Effects of Potato-Psyllid-Vectored '*Candidatus Liberibacter solanacearum*' Infection on Potato Leaf and Stem Physiology

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ABSTRACT

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The bacterium '*Candidatus Liberibacter solanacearum*' is associated with zebra chip disease (ZC), a threat to potato production in North America and New Zealand. It is vectored by potato psyllids. Previous studies observed that '*Ca. L. solanacearum*' infection causes potato tubers to undergo ZC-symptom-associated shifts in physiology, such as increased levels of amino acids, sugars, and phenolics. However, little is known about how '*Ca. L. solanacearum*' infections caused by psyllid vector feeding may affect metabolism in potato foliage and stems. This study compared metabolism in potato plants fed upon by '*Ca. L. solanacearum*'-

positive psyllids with potato plants not exposed to psyllids. Foliar levels of asparagine, aspartic acid, glutamine, fructose, glucose, sucrose, a ferulic acid derivative, and quinic acid were lower in '*Ca. L. solanacearum*'-inoculated than noninfected plants. However, foliar levels of proline, serine, four phenolic compounds, and most terpenoids were greater in '*Ca. L. solanacearum*'-inoculated than noninfected plants. Upper stem levels of asparagine and aspartic acid, upper and lower stem levels of ellagitannins and most monoterpenoids, and lower stem level of sesquiterpenoids were greater in '*Ca. L. solanacearum*'-inoculated than noninfected plants. These results suggest that many defense-related terpenoid compounds might increase in plants which had psyllids inoculate '*Ca. L. solanacearum*'. This could impact progression and spread of ZC.

Additional keyword: carbohydrates.

Potato (*Solanum tuberosum* L.) production in Central America, western North America, and New Zealand is threatened by zebra chip disease (ZC), putatively caused by '*Candidatus Liberibacter solanacearum*' (syn. '*Ca. L. psyllaurosus*') (10,11,21,29,35,46). Other potato-growing regions could have ZC spread to them as well (18). Effective ZC management currently involves costly (≈\$700/ha) chemical control programs for the potato psyllid vector, *Bactericera cockerelli* (Sulc) (16). Long-term management would need to exploit natural host resistance, including discovering resistance markers for reduced symptom development when plants are infected with '*Ca. L. solanacearum*' or potato plants where vectoring efficiency is reduced (27,28).

Characteristic ZC symptoms include increased oxidation (browning) of freshly cut tubers and brown striping ("zebra stripes") when tuber slices are fried (27,36,46). ZC also causes symptoms in aerial tissues of potato plants, including plant dwarfing, formation of aerial tubers, leaf scorch, chlorosis, and plant collapse (27,36,46).

Tuber ZC symptoms are associated with increased levels of phenolics (1,30,33,41,44), reducing sugars (glucose and fructose) (7,8,15,33,44), defense-associated enzymes (1,33,41), amino acids (e.g., isoleucine, leucine, serine, tyrosine, and valine) (30,33,41, 44), and minerals (e.g., phosphorus, potassium, and calcium) (24).

All of these physiological changes could be attributed to host attempts to combat infection via a hypersensitive response (25). In particular, increased levels of phenolics and terpenoids are

often associated with hypersensitive responses and other general coordinated host responses to pathogens (5,17,31,42). Phenolic compounds are precursors to the cell-wall-thickening lignin and tannins, which could make host tissues harder to penetrate by microorganisms (42,43). Likewise, both phenolics and terpenoids have putative antibiotic effects against pathogens (19,20). Changes in levels of other compounds may also occur as part of a host defense response. For instance, certain amino acids might become precursors to particular antibiotic or antiherbivory compounds (6,26). However, in the case of '*Ca. L. solanacearum*', it remains unclear whether or not the bacterium is negatively impacted by host physiological changes, although some evidence exists that it is impacted somewhat because lower titers may be present where host physiological changes are greatest (34,41,44).

Regardless, it is hypothesized that changes in host physiology result directly in observed ZC symptoms (41). Browning of freshly cut potato tubers would be expected to increase with greater phenolic and polyphenol oxidase levels, because polyphenol oxidases utilize chlorogenic acid and similar phenolics to form brown-colored products upon exposure to air (23,41). Greater browning of fried potato tuber slices would be expected with increased sugar and amino acid levels in tubers, because these compounds cross-link to form a brown acrylamide product at frying temperatures via the Maillard reaction (14,39,41).

