 3.778$; $F = 1.889$; $P = 0.006$; $n = 30$), and 2013 ($\Delta = 4.381$; $F = 3.929$; $P < 0.001$; $n = 29$). Levels of most amino acids for both years (with the exception of alanine and tyrosine), 12 amino acids in 2012 (aspartic acid, glutamic acid, glycine, histidine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine) and 15 amino acids in 2013 (asparagine, aspartic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, and valine) had greater fold increases in Atlantic tubers than tubers from at least one other entry (Table 2). For both years ($F = 23.278$; $P < 0.001$; $n = 59$ for entry; $F = 28.905$; $P < 0.001$; $n = 59$ for year; and $F = 8.807$; $P < 0.001$; $n = 59$ for the entry and year interaction), 2012 ($F = 9.600$; $P < 0.001$; $n = 30$), and 2013 ($F = 16.533$; $P < 0.001$; $n = 29$), the mean fold change of amino acids was significant greater in Atlantic tubers than tubers from all other entries (Fig. 4).

Levels of glycine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, and valine were all positively correlated ($P < 0.05$) with both fresh and fried symptom ratings. Asparagine, aspartic acid, histidine, methionine, and tryptophan were positively correlated ($P < 0.05$) with only fresh symptoms, whereas tyrosine was positively correlated ($P < 0.05$) with only fried symptoms. Lso titers were significantly positively correlated ($P < 0.05$) with asparagine, glutamine, isoleucine, lysine, methionine, phenylalanine, serine, and tryptophan levels.

Sugar responses in susceptible and tolerant entries. Fructose and glucose levels were examined in tuber tissues. Entry

and infection status significantly affected overall levels of sugars in both years ($\Delta = 0.804$; $F = 16.927$; $P < 0.001$; $n = 150$ for entry; $\Delta = 0.405$; $F = 42.556$; $P < 0.001$; $n = 150$ for infection status; $\Delta = 0.553$; $F = 77.363$; $P < 0.001$; $n = 150$ for year; $\Delta = 0.534$; $F = 9.186$; $P < 0.001$; $n = 150$ for the entry and infection status interaction; $\Delta = 0.459$; $F = 7.513$; $P < 0.001$; $n = 150$ for the entry and year interaction; $\Delta = 0.340$; $F = 32.163$; $P < 0.001$; $n = 150$ for the infection status and year interaction; and $\Delta = 0.309$; $F = 4.605$; $P < 0.001$; $n = 150$ for the entry, infection status, and year interaction), 2012 ($\Delta = 0.475$; $F = 4.864$; $P < 0.001$; $n = 90$ for entry; $\Delta = 0.119$; $F = 5.194$; $P = 0.008$; $n = 90$ for infection status; and $\Delta = 0.301$; $F = 2.759$; $P = 0.004$; $n = 90$ for the entry and infection status interaction), and 2013 ($\Delta = 1.113$; $F = 12.045$; $P < 0.001$; $n = 60$ for entry; $\Delta = 0.565$; $F = 30.476$; $P < 0.001$; $n = 60$ for infection status; and $\Delta = 0.888$; $F = 7.592$; $P < 0.001$; $n = 60$ for the entry and infection status interaction). Because MANOVAs suggested significant differences due to year, entry, and infection interactions, ANOVAs were repeated with treatment combinations separated out. ANOVAs suggested significant ($P < 0.05$) effects due to treatment combination for both sugars and both years, 2012, and 2013. For both years, tubers from the BS2 entry had consistently greater amounts of sugars than all other cultivars, except Atlantic regardless of infection status (Table 3). Infected Atlantic tubers had similar fructose levels to infected BS2 tubers, but noninfected BS2 had greater levels of both fructose and glucose than Atlantic tubers (Table 3). For 2012, infected Atlantic tuber levels of fructose were greater than other entry levels (Table 3). However, fructose and glucose levels did not differ between entries when tubers were not infected (Table 3). For 2013, infected BS2 had greater levels of fructose than all other combinations except infected Atlantic tubers, and glucose levels were greater in infected BS2 than all other treatment combinations (Table 3). BS2 levels of fructose and glucose were greater than most other entries when noninfected, with the exception of no significant difference between BS2 and ZC73 fructose levels (Table 3).

Regarding variations in fold increases between entries, MANOVA suggested significant differences between entries for both years ($\Delta = 0.589$; $F = 4.008$; $P < 0.001$; $n = 60$ for entry; $\Delta = 0.266$; $F = 8.496$; $P < 0.001$; $n = 60$ for year; and $\Delta = 0.669$; $F = 4.824$; $P < 0.001$; $n = 60$ for the entry and year interaction), 2012 ($\Delta = 0.670$; $F = 2.415$; $P = 0.020$; $n = 30$), and 2013 ($\Delta = 1.091$; $F = 5.762$; $P < 0.001$; $n = 30$). For fructose fold changes were not significantly different among entries for 2012 ($F = 1.817$; $P = 0.148$; $n = 30$), but were significant for 2013 ($F = 4.982$; $P = 0.003$; $n = 30$) and when both years were analyzed together ($F = 5.642$; $P < 0.001$; $n = 60$ for entry; $F = 16.854$; $P < 0.001$; $n = 60$ for year; and $F = 4.003$; $P < 0.001$; $n = 60$ for the entry and year interaction). Greater differences were observed in Atlantic than ARS1, BS2, ZC73, and ZC74 in 2013 (Fig. 5), and in Atlantic compared with ARS1 and ZC73 when both years were analyzed together. Likewise, for glucose

Fig. 4. Fold differences of amino acid levels for '*Candidatus Liberibacter solanacearum*'-infected tubers over control tubers of each entry for both years and each year separately. Bars represent standard errors. Different letters represent means that are significantly different by Tukey's honest significant difference test.