However, what physiological changes may occur in leaves and stems of '*Ca. L. solanacearum*'-infected potato plants remains unclear. Physiological shifts could be hypothesized to be associated with the aerial symptoms of ZC such as dwarfing, aerial tuber formation, chlorosis, leaf scorching, and plant collapse (30,36). Because '*Ca. L. solanacearum*' is vectored by potato psyllids, the combination of host responses to potato psyllid vectors as well as host responses to '*Ca. L. solanacearum*' itself

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could result in these symptoms. It has been shown previously that the combination of '*Ca. L. solanacearum*' infection and psyllid feeding together inflicted much greater symptom formation on potato hosts, and likely changed physiology greater, than when psyllid feeding occurred alone (37). In terms of previous studies examining shifts in host physiology associated with ZC, preliminary work focused on examining foliar sugar and starch content from plants fed upon by psyllids that were likely '*Ca. L. solanacearum*' positive (15). Leaves from psyllid-infested potato plants had lower sugar and greater starch levels than noninfested controls (15). Furthermore, plants with previous '*Ca. L. solanacearum*'-positive potato psyllid infestations had greater terpenoid levels than those that were previously noninfested (13).

Expanded knowledge about the physiological changes that potato leaves and stems undergo when infected with '*Ca. L. solanacearum*' via psyllid inoculation could aid in the development of effective ZC management strategies. For instance, if there is reduced amino acid and sugar production as a result of '*Ca. L. solanacearum*' infection, then fewer and smaller tubers would develop in ZC diseased plants. This is important if management strategies reduce the expression of ZC-associated browning in tubers but fail to address the effects of '*Ca. L. solanacearum*' infection and potato psyllid feeding on aerial portions of the host. Furthermore, increased understanding of the host-pathogen interactions of potato infected with '*Ca. L. solanacearum*' could be useful in development of '*Ca. L. solanacearum*' resistance or ZC tolerance markers for potato breeding programs.

In addition to potential effects on '*Ca. L. solanacearum*' success, infection-induced changes in potato host leaf or stem physiology due to '*Ca. L. solanacearum*' infections could manipulate potato host attractiveness to additional feeding by potato psyllids. This, in turn, could affect the likelihood of '*Ca. L. solanacearum*' spread beyond initially infected plants. Often, plants may respond to pathogen infections and insect infestations by producing greater levels of secondary metabolites such as certain phenolics and terpenoids, both of which could influence insect behaviors (42). Davis et al. (13) observed that potato psyllids were more likely to settle on previously '*Ca. L. solanacearum*'-positive psyllid-infested potato plants than those that were not previously infested, although this effect became nonsignificant later. This observation might be the result of increased terpenoid levels that occurred in psyllid-infested host plants (13). Alternatively, differences in psyllid preference may have been the result of other, nonexamined changes in leaf and stem physiology.

This study examined levels of both primary (amino acids and carbohydrates) and secondary (phenolics and terpenoids) metabolites in healthy potato plants ('Russet Norkotah') and those infected with '*Ca. L. solanacearum*' by potato psyllid vectors. This was done to address the hypothesis that '*Ca. L. solanacearum*' infections started by psyllid vectors significantly alter potato leaf and stem physiology. Furthermore, relationships were determined between levels of these host compounds with ZC symptoms and '*Ca. L. solanacearum*' titers. Knowledge gained from this study is useful in understanding the effects that '*Ca. L. solanacearum*' infection has on ZC symptom progression of aerial parts of the potato host, with implications for ZC management.

MATERIALS AND METHODS

Experimental design. Russet Norkotah seed potato plants were planted on 19 April 2012 at Texas A&M AgriLife Research and Extension Center in Bushland, TX, and covered with tent-shaped mesh cages (100 by 100 by 100 cm) before plant emergence. In May, plant thinning was conducted to reduce the number of plants to four in each cage. Treatments were assigned to the plants in a randomized, complete block design.

Plants were infected with '*Ca. L. solanacearum*' according to the methods of Wallis et al. (44). In brief, potato psyllids of a

central haplotype (40), randomly selected from a '*Ca. L. solanacearum*'-positive greenhouse colony, were used to infest 16 potato plants (4 plants in each of four replicate cages). To confirm the '*Ca. L. solanacearum*'-positive status of the colony, three composite samples of 10 psyllids were tested prior to our experiments. In addition, the greenhouse colony was tested for other inoculation studies and on a weekly basis. It never tested negative for '*Ca. L. solanacearum*'. The biotype of '*Ca. L. solanacearum*' in the potato psyllid colony was determined to be a mix of '*Ca. L. solanacearum*' A and B (45). '*Ca. L. solanacearum*' inoculations involved releasing 30 psyllids from the '*Ca. L. solanacearum*'-positive colony at the base of a single plant in each 100-cm³ cage containing four plants 4 weeks prior to harvest. Another 16 potato plants were left noninfested as controls (4 plants in each of four replicate cages). After 1 week, both infested and noninfested plants were thoroughly sprayed with a mix of the insecticides Movento (Bayer CropScience), Agri-Mek (Syngenta Crop Protection, LLC), and Fulfill (Syngenta) to remove the potato psyllids. The pesticide mix was reapplied a week later to both infested and noninfested plants to assure insect removal.

Plants were harvested on 26 July 2012. Plants were cut at soil level, bagged, labeled, and placed immediately on dry ice. These plants were then shipped overnight on dry ice to San Joaquin Valley Agricultural Sciences Center, United States Department of Agriculture-Agricultural Research Service, in Parlier, CA. Overall aerial ZC symptoms were assessed on a scale of 0 to 3, where 0 = healthy; 1 = lightly symptomatic, less than 25% discolored tissue; 2 = moderately symptomatic, 25 to 75% discolored tissue; and 3 = severely infected, greater than 75% tissue discoloration. The ratings are equivocal to the 0-to-4 scale visualized within Rashed et al. (31), with plants rated 4 = dead not sampled for this study.

'*Ca. L. solanacearum*' titer and biochemical analyses. Potato leaf and stem samples were stored at -20°C before biochemical analyses. Upon removal from storage, the samples were separated by leaf, upper stem material (within ≈10 cm of the apical end of the plant), and lower stem material (within ≈10 cm of the soil line). Regardless of leaf or stem material, the tissues were pulverized via mortar and pestle in liquid nitrogen. For each sample, three 0.10-g aliquots were weighed out and placed into separate, labeled 1.5-ml microcentrifuge tubes, and flash-frozen in liquid nitrogen to prevent oxidation.

Additional tissue aliquots had DNA extracted using the DNeasy Plant mini kit (Qiagen, Valencia, CA) for titer analyses. The methods of Wallis et al. (41) were used to conduct quantitative polymerase chain reaction (PCR) for the determination of '*Ca. L. solanacearum*' titers. In brief, each quantitative PCR 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 (21), and 9.5 µl of water for a total reaction solution of 25 µl (41). A DNA Engine Opticon 2 Real Time PCR Detection System (Bio-Rad) was used to monitor the reactions, Cycle threshold values were calculated by the system software, and standard curves of known DNA amounts were performed to tabulate '*Ca. L. solanacearum*' titers of each sample (41).

The methods of Wallis et al. (42), Rashed et al. (33), and Wallis et al. (44) were employed to analyze plant biochemistry. In brief, the 0.10-g aliquots were twice-extracted overnight at 4°C in 0.5 ml of phosphate-buffered saline (PBS) solution (pH 7.8) to analyze amino acids and carbohydrates, 0.5 ml of methanol (Sigma-Aldrich, St. Louis) to analyze phenolic compounds, or 0.5 ml of methyl tert-butyl ether (MTBE) (Sigma-Aldrich) to analyze terpenoids (for a total of 1 ml of each extract).

A total of 100 µl of the PBS buffer extract was used to assess amino acids using the commercially available kit from Phomenex (Torrance, CA). Processed amino acid extracts then were analyzed with a Shimadzu (Columbia, MD) GC-2010 gas chro-

matograph (GC) with a flame ionization detector (FID) and a Zebtron AAA column, with identification and quantification performed using standard preparations from the Phenomenex amino acid kit.

To analyze carbohydrates, 50 µl of the PBS buffer extract was injected into a Shimadzu high-performance liquid chromatograph (HPLC) equipped with Supelco C-611 carbohydrate column (300 by 7.8 mm) (Sigma-Aldrich) and a refractive index detector (RID-10 from Shimadzu) (33). Fructose, glucose, and sucrose commercial standards (all from Sigma-Aldrich) were used to confirm peak identities and to convert peak areas to gram amounts.

To analyze phenolics, 50 µl of methanol extract 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) (33,44). A binary solvent program was used, proceeding for 95% solvent A (water with 0.2% [vol/vol] acetic acid) (Sigma-Aldrich) to 100% solvent B (methanol with 0.2% [vol/vol] acetic acid) and back to reset for the following injection over 40 min. Most compounds were previously identified by Wallis et al. (44). However, additional phenolic compounds were identified and all compounds quantified into milligrams per gram fresh weight (FW) by matching retention times with commercial standards (from Sigma-Aldrich), matching UV/Vis spectra of peaks, or determining molecular weights by running representative samples on a Shimadzu LCMS-2020 liquid chromatograph-mass spectrometer with the same binary gradient program. Peaks were converted to gram amounts using compounds within the same compound class when commercial standards were unavailable (33).