TABLE 3. Mean ($\pm$ SE) sugar levels (μ g/g FW) separated by entry for both years and each year separately^z

Compound	Year	Treatment	Atlantic	ARS1	ARS2	BS2	ZC73	ZC74	F		
									Within treatment	Between year	Between treatment
Fructose	Both	Control	262 $\pm$ 67 b	306 $\pm$ 74 b	200 $\pm$ 58 b	917 $\pm$ 337 a	223 $\pm$ 84 b	138 $\pm$ 34 b	5.572***	6.338*	68.044***
		Lso+	1800 $\pm$ 280 a	308 $\pm$ 57 b	783 $\pm$ 222 b	1490 $\pm$ 360 a	319 $\pm$ 95 b	327 $\pm$ 102 b	31.015***	99.349***	
	2012	Control	321 $\pm$ 114	266 $\pm$ 116	241 $\pm$ 104	353 $\pm$ 63	43 $\pm$ 6	86 $\pm$ 39	2.239		10.484**
		Lso+	1310 $\pm$ 260 a	222 $\pm$ 67 bc	261 $\pm$ 82 bc	646 $\pm$ 120 b	178 $\pm$ 65 bc	60 $\pm$ 9 c	13.403***		
	2013	Control	190 $\pm$ 54 b	354 $\pm$ 97 b	150 $\pm$ 29 b	1590 $\pm$ 640 a	438 $\pm$ 133 ab	200 $\pm$ 49 b	4.163**		42.941***
		Lso+	2690 $\pm$ 390 ab	462 $\pm$ 65 d	1720 $\pm$ 280 bc	3000 $\pm$ 460 a	572 $\pm$ 206 cd	806 $\pm$ 70 cd	14.959***		
Glucose	Both	Control	260 $\pm$ 87 b	142 $\pm$ 37 b	190 $\pm$ 62 b	1390 $\pm$ 51 a	220 $\pm$ 76 b	505 $\pm$ 202 b	6.973***	15.122***	80.768***
		Lso+	1620 $\pm$ 400 b	1040 $\pm$ 390 bc	1340 $\pm$ 450 bc	2680 $\pm$ 910 a	413 $\pm$ 139 c	752 $\pm$ 274 bc	16.087***	176.145***	
	2012	Control	44.5 $\pm$ 30.7	48.6 $\pm$ 18.4	28.7 $\pm$ 14.6	436 $\pm$ 348	21.9 $\pm$ 9.2	460 $\pm$ 376	1.034		1.539
		Lso+	790 $\pm$ 231 a	21.0 $\pm$ 4.0 b	272 $\pm$ 246 ab	564 $\pm$ 185 ab	195 $\pm$ 166 ab	20.1 $\pm$ 3.9 b	3.254*		
	2013	Control	518 $\pm$ 98 b	254 $\pm$ 35 b	384 $\pm$ 60 b	2530 $\pm$ 820 a	458 $\pm$ 74 b	558 $\pm$ 102 b	6.443***		61.740***
		Lso+	3100 $\pm$ 610 b	2880 $\pm$ 31 b	3270 $\pm$ 45 b	6490 $\pm$ 1320 a	806 $\pm$ 132 b	2070 $\pm$ 80 b	8.815***		

^z F statistics are provided. Different letters represent significant mean separations by Tukey's honest significant difference test. * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

fold changes were significantly different among entries for both years ($F = 6.623$; $P < 0.001$; $n = 30$ for entry; $F = 0.294$; $P = 0.590$; $n = 60$ for year; and $F = 5.699$; $P < 0.001$; $n = 60$ for the entry and year interaction), 2012 ($F = 6.174$; $P < 0.001$; $n = 30$), and 2013 ($F = 6.090$; $P = 0.001$; $n = 30$), with greater differences in Atlantic than ARS2, BS2, ZC73, and ZC74 when both years analyzed together; greater differences in Atlantic than all other entries in 2012; and greater differences in ARS1 and ARS2 than ZC73 in 2013 (Fig. 5).

Fructose and glucose levels were significantly positively correlated ($P < 0.05$) with fresh symptoms, fried symptoms, and Lso titers.

Phenolic responses in susceptible and tolerant entries. A total of 12 phenolics were quantified including chlorogenic acids (chlorogenic acid, a chlorogenic acid derivative, cryptochlorogenic acid, and a cryptochlorogenic acid derivative), flavonoids (four flavonoid glycoside derivatives and one flavonoid methyl ester glycoside), and phenolic acids (protocatechuic acid glycoside and a quinic acid derivative).

Entry and infection status significantly affected overall levels of phenolics in both years ($\Delta = 2.023$; $F = 6.739$; $P < 0.001$; $n = 150$ for entry; $\Delta = 0.710$; $F = 23.411$; $P < 0.001$; $n = 150$ for infection status; $\Delta = 0.747$; $F = 28.314$; $P < 0.001$; $n = 150$ for year; $\Delta = 1.648$; $F = 4.875$; $P < 0.001$; $n = 150$ for the entry and infection status interaction; $\Delta = 2.052$; $F = 6.901$; $P < 0.001$; $n = 150$ for the entry and year interaction; $\Delta = 0.610$; $F = 14.986$; $P < 0.001$; $n = 150$ for the infection status and year interaction; and $\Delta = 1.434$; $F = 3.988$; $P < 0.001$; $n = 150$ for the entry, infection status, and year interaction), 2012 ($\Delta = 2.514$; $F = 6.621$; $P < 0.001$; $n = 90$ for entry; $\Delta = 0.778$; $F = 21.668$; $P < 0.001$; $n = 90$ for infection status; and $\Delta = 1.659$; $F = 3.250$; $P < 0.001$; $n = 90$ for the entry and infection status interaction), and 2013 ($\Delta = 3.225$; $F = 6.939$; $P < 0.001$; $n = 60$ for entry; $\Delta = 0.941$; $F = 55.231$; $P < 0.001$; $n = 60$ for infection status; and $\Delta = 3.016$; $F = 5.806$; $P < 0.001$; $n = 60$ for the entry and

infection status interaction). Because of significant interactions, ANOVAs were repeated with treatment combinations separated out.