To analyze terpenoids and volatile compounds, 1 µl of the MTBE extract was injected into a Shimadzu GC-2010 GC system with a βDex column (Restek, Bellefonte, PA) and an FID. At a constant linear velocity for helium of 30 ml/min, the oven program changed from 40 to 210°C over 40 min (with an original hold of 5 min at 40°C). Commercial standards (all from Sigma-Aldrich) were used to identify compound peaks and to convert amounts into micrograms per gram FW amounts. For the compounds that did not have commercial standards, identifications were made using a Shimadzu GC system equipped with the same column and a GC-MS2010S mass spectrometer detector running the same GC-FID program. Standards curves from similar, commercially available compounds were used to convert unknowns into nanogram per gram FW amounts, with α-pinene used as the external standard for conversion of monoterpenoids and β-caryophellene used as the external standard for conversion of sesquiterpenoids.

Statistical analyses. All statistics were performed using IBM-SPSS software (version 19.0; IBM, Armonk, NY) and $\alpha = 0.05$. Normality tests were performed using the SPSS EXPLORE feature. For each compound class (and subclasses for terpenoids and volatiles), multivariate analyses of variance (MANOVA) was utilized to examine differences between noninfected and infected plants for each tissue type. Blocks were originally included but later removed from the final model because they were always nonsignificant ($P > 0.05$). In addition to blocks, the plant number within the cage also was included due to the psyllids being released on only one plant (which was designed as “plant 1” in all cages). However, plant number was also removed from all tests because it was always nonsignificant ($P > 0.05$). If the MANOVA Pillai’s trace statistic was significant, follow-up analyses of variance (ANOVAs) were performed on individual compounds. Spearman’s rank correlations were used to determine associations between aerial and tuber ZC symptoms using both noninfected and ‘*Ca. L. solanacearum*’-infected plants, to determine whether changes in aerial symptoms related to ZC belowground. Likewise, Spearman’s correlations were used to determine correlations between compound levels in infected plants and aerial ZC symptoms and ‘*Ca. L. solanacearum*’ titers. Positive correlations indi-

cated that greater compound levels were associated with greater symptoms or greater ‘*Ca. L. solanacearum*’ titers.

RESULTS

Associations between aerial symptoms with tuber symptoms, aerial symptoms, and ‘*Ca. L. solanacearum*’ titers.

Symptom ratings for leaves (Spearman $\rho = 0.659$; $P < 0.001$; $N = 29$), lower stems ($\rho = 0.611$; $P < 0.001$; $N = 32$), and upper stems ($\rho = 0.589$; $P < 0.001$; $N = 32$) were positively associated with tuber symptoms.

There were no significant associations between symptoms and ‘*Ca. L. solanacearum*’ titers in leaves ($\rho = -0.382$; $P = 0.178$; $N = 14$), upper stems ($\rho = -0.243$; $P = 0.364$; $N = 16$), or lower stems ($\rho = -0.070$; $P = 0.797$; $N = 16$).

‘*Ca. L. solanacearum*’ infection effects on levels of amino acids. In total, 17 amino acids (alanine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, tryptophan, tyrosine, and valine) were analyzed in both leaf and stem tissue. In leaf tissue, infected plants had significantly different amino acid levels than noninfected plants according to MANOVA (Pillai’s trace $\Delta = 0.944$; $F = 5.631$; $P = 0.021$; $N = 25$). Serine and proline foliar levels were significantly greater in ‘*Ca. L. solanacearum*’-infected than noninfected plants (Table 1). In contrast, noninfected plants had significantly greater foliar levels of asparagine, aspartic acid, and glutamine than ‘*Ca. L. solanacearum*’-infected plants (Table 1). Tyrosine levels were negatively correlated with leaf symptoms (Table 1).

Amino acids in upper stems were significantly different, on average, in ‘*Ca. L. solanacearum*’-infected plants than noninfected plants, according to MANOVA ($\Delta = 0.950$; $F = 5.328$; $P = 0.037$; $N = 24$). In upper stems, levels of asparagine, aspartic acid, and threonine were significantly greater in ‘*Ca. L. solanacearum*’-infected than noninfected plants (Table 1). Tyrosine levels in upper stem tissue were negatively correlated with upper stem symptoms (Table 1).