For both years and each year individually, all compounds were significantly different due to infection status with greater phenolic compound levels present in Lso-infected than noninfected tubers (Table 4). With a few exceptions, such as for flavonoid glycoside 1 and quinic acid derivative levels in 2013 only, Atlantic tubers that were Lso-infected had greater phenolic compound levels than Lso-infected tubers from the other entries (Table 4). Differences between entries when tubers were noninfected were much more variable and dependent on the particular phenolic compound analyzed (Table 4). However, noninfected BS2 tubers had greater levels of chlorogenic acid, the chlorogenic acid derivative, and protocatechuic acid glycoside when both years were analyzed as well as for each year individually (Table 4).

Regarding differences in fold changes due to entries, MANOVA suggested that overall phenolic levels significantly differed between entries for both years ($\Delta = 2.043$; $F = 2.637$; $P < 0.001$; $n = 60$ for entry; $\Delta = 0.709$; $F = 8.415$; $P < 0.001$; $n = 60$ for year; and $\Delta = 1.932$; $F = 2.404$; $P < 0.001$; $n = 60$ for the entry and year interaction), 2012 ($\Delta = 2.708$; $F = 1.934$; $P = 0.003$; $n = 30$), and 2013 ($\Delta = 3.502$; $F = 3.313$; $P < 0.001$; $n = 30$). When both years were analyzed together, almost every phenolic compound, with the exception of flavonoid glycoside 1, was greater in Atlantic in fold change than the ARS1, ARS2, ZC73, and ZC74 entries (Table 5). Levels of all analyzed phenolics in 2012 and all but three (a cryptochlorogenic acid derivate, flavonoid glycoside 1, and a quinic acid derivative) in 2013 had greater fold increases in Atlantic tubers than tubers from at least one other entry (Table 5). For both years ($F = 60.481$; $P < 0.001$; $n = 60$ for entry; $F = 12.048$; $P < 0.001$; $n = 60$ for year; and $F = 2.089$; $P = 0.083$; $n = 60$ for the entry and year interaction), 2012 ($F = 20.169$; $P < 0.001$; $n = 30$), and 2013 ($F = 49.460$; $P < 0.001$; $n = 29$), the mean fold change of phenolic compounds was significantly greater in Atlantic tubers than tubers from all other entries (Fig. 6).

The levels of all analyzed individual phenolics except the flavonoid methyl ester glycoside were significantly positively correlated ($P < 0.05$) with both fresh and fried symptoms. The flavonoid methyl ester was significantly positively correlated ($P < 0.05$) with only fresh symptoms. Lso titers were significantly positively correlated ($P < 0.05$) with levels of chlorogenic acid, cryptochlorogenic acid, cryptochlorogenic acid derivative, flavonoid glycoside 2, and protocatechuic acid glycoside.

DISCUSSION

After multiple years of screening for tolerance, a total of five potato advanced breeding clones exhibited little to no fresh and fried ZC symptoms when infected with Lso. Lso titers were also significantly reduced for at least some of the breeding clones relative to susceptible Atlantic, albeit only marginally statistically so in 2013.

Year-to-year variations were likely attributed to uncontrolled climatic factors which would affect vectoring efficacy and well as infection progression and tuber development. Indeed, much more variability in pathogen titers occurred in 2013 overall, but generally titers were much greater in that year, suggesting more favorable conditions for Lso infections to progress than 2012. Furthermore, climate likely influenced differences in symptoms and physiology between years, with two cultivars, BS2 in 2012 and ARS2 in 2013, having no statistical difference in evaluation of fried slices taken at the time of chemical analyses in one of the two years (albeit both did consistent differ when a larger number of fried slices were evaluated upon harvest). Greater tuber levels for many of the observed compounds were present in Lso-infected tubers overall in 2013, implying, with generally greater Lso titers, that infections proceeded further than in 2012 as well.

However, despite variations between years, overall findings demonstrate that Lso tolerance and possible resistance to Lso likely

Fig. 5. Fold differences of **A**, fructose or **B**, glucose levels for '*Candidatus Liberibacter solanacearum*'-infected tubers over control tubers of each entry for both years and each year separately. Bars represent standard errors. Different letters represent means that are significantly different by Tukey's honest significant difference test.

exists in wild species of potato, and can be exploited to create commercially viable, ZC-resistant cultivars. Additional breeding clones are being screened to discover even more possibly Lso-infection tolerant potato lines.

Unique to this study is an examination of host chemistry in an effort to find a potential mechanism of tolerance. Regarding differences in noninfected, constitutive (baseline) levels of metabolites between entries, few if any relationships to ZC progression and Lso titers were observed. Indeed, the majority of

differences between entries in noninfected tubers where greater levels of certain amino acids, sugars, and phenolics in BS2 compared with all other entries (including the susceptible Atlantic and putative tolerant varieties). This was likely the result of BS2 being bred to be a red marketing type of potato, whereas the others were chipping marketing types. Regardless, because of few differences in the physiology of Atlantic from the tested breeding lines, it seems unlikely that physiological markers of tolerance could be used prior to actual infection by Lso.

TABLE 4. Mean ($\pm$ SE) phenolic compound levels (μ g/g FW) separated by entry for both years and each year separately^z

									F		
Compound	Year	Treatment	Atlantic	ARS1	ARS2	BS2	ZC73	ZC74	Within treatment	Between year	Between treatment
Chlorogenic acid	Both	Control	4.58 ± 1.09 bc	2.42 ± 0.24 c	8.42 ± 2.18 abc	13.2 ± 3.2 a	6.67 ± 2.14 bc	10.6 ± 2.5 ab	6.454***	56.472***	81.091***
		Lso+	479 ± 82 a	29.0 ± 4.1 b	50.6 ± 9.2 b	66.9 ± 12.6 b	17.8 ± 3.6 b	30.3 ± 4.6 b	47.046***	28.432***	
	2012	Control	5.89 ± 1.88 bc	2.92 ± 0.21 c	13.3 ± 2.6 abc	20.5 ± 3.8 a	11.1 ± 2.9 abc	16.6 ± 2.7 ab	6.400***		69.520***
		Lso+	657 ± 76 a	21.5 ± 4.1 b	70.8 ± 8.3 b	85.2 ± 16.3 b	26.2 ± 2.7 b	20.4 ± 2.3 b	61.881***		
Chlorogenic acid derivative	Both	Control	7.65 ± 2.32 bc	5.28 ± 1.43 c	10.7 ± 2.6 bc	21.7 ± 5.7 a	3.76 ± 1.08 c	14.1 ± 3.7 b	13.060***	121.779***	126.776***
		Lso+	389 ± 59 a	26.0 ± 3.2 c	39.6 ± 7.3 bc	93.9 ± 19.2 b	18.6 ± 4.1 c	31.4 ± 3.4 c	69.234***	60.387***	
	2012	Control	13.2 ± 2.5 bc	9.17 ± 0.90 c	17.3 ± 2.4 bc	35.5 ± 5.9 a	6.08 ± 1.37 c	23.9 ± 2.4 ab	12.615***		135.768***
		Lso+	526 ± 42 a	23.4 ± 3.7 c	56.3 ± 5.9 bc	130 ± 22 b	28.2 ± 3.0 c	37.0 ± 2.1 c	100.785***		
Cryptochlorogenic acid	Both	Control	1.06 ± 0.23 b	0.600 ± 0.101 b	2.66 ± 0.42 ab	5.11 ± 0.72 a	0.978 ± 0.307 b	2.18 ± 1.51 ab	5.347**		25.242***
		Lso+	143 ± 43 a	30.7 ± 5.9 b	9.50 ± 2.82 b	29.6 ± 6.6 b	1.31 ± 0.19 b	21.2 ± 6.9 b	8.307***		
	2012	Control	1.68 ± 0.15 b	2.44 ± 0.30 ab	3.53 ± 0.25 ab	5.58 ± 0.91 a	2.76 ± 0.24 ab	5.04 ± 1.53 a	3.864**	0.435	104.081***
		Lso+	220 ± 31 a	26.0 ± 7.1 b	22.0 ± 2.9 b	39.6 ± 6.7 b	9.12 ± 1.85 b	24.4 ± 5.3 b	30.516***	1.565	
Cryptochlorogenic acid derivative	Both	Control	1.72 ± 0.18 b	3.21 ± 0.19 ab	3.13 ± 0.34 ab	5.51 ± 1.64 a	2.90 ± 0.19 ab	5.71 ± 1.00 a	3.814**		33.045***
		Lso+	229 ± 40 a	8.20 ± 1.79 b	20.6 ± 3.8 b	26.4 ± 4.6 b	12.2 ± 2.3 b	13.9 ± 1.8 b	27.857***		
	2013	Control	1.63 ± 0.26	1.51 ± 0.20	4.01 ± 0.26	5.66 ± 0.75	2.59 ± 0.49	4.24 ± 3.32	1.344		41.744***
		Lso+	204 ± 56 a	58.0 ± 6.8 b	24.4 ± 4.9 b	63.2 ± 10.7 b	3.66 ± 0.36 b	43.3 ± 10.5 b	8.941***		
Flavonoid glycoside 1	Both	Control	1.80 ± 0.36	2.54 ± 0.49	1.08 ± 0.11	2.68 ± 1.66	1.42 ± 0.25	7.19 ± 4.02	2.243	2.332	25.117***
		Lso+	168 ± 51 a	38.8 ± 14.0 b	13.5 ± 3.3 b	32.8 ± 13.1 b	5.36 ± 2.44 b	33.9 ± 12.9 b	5.499***	2.06	
	2012	Control	0.820 ± 0.146	1.74 ± 0.16	1.21 ± 0.11	4.08 ± 3.03	1.96 ± 0.29	1.28 ± 0.15	0.868		6.098*
		Lso+	217 ± 74 a	2.41 ± 0.64 b	5.52 ± 1.04 b	1.59 ± 0.46 b	8.18 ± 3.50 b	3.40 ± 2.06 b	8.253***		
Flavonoid glycoside 2	Both	Control	2.98 ± 0.21	3.51 ± 0.92	0.919 ± 0.191	1.01 ± 0.16	0.776 ± 0.173	14.3 ± 8.1	2.442		68.330***
		Lso+	79.5 ± 33.5 a	104 ± 10 a	28.0 ± 3.6 ab	89.0 ± 18.3 a	0.274 ± 0.075 b	88.8 ± 17.9 a	5.348**		
	2012	Control	5.48 ± 2.73 b	2.67 ± 0.73 b	1.77 ± 0.85 b	15.1 ± 5.0 a	6.13 ± 1.71 b	9.27 ± 3.32 ab	4.663**	35.239***	72.224***
		Lso+	35.2 ± 5.7 a	19.9 ± 4.1 bc	11.2 ± 1.8 cd	24.7 ± 2.5 b	9.27 ± 2.55 d	8.77 ± 1.33 d	15.723***	2.263	
Flavonoid glycoside 3	Both	Control	9.73 ± 4.40 b	4.39 ± 0.75 b	2.43 ± 1.48 b	26.8 ± 5.6 a	10.9 ± 0.9 ab	13.9 ± 5.3 ab	5.453**		14.482***
		Lso+	45.9 ± 6.2 a	9.59 ± 1.73 c	8.08 ± 1.77 c	27.9 ± 2.9 b	13.9 ± 3.0 c	10.1 ± 1.9 c	20.365***		
	2013	Control	0.391 ± 0.080	0.601 ± 0.386	0.973 ± 0.644	1.08 ± 0.17	0.453 ± 0.205	3.71 ± 2.09	1.904		183.605***
		Lso+	15.8 ± 3.6 bc	38.5 ± 2.9 a	16.8 ± 2.2 bc	19.0 ± 3.4 b	0.953 ± 0.515 d	6.44 ± 1.25 cd	25.391***		
Flavonoid glycoside 4	Both	Control	0.181 ± 0.039 b	0.505 ± 0.143 a	0.339 ± 0.068 ab	0.113 ± 0.029 b	0.292 ± 0.079 ab	0.320 ± 0.070 ab	6.440***	1.314	15.075***