There were no significant overall effects of ‘*Ca. L. solanacearum*’ infection on lower stem levels of amino acids according to MANOVA ($\Delta = 0.744$; $F = 1.455$; $P = 0.288$; $N = 28$). However, asparagine, aspartic acid, and leucine levels in lower stem tissue were significantly negatively correlated with lower stem symptoms (Table 1). Asparagine, aspartic acid, glutamine, histidine, and lysine levels in lower stem tissue were significantly positively correlated with lower stem ‘*Ca. L. solanacearum*’ titers (Table 1).

‘*Ca. L. solanacearum*’ infection effects on carbohydrates.

Fructose, glucose, and sucrose levels were examined in all tissues. In leaf ($\Delta = 0.625$; $F = 15.582$; $P < 0.001$; $N = 32$), upper stem ($\Delta = 0.399$; $F = 3.324$; $P = 0.048$; $N = 19$), and lower stem ($\Delta = 0.512$; $F = 7.330$; $P = 0.002$; $N = 25$) tissues, overall sugar levels were significantly greater in noninfected than ‘*Ca. L. solanacearum*’-infected plants. Levels of fructose, glucose, and sucrose alone were also significantly greater in noninfected plants in all tissue types, with the exception of fructose levels in upper stem tissue (Fig. 1). Fructose levels in leaves were negatively associated with ‘*Ca. L. solanacearum*’ titers ($\rho = -0.532$; $P = 0.034$; $N = 16$). Fructose levels in upper stem tissue were negatively associated with symptoms ($\rho = -0.880$; $P = 0.021$; $N = 6$). Sucrose levels in lower stem tissue were positively associated with lower stem symptoms ($\rho = 0.661$; $P = 0.005$; $N = 16$). No other significant correlations were observed between sugar levels, symptoms, or ‘*Ca. L. solanacearum*’ titers.

‘*Ca. L. solanacearum*’ infection effects on phenolics. In total, 22 phenolics were quantified in leaf tissue and 14 phenolics were quantified in stem tissues (Table 2). Overall, noninfected plant phenolics levels were different than ‘*Ca. L. solanacearum*’-infected plant levels for leaf ($\Delta = 0.993$; $F = 54.490$; $P < 0.001$;

$N = 32$), upper stem ($\Delta = 0.696$; $F = 2.774$; $P = 0.024$; $N = 32$), and lower stem ($\Delta = 0.999$; $F = 936.611$; $P < 0.001$; $N = 32$) tissues.

In leaf tissue, '*Ca. L. solanacearum*'-infected plants had lower levels of betuloside, diflavone, ferulic acid derivative, quercetin diglycoside 2, and quinic acid than noninfected controls (Table 3). In contrast, '*Ca. L. solanacearum*'-infected plants had greater levels of dichlorogenic acid, epicatechin gallate, procyanidin A, procyanidin B isomer 4, and procyanidin digalloyl dimer than noninfected plants (Table 3). In the upper stem, '*Ca. L. solanacearum*'-infected plants had greater levels of a flavonoid rhamnoside glycoside, ellagitannin 1, ellagitannin 2, and quinic acid than noninfected plants (Table 3). In the lower stem, '*Ca. L. solana-*

cearum'-infected plants had significantly greater levels of chlorogenic acid, ellagitannin 1, ellagitannin 2, a flavonoid glycoside, and a flavonoid rhamnoside glycoside than noninfected plants (Table 3). However, noninfected plants had greater lower stem levels of a syringic acid derivative than '*Ca. L. solanacearum*'-infected plants (Table 3).

Leaf tissue levels of epicatechin gallate, ferulic acid derivative, flavanol methyl ester, procyanidin B isomer 1, procyanidin B isomer 2, procyanidin B isomer 3, and procyanidin C isomer 1 were positively associated with foliar symptoms (Table 3). Leaf tissue levels of betuloside, diflavone, ferulic acid derivative, and flavonoid glycoside 3 were negatively associated with foliar '*Ca. L. solanacearum*' titers (Table 3). Upper stem levels of apigenin

TABLE 1. Mean ($\pm$ standard error) levels ($\mu\text{mol/g}$ FW) of amino acids in noninfected and infected leaf, upper stem, and lower stem tissue^a