		Lso+	19.8 ± 5.6 a	3.99 ± 1.77 b	1.06 ± 0.26 b	1.81 ± 0.60 b	0.391 ± 0.068 b	1.32 ± 0.22 b	7.942***	0.553	
	2012	Control	0.232 ± 0.061 a	0.171 ± 0.029 a	0.503 ± 0.067 a	0.176 ± 0.035 a	0.431 ± 0.118 a	0.387 ± 0.120 a	3.124*		8.592**
		Lso+	24.9 ± 8.1 a	0.454 ± 0.113 b	1.48 ± 0.33 b	2.50 ± 0.85 b	0.530 ± 0.066 b	1.65 ± 0.28 b	8.426***		
Flavonoid methyl ester glycoside	Both	Control	0.119 ± 0.036 b	0.906 ± 0.195 a	0.143 ± 0.030 b	0.0366 ± 0.0071 b	0.126 ± 0.024 b	0.240 ± 0.043 b	14.562***		16.865***
		Lso+	10.7 ± 3.7 a	10.4 ± 3.6 a	0.315 ± 0.078 b	0.564 ± 0.276 b	0.139 ± 0.034 b	0.708 ± 0.144 b	6.134***		
	2012	Control	0.246 ± 0.041 b	0.374 ± 0.040 b	1.42 ± 0.35 a	0.241 ± 0.060 b	0.609 ± 0.210 b	0.500 ± 0.113 b	9.955***	15.088***	61.798***
		Lso+	13.3 ± 3.0 a	3.68 ± 0.42 b	6.04 ± 1.19 b	5.61 ± 1.02 b	2.77 ± 0.55 b	3.96 ± 0.70 b	14.641***	6.196***	
Protocatechuic acid glycoside	Both	Control	0.189 ± 0.050 b	0.449 ± 0.053 b	2.31 ± 0.33 a	0.224 ± 0.106 b	0.729 ± 0.393 b	0.612 ± 0.183 b	12.017***		49.083***
		Lso+	17.5 ± 4.1 a	4.05 ± 0.42 b	8.71 ± 1.03 b	5.77 ± 1.39 b	4.15 ± 0.31 b	4.03 ± 0.86 b	8.040***		
	2013	Control	0.314 ± 0.057	0.284 ± 0.032	0.357 ± 0.072	0.261 ± 0.054	0.465 ± 0.038	0.365 ± 0.110	1.229		46.600***
		Lso+	5.71 ± 1.12 a	3.02 ± 0.89 ab	1.24 ± 0.20 ab	5.32 ± 1.57 a	0.271 ± 0.040 b	3.83 ± 1.37 ab	4.410**		
Quinic acid derivative	Both	Control	0.674 ± 0.292 b	1.16 ± 0.24 ab	1.95 ± 0.61 a	0.939 ± 0.159 b	0.470 ± 0.119 b	0.604 ± 0.155 b	4.802**	37.871***	32.348***
		Lso+	59.8 ± 15.8 a	12.2 ± 1.88 b	12.8 ± 2.6 b	13.6 ± 2.7 b	5.72 ± 1.28 b	6.57 ± 1.30 b	8.706***	17.993***	
	2012	Control	1.19 ± 0.44 b	1.79 ± 0.19 ab	3.20 ± 0.83 a	1.11 ± 0.19 b	0.715 ± 0.155 b	0.984 ± 0.154 b	4.919**		32.589***
		Lso+	86.2 ± 19.6 a	12.3 ± 2.8 b	18.7 ± 2.4 b	18.3 ± 3.2 b	8.84 ± 0.85 b	8.61 ± 1.62 b	13.135***		
Quinic acid derivative	Both	Control	0.0534 ± 0.0238 b	0.412 ± 0.072 ab	0.460 ± 0.139 ab	0.733 ± 0.256 a	0.175 ± 0.045 b	0.147 ± 0.038 b	4.091**		118.960***
		Lso+	12.3 ± 1.9 a	12.1 ± 1.9 a	2.37 ± 0.45 b	5.05 ± 0.92 b	0.117 ± 0.033 b	2.91 ± 0.866 b	18.001***		
	2012	Control	14.0 ± 7.4 b	11.4 ± 1.9 b	6.93 ± 2.31 b	43.9 ± 10.0 a	21.6 ± 4.4 b	17.9 ± 6.0 b	6.033***	19.619***	47.276***
		Lso+	78.9 ± 7.1 a	29.3 ± 4.3 b	22.8 ± 3.8 b	60.3 ± 7.5 a	26.2 ± 6.9 b	21.3 ± 2.6 b	21.759***	12.308***	
Quinic acid derivative	Both	Control	23.4 ± 12.7 b	13.2 ± 2.2 b	6.19 ± 4.0 b	64.1 ± 13.6 a	33.4 ± 2.2 ab	25.1 ± 10.2 b	5.162**		14.489***
		Lso+	90.5 ± 8.6 a	20.0 ± 3.9 b	21.2 ± 6.0 b	74.4 ± 8.4 a	39.1 ± 7.8 b	22.6 ± 3.8 b	20.672***		
	2013	Control	2.58 ± 0.27 b	9.30 ± 3.22 b	7.82 ± 2.27 b	19.6 ± 1.6 a	7.55 ± 2.43 b	9.27 ± 2.47 b	6.168***		197.335***
		Lso+	58.1 ± 4.9 a	46.1 ± 2.6 ab	25.7 ± 1.1 cd	35.0 ± 3.6 bc	2.79 ± 0.73 c	18.9 ± 3.1 d	41.777***		
Quinic acid derivative	Both	Control	3.74 ± 1.40 b	1.63 ± 0.11 b	1.95 ± 0.31 b	12.9 ± 3.9 a	3.24 ± 0.35 b	2.20 ± 0.32 b	14.245***	21.564***	55.438***
		Lso+	33.2 ± 6.7 a	2.40 ± 0.26 c	2.71 ± 0.24 c	21.2 ± 4.6 b	7.17 ± 0.83 c	3.48 ± 0.41 c	43.936***	115.526***	
	2012	Control	5.22 ± 2.50 b	1.69 ± 0.13 b	1.08 ± 0.10 b	22.8 ± 3.7 a	2.85 ± 0.32 b	2.72 ± 0.44 b	20.165***		68.010**
		Lso+	50.1 ± 3.6 a	2.99 ± 0.21 c	2.57 ± 0.36 c	32.0 ± 3.6 b	7.35 ± 1.29 c	3.02 ± 0.40 c	86.728***		
2013	Control	1.97 ± 0.09 bc	1.55 ± 0.19 c	2.99 ± 0.15 ab	1.03 ± 0.14 c	3.72 ± 0.65 a	1.57 ± 0.32 c	10.149***		27.822***	
	Lso+	2.92 ± 0.44 bc	1.34 ± 0.11 c	2.96 ± 0.21 bc	1.70 ± 0.29 c	6.85 ± 0.48 a	4.32 ± 0.85 b	18.959***			