Tissue, compound	Noninfected	Infected	F ($N = 24-28$)	Symptom ρ ($N = 14-16$)	Titer ρ ($N = 15-16$)
Leaf					
Alanine	1.37 $\pm$ 0.19	3.36 $\pm$ 0.82	3.819	0.398	0.094
Asparagine	5.07 $\pm$ 1.23	2.53 $\pm$ 0.41	5.254*	-0.416	0.303
Aspartic acid	1.78 $\pm$ 0.21	0.599 $\pm$ 0.103	31.658***	0.117	-0.100
Glutamic acid	1.12 $\pm$ 0.10	0.673 $\pm$ 0.216	2.544	-0.046	-0.229
Glutamine	3.97 $\pm$ 0.53	1.86 $\pm$ 0.30	13.888***	-0.268	0.021
Glycine	0.198 $\pm$ 0.05	0.391 $\pm$ 0.072	3.814	-0.089	-0.150
Histidine	0.340 $\pm$ 0.045	0.356 $\pm$ 0.035	0.083	0.064	0.297
Isoleucine	0.854 $\pm$ 0.099	1.00 $\pm$ 0.10	0.979	-0.105	0.009
Leucine	0.769 $\pm$ 0.096	0.786 $\pm$ 0.072	0.022	0.025	0.100
Lysine	0.708 $\pm$ 0.83	0.568 $\pm$ 0.050	2.362	0.041	0.085
Methionine	2.17 $\pm$ 0.36	1.43 $\pm$ 0.46	1.351	0.105	-0.100
Phenylalanine	0.787 $\pm$ 0.097	0.762 $\pm$ 0.070	0.044	-0.364	0.279
Proline	0.688 $\pm$ 0.130	1.83 $\pm$ 0.42	4.725*	-0.311	-0.085
Serine	7.58 $\pm$ 0.76	14.9 $\pm$ 2.0	8.352**	0.117	-0.015
Threonine	0.189 $\pm$ 0.028	0.299 $\pm$ 0.063	1.846	-0.078	0.035
Tryptophan	0.362 $\pm$ 0.024	0.431 $\pm$ 0.035	2.128	-0.380	0.129
Tyrosine	0.218 $\pm$ 0.051	0.249 $\pm$ 0.048	0.186	-0.542*	0.285
Valine	1.79 $\pm$ 0.25	1.80 $\pm$ 0.16	0.001	0.048	0.062
Upper stem					
Alanine	2.24 $\pm$ 0.45	4.42 $\pm$ 1.28	2.932	0.116	0.041
Asparagine	2.61 $\pm$ 0.59	6.00 $\pm$ 1.44	5.289*	-0.109	0.194
Aspartic acid	0.449 $\pm$ 0.127	1.16 $\pm$ 0.203	9.300**	-0.188	0.136
Glutamic acid	0.740 $\pm$ 0.116	0.841 $\pm$ 0.110	0.384	-0.105	0.326
Glutamine	1.53 $\pm$ 0.48	2.32 $\pm$ 0.45	1.405	-0.123	0.325
Glycine	0.221 $\pm$ 0.046	0.214 $\pm$ 0.054	0.009	0.398	0.076
Histidine	0.286 $\pm$ 0.074	0.374 $\pm$ 0.078	0.659	-0.259	0.215
Isoleucine	0.945 $\pm$ 0.207	1.31 $\pm$ 0.28	1.127	-0.248	0.221
Leucine	0.643 $\pm$ 0.080	0.715 $\pm$ 0.123	0.257	-0.295	0.194
Lysine	0.376 $\pm$ 0.075	0.511 $\pm$ 0.090	1.366	-0.342	0.268
Methionine	0.766 $\pm$ 0.246	0.584 $\pm$ 0.132	0.380	0.252	0.144
Phenylalanine	0.427 $\pm$ 0.059	0.540 $\pm$ 0.113	0.847	-0.266	0.059
Proline	3.24 $\pm$ 2.19	3.48 $\pm$ 1.14	0.008	-0.052	0.297
Serine	5.96 $\pm$ 0.72	8.46 $\pm$ 2.02	1.544	0.103	0.012
Threonine	0.291 $\pm$ 0.036	0.576 $\pm$ 0.133	4.980*	0.272	-0.268
Tryptophan	0.159 $\pm$ 0.039	0.167 $\pm$ 0.030	0.023	-0.395	0.315
Tyrosine	0.205 $\pm$ 0.045	0.387 $\pm$ 0.126	2.110	-0.570*	0.011
Valine	1.73 $\pm$ 0.39	2.30 $\pm$ 0.55	0.749	-0.113	0.185
Lower stem					
Alanine	3.21 $\pm$ 0.66	4.35 $\pm$ 0.86	1.065	0.166	0.059
Asparagine	2.92 $\pm$ 0.69	7.04 $\pm$ 1.77	4.195	-0.514*	0.624**
Aspartic acid	1.29 $\pm$ 0.29	1.88 $\pm$ 1.67	1.196	-0.529*	0.568*
Glutamic acid	0.850 $\pm$ 0.194	0.897 $\pm$ 0.194	0.030	-0.205	0.441
Glutamine	3.36 $\pm$ 0.831	4.35 $\pm$ 0.970	0.575	-0.456	0.564*
Glycine	0.125 $\pm$ 0.035	0.205 $\pm$ 0.042	2.049	0.026	0.315
Histidine	0.359 $\pm$ 0.060	0.449 $\pm$ 0.073	0.878	-0.418	0.535*
Isoleucine	1.14 $\pm$ 0.22	1.64 $\pm$ 0.33	1.545	-0.379	0.382
Leucine	0.721 $\pm$ 0.087	0.805 $\pm$ 0.140	0.237	-0.529*	0.353
Lysine	0.413 $\pm$ 0.069	0.494 $\pm$ 0.085	0.534	-0.412	0.526*
Methionine	0.667 $\pm$ 0.317	0.529 $\pm$ 0.138	0.175	-0.210	0.471
Phenylalanine	0.391 $\pm$ 0.057	0.473 $\pm$ 0.087	0.579	-0.374	0.485
Proline	2.22 $\pm$ 1.35	5.67 $\pm$ 1.62	2.571	-0.169	0.418
Serine	6.27 $\pm$ 0.80	9.01 $\pm$ 1.59	2.163	0.044	0.006
Threonine	0.563 $\pm$ 0.107	0.724 $\pm$ 0.160	0.654	-0.202	-0.065
Tryptophan	0.144 $\pm$ 0.030	0.169 $\pm$ 0.024	0.428	-0.453	0.368
Tyrosine	0.193 $\pm$ 0.046	0.275 $\pm$ 0.051	1.357	-0.383	0.274
Valine	1.99 $\pm$ 0.33	2.97 $\pm$ 0.59	1.939	-0.324	0.376