Regarding tuber levels of metabolites in Lso-inoculated plants, many more differences were observed between entries for both years of this study. This was important because changes in host chemistry have been consistently linked to ZC symptom development in tubers. This study examined shifts in amino acids, sugars, and phenolics that occurred after being infected with Lso for a substantial period (Rashed et al. 2013; Wallis et al. 2014). Positive correlations were indeed observed between the levels of many of these compounds with observed symptoms, again confirming previous reports that shifts in host chemistry were associated with ZC progression (Rashed et al. 2013).

For the majority of compounds in both years of this study, infected Atlantic had greater levels than any other entry. This was despite that, in many cases, healthy tubers of Atlantic did not initially chemically differ from those of tubers of breeding clones. Especially apparent was that when comparing fold increases in compound levels of infected tuber levels compared with non-infected tubers, Atlantic tubers generally underwent far greater increases when infected compared with any other of the entries. Since most compounds were positively correlated with symptoms, it therefore makes sense that such large increases in compound levels of Atlantic tubers following Lso infection resulted in very apparent ZC symptoms, whereas in the other entries symptoms were missing or less apparent. Since a very clear induction of many analyzed compounds occurred in Atlantic versus other entries following Lso infection, the possibility exists that transcripts or proteins might be discovered that reduce host responses to Lso, which in turn could be useful resistance markers for breeding programs. Such hypothetical proteins may be expressed on the surface (with leucine-rich repeat domains) and suppress or block host recognition of the pathogen instead of triggering a host response. This study therefore suggests that if an Lso tolerance

marker is found through genomic analyses it should be screened to see if it has putative host physiology regulatory functions in addition to suppression of pathogen growth.

It should be noted that this study, due to the complexities of chemical analyses as well as a limited amount of field space for repeated screenings, omitted breeding lines exhibiting no to light ZC symptoms when freshly cut, which was an additional 24 lines compared with the five selected for analyses here. The study also did not include breeding lines exhibiting moderate and not severe symptoms. Inclusion of these additional lines should be considered in future studies as these could provide further support that a gradient of host physiological responses results in a gradient of ZC symptom development. In addition, physiological responses of one

Fig. 6. Fold differences of phenolic compound levels for ‘*Candidatus Liberibacter solanacearum*’-infected tubers over control tubers of each entry for both years and each year separately. Bars represent standard errors. Different letters represent means that are significantly different by Tukey’s honest significant difference test.

TABLE 5. Mean ($\pm$ SE) fold differences of ‘*Candidatus Liberibacter solanacearum*’ (Lso)-infected over control levels of each individual phenolic compound by entry for both years and each year separately^z