^a F test and Spearman's ρ correlation statistics are given; *, **, and *** indicate $P < 0.05$, 0.01, and 0.001, respectively.

glucoside, chlorogenic acid, and rutin were positively associated with upper stem symptoms (Table 3). Lower stem levels of chichoric acid, chlorogenic acid, and cryptochlorogenic acid ($p = 0.546$; $P = 0.029$; $N = 16$) were positively associated with lower stem symptoms (Table 3). Lower stem levels of a flavonoid rhamnoside glycoside and quinic acid were positively associated with '*Ca. L. solanacearum*' titers (Table 3). No other significant correlations were observed between phenolic levels, symptoms, or '*Ca. L. solanacearum*' titers.

'*Ca. L. solanacearum*' infection effects on terpenoid levels. In total, 17 monoterpenoids (Table 4) and 14 sesquiterpenoids (Table 5) were quantified in potato tissues.

Leaf levels of monoterpenoids ($\Delta = 0.912$; $F = 6.104$; $P = 0.003$; $N = 28$) and sesquiterpenoids ($\Delta = 0.736$; $F = 2.595$; $P = 0.047$; $N = 28$) were greater in '*Ca. L. solanacearum*'-infected plants than those noninfected. Most individual monoterpenoids, with the exception of the two β -pinene enantiomers, α -terpinolene, and allo-ocimene (Table 4), and most sesquiterpenoids, with the exception of β -gurjunene, β -farnesene, and Δ -cadinene (Table 5), were greater in '*Ca. L. solanacearum*'-infected plants than noninfected plants. There were no significant associations between leaf terpenoid levels with foliar symptoms or '*Ca. L. solanacearum*' titers.

Upper stem levels of monoterpenoids were greater in '*Ca. L. solanacearum*'-infected plants than those noninfected ($\Delta = 0.962$; $F = 20.685$; $P < 0.001$; $N = 32$). However, levels of sesquiterpenoids were observed to be greater in noninfected plants than in '*Ca. L. solanacearum*'-infected plants ($\Delta = 0.902$; $F = 11.169$; $P < 0.001$; $N = 32$). With the exceptions of allo-ocimene, bornyl acetate, and terpin-4-ol, individual monoterpenoid levels in the upper stem were greater in '*Ca. L. solanacearum*'-infected plants than noninfected plants (Table 4). In contrast, the levels of the sesquiterpenoids Δ -cadinol and germacrene D were greater in noninfected than '*Ca. L. solanacearum*'-infected plants (Table 5). Upper stem levels of the monoterpenoids α -terpinene, β -linalool, bornyl acetate, terpin-4-ol, and neryl acetate (Table 4), as well as all sesquiterpenoids, except β -gurjunene (Table 5), were positively correlated with '*Ca. L. solanacearum*' titers. No significant associations were observed between upper stem terpenoid levels and symptoms.

Lower stem levels of monoterpenoids ($\Delta = 0.949$; $F = 14.372$; $P < 0.001$; $N = 31$) and sesquiterpenoids ($\Delta = 0.785$; $F = 4.177$; $P = 0.004$; $N = 31$) overall were greater in '*Ca. L. solanacearum*'-infected plants than those noninfected. With the exception of allo-ocimene and β -gurjunene, all individual monoterpenoids (Table 4) and sesquiterpenoids (Table 5) were at greater levels in '*Ca. L. solanacearum*'-infected plants than noninfected plants. No significant correlations were observed between lower stem levels of monoterpenoids with symptoms or '*Ca. L. solanacearum*' titers (Table 4). Lower stem levels of β -gurjunene, both α -farnesene enantiomers, α -humulene, α -muurolene, Δ -cadinol, and germacrene D-4-ol were negatively associated with ZC symptoms (Table 5).

Lower stem sesquiterpenoid levels were not significantly associated with '*Ca. L. solanacearum*' titers (Table 5).

DISCUSSION

'*Ca. L. solanacearum*'-infected plants had significantly different levels of just a few particular amino acids and phenolics compared with noninfected plants in this study, with increases or decreases depending on the compound and tissue type. However, most terpenoid compound levels were generally greater in '*Ca. L. solanacearum*'-infected plants than controls, although an ex-

TABLE 2. Putative identifications of phenolic compounds quantified in potato leaf or stem tissues with identification criteria given

Tissue, compound	Retention time (min) ^a	UV/Vis spectra maxima (nm)	Molecular weight
Leaf			
Betuloside	27.65	279	327
Chichoric acid ^a	29.46	277	473
Dichlorogenic acid	20.76	277	705
Diflavone	25.94	277	538
Epicatechin gallate ^a	10.92	277	442
Ferulic acid derivative	30.26	279	308
Flavanol methyl ester	9.79	277	434
Flavonoid glycoside 1	15.17	278	446
Flavonoid glycoside 2	15.35	278	446
Flavonoid glycoside 3	25.67	284, 310	429
Procyanidin A	15.64	280	574
Procyanidin B isomer 1 ^a	13.88	278	566
Procyanidin B isomer 2 ^a	14.24	278	596
Procyanidin B isomer 3	18.87	278	579
Procyanidin B isomer 4	20.15	278	609
Procyanidin C isomer 1	13.70	277	866
Procyanidin C isomer 2	17.79	278	866
Procyanidin digalloyl dimer	17.30	275	720
Protocatechuic acid hexoside	6.15	260	316
Quercetin diglycoside 1	16.11	277	772
Quercetin diglycoside 2	17.09	275	772
Quinic acid ^a	1.62	264	192
Stem			
Apigenin glucoside	18.91	281, 320	430
Chichoric acid ^a	29.44	264	474
Chlorogenic acid ^a	9.16	323	354
Cryptochlorogenic acid ^a	13.90	323	354
Ellagitannin 1	20.62	283, 307	926
Ellagitannin 2	20.80	267, 313	910
Flavonoid diglycoside	27.41	280, 314	624
Flavonoid glycoside	15.29	280	447
Flavonoid rhamnoside glycoside	20.19	273, 314	579
Protocatechuic acid hexoside	6.16	278	316
Quercetin diglycoside	17.13	265, 358	772
Quinic acid ^a	1.50	264	192
Rutin ^a	19.65	255, 347	610
Syringic acid derivative	27.91	273, 313	525

^a Compound identity confirmed by matching retention time with commercial standard.

Fig. 1. Mean ($\pm$ standard error) levels of fructose, glucose, and sucrose in **A**, leaves; **B**, upper stems; and **C**, lower stems of both noninfected and '*Candidatus Liberibacter solanacearum*'-infected potato plants. Pairwise comparisons made by follow-up analyses of variance (F values) are given for each compound and tissue; *, **, and *** indicate