Compound	Year	Atlantic	ARS1	ARS2	BS2	ZC73	ZC74	<i>F</i>	
								Between entry	Between year
Chlorogenic acid	Both	191.39 $\pm$ 86.37 a	15.16 $\pm$ 3.80 b	6.66 $\pm$ 1.03 b	8.17 $\pm$ 2.13 b	3.37 $\pm$ 0.83 b	10.03 $\pm$ 3.74 b	5.513***	2.302
	2012	328.18 $\pm$ 155.26 a	5.10 $\pm$ 1.80 b	6.88 $\pm$ 1.63 b	7.71 $\pm$ 3.89 b	3.50 $\pm$ 0.78 b	1.66 $\pm$ 0.76 b	4.330**	
	2013	54.61 $\pm$ 10.33 a	25.22 $\pm$ 3.37 b	6.45 $\pm$ 1.44 cd	8.64 $\pm$ 2.28 cd	3.23 $\pm$ 1.58 d	18.41 $\pm$ 5.23 bc	14.056***	
Chlorogenic acid derivative	Both	128.62 $\pm$ 58.34 a	27.74 $\pm$ 9.47 b	3.67 $\pm$ 0.61 b	5.20 $\pm$ 0.93 b	4.30 $\pm$ 1.15 b	24.19 $\pm$ 16.01 b	4.544**	5.957*
	2012	39.78 $\pm$ 5.47 a	2.49 $\pm$ 0.76 b	3.34 $\pm$ 0.55 b	4.15 $\pm$ 1.05 b	6.59 $\pm$ 1.73 b	1.68 $\pm$ 0.29 b	37.870***	
	2013	217.45 $\pm$ 106.48 a	52.99 $\pm$ 9.18 b	4.00 $\pm$ 1.14 b	6.25 $\pm$ 1.50 b	2.00 $\pm$ 0.59 b	46.70 $\pm$ 30.01 b	3.334*	
Cryptochlorogenic acid	Both	124.75 $\pm$ 19.37 a	21.37 $\pm$ 6.63 b	6.55 $\pm$ 1.09 b	9.81 $\pm$ 2.06 b	3.72 $\pm$ 1.01 b	54.31 $\pm$ 35.72 b	8.623***	3.444
	2012	122.31 $\pm$ 28.92 a	2.83 $\pm$ 0.83 b	7.06 $\pm$ 2.05 b	7.43 $\pm$ 2.98 b	5.72 $\pm$ 1.55 b	2.48 $\pm$ 0.52 b	16.135***	
	2013	127.19 $\pm$ 29.14 a	39.92 $\pm$ 5.05 bc	6.03 $\pm$ 0.99 c	12.19 $\pm$ 2.71 c	1.72 $\pm$ 0.43 c	106.13 $\pm$ 66.31 ab	3.386*	
Cryptochlorogenic acid derivative	Both	103.80 $\pm$ 39.22 a	21.79 $\pm$ 9.60 b	19.11 $\pm$ 6.23 b	50.06 $\pm$ 18.19 ab	4.92 $\pm$ 3.21 b	12.19 $\pm$ 6.31 b	6.652***	0.182
	2012	178.60 $\pm$ 62.83 a	1.07 $\pm$ 0.53 b	3.87 $\pm$ 1.38 b	2.94 $\pm$ 1.28 b	9.46 $\pm$ 6.00 b	0.97 $\pm$ 0.30 b	7.690***	
	2013	29.00 $\pm$ 13.37 bc	42.51 $\pm$ 14.12 ab	34.36 $\pm$ 7.51 bc	97.17 $\pm$ 19.43 a	0.37 $\pm$ 0.09 c	23.41 $\pm$ 10.78 bc	6.780***	
Flavonoid glycoside 1	Both	28.02 $\pm$ 9.92 b	101.64 $\pm$ 46.43 a	28.61 $\pm$ 11.06 b	11.11 $\pm$ 4.78 b	3.20 $\pm$ 2.10 b	13.01 $\pm$ 8.03 b	5.520***	19.806***
	2012	5.13 $\pm$ 1.00 a	3.33 $\pm$ 0.77 ab	7.00 $\pm$ 2.84 a	0.95 $\pm$ 0.28 b	1.11 $\pm$ 0.28 b	0.83 $\pm$ 0.39 b	3.982**	
	2013	50.90 $\pm$ 13.40 b	199.95 $\pm$ 69.76 a	50.23 $\pm$ 17.58 b	21.27 $\pm$ 7.14 b	5.29 $\pm$ 4.19 b	25.19 $\pm$ 14.69 b	5.418**	
Flavonoid glycoside 2	Both	93.94 $\pm$ 20.68 a	9.74 $\pm$ 4.24 b	3.36 $\pm$ 0.85 b	33.15 $\pm$ 20.10 b	1.59 $\pm$ 0.35 b	10.17 $\pm$ 5.73 b	7.891***	0.436
	2012	98.17 $\pm$ 35.81 a	4.07 $\pm$ 1.55 b	4.00 $\pm$ 1.53 b	47.36 $\pm$ 40.06 ab	1.75 $\pm$ 0.51 b	17.01 $\pm$ 11.11 b	2.887*	
	2013	89.71 $\pm$ 25.16 a	15.42 $\pm$ 7.89 b	2.71 $\pm$ 0.85 b	18.94 $\pm$ 10.58 b	1.44 $\pm$ 0.53 b	3.32 $\pm$ 0.91 b	8.572***	
Flavonoid glycoside 3	Both	54.17 $\pm$ 23.56 a	9.03 $\pm$ 1.23 b	4.02 $\pm$ 0.88 b	31.97 $\pm$ 6.99 ab	6.54 $\pm$ 3.17 b	12.02 $\pm$ 4.76 b	4.038**	3.133
	2012	87.36 $\pm$ 43.81 a	7.92 $\pm$ 0.88 b	3.27 $\pm$ 0.39 b	36.83 $\pm$ 7.75 ab	12.46 $\pm$ 5.25 b	12.34 $\pm$ 9.39 b	2.916*	
	2013	20.98 $\pm$ 5.36 ab	10.15 $\pm$ 2.32 bc	4.78 $\pm$ 1.74 bc	27.12 $\pm$ 12.16 a	0.62 $\pm$ 0.14 c	11.69 $\pm$ 3.71 abc	2.971*	
Flavonoid glycoside 4	Both	429.03 $\pm$ 215.07 a	19.23 $\pm$ 4.82 b	10.40 $\pm$ 4.45 b	17.91 $\pm$ 4.78 b	8.17 $\pm$ 2.69 b	27.28 $\pm$ 15.87 b	4.844**	4.220*
	2012	58.71 $\pm$ 12.87 a	6.65 $\pm$ 0.57 b	14.62 $\pm$ 8.83 b	24.34 $\pm$ 7.83 b	15.41 $\pm$ 2.51 b	6.99 $\pm$ 1.24 b	7.305***	
	2013	799.36 $\pm$ 373.38 a	31.80 $\pm$ 5.01 b	6.18 $\pm$ 1.45 b	11.48 $\pm$ 4.57 b	0.93 $\pm$ 0.30 b	47.56 $\pm$ 30.44 b	4.344**	
Flavonoid methyl ester glycoside	Both	54.35 $\pm$ 14.76 a	6.15 $\pm$ 1.78 b	4.90 $\pm$ 1.34 b	2.39 $\pm$ 0.31 b	2.49 $\pm$ 0.99 b	1.75 $\pm$ 0.32 b	14.535***	2.673
	2012	30.45 $\pm$ 4.75 a	4.46 $\pm$ 1.23 b	7.42 $\pm$ 2.18 b	2.17 $\pm$ 0.48 b	4.31 $\pm$ 1.66 b	1.36 $\pm$ 0.18 b	22.914***	
	2013	78.26 $\pm$ 25.92 a	7.84 $\pm$ 3.35 b	2.38 $\pm$ 0.34 b	2.62 $\pm$ 0.42 b	0.67 $\pm$ 0.19 b	2.14 $\pm$ 0.59 b	8.310***	
Protocatechuic acid glycoside	Both	14.86 $\pm$ 3.74 a	4.84 $\pm$ 1.37 b	6.38 $\pm$ 1.83 b	1.53 $\pm$ 0.26 b	0.94 $\pm$ 0.29 b	2.06 $\pm$ 0.57 b	16.045***	15.079***
	2012	5.52 $\pm$ 1.32 ab	2.18 $\pm$ 0.51 bc	7.13 $\pm$ 3.23 a	1.16 $\pm$ 0.32 c	1.01 $\pm$ 0.26 c	1.22 $\pm$ 0.58 c	3.202*	
	2013	24.20 $\pm$ 4.20 a	7.49 $\pm$ 2.15 bc	5.64 $\pm$ 2.09 c	1.90 $\pm$ 0.35 bc	0.87 $\pm$ 0.56 c	2.91 $\pm$ 0.88 bc	16.313***	
Quinic acid derivative	Both	9.92 $\pm$ 3.95 a	1.38 $\pm$ 0.20 b	1.58 $\pm$ 0.31 b	1.55 $\pm$ 0.25 b	2.12 $\pm$ 0.28 b	2.11 $\pm$ 0.61 b	7.347***	7.222**
	2012	18.33 $\pm$ 5.89 a	1.85 $\pm$ 0.24 b	2.14 $\pm$ 0.51 b	1.31 $\pm$ 0.34 b	2.23 $\pm$ 0.53 b	0.91 $\pm$ 0.16 b	7.855***	
	2013	1.50 $\pm$ 0.23 b	0.91 $\pm$ 0.13 b	1.01 $\pm$ 0.12 b	1.78 $\pm$ 0.36 b	2.00 $\pm$ 0.26 b	3.31 $\pm$ 0.96 a	3.837*	

^z Values over 1 represent greater levels in Lso-infected tubers, whereas values under 1 represent levels greater in noninfected control. *F* statistics are provided. Different letters represent significant mean separations by Tukey’s honest significant difference test. **P* < 0.05; ***P* < 0.01; and ****P* < 0.001.

type of compound, such as phenolics, may be distinct from those of other types, such as sugars and amino acids, leading to the reduction of some visible symptoms (such as fresh symptoms) but not others (such as fried symptoms).

Results from this study support a hypothesis that *Liberibacter* bacteria such as Lso may exist as non-disease-causing endophytic parasites in wild host species. With the expansion of commercial agriculture, these bacteria were able to parasitize commercial cultivars of plants that displayed increased sensitivity to infections and therefore greater symptom development, likely because when commercial cultivars were developed traits beneficial to commercial success were selected and not traits providing increased resistance to unforeseen parasites. If true, larger-scale studies aimed at purposely identifying and characterizing endophytic bacteria and other microorganisms appear warranted in wild plant relatives of many commercial crops, as these might eventually become important emerging pathogens in the future when finally given access to susceptible hosts, efficient vectors, or if they are moved into new areas.

These results also hypothetically could be applied to additional *Liberibacter*-caused diseases, such as citrus greening (huanglongbing, caused by ‘*Candidatus Liberibacter asiaticum*’, ‘*Candidatus Liberibacter americanus*’, or ‘*Candidatus Liberibacter africanus*’) or recent *Liberibacter*-caused diseases of carrots and celery in Europe (with European strains of Lso as putative causal agents) (Munyaneza 2012). For those diseases, it remains relatively unknown what changes in host physiology occur throughout the infection process. However, it is plausible that because *Liberibacter* bacteria live an endophytic lifestyle within plant hosts, they likely evolved in wild host species and, as a result, germplasm from such sources may provide tolerance in the form of milder host responses to infection.

In conclusion, this study demonstrated that Lso tolerance in potato germplasm is present and is characterized by reduced Lso titers or even changes in tuber biochemistry relative to susceptible potato cultivars such as Atlantic. This potato germplasm as well as other wild solanaceous species-derived germplasm can be used in developing new potato varieties for those potato growing regions where ZC is a major concern. This study also observed that a lack of biochemical changes upon Lso infection might be a potential mechanism for ZC resistance in addition to reductions in Lso progression, and this trait can be screened for in future trials to develop new varieties. Alleles that control the intensity of host response to Lso could eventually be decoded and used as genetic markers of ZC resistance to speed selections and ultimately mitigate the threat ZC disease poses to potato production everywhere.

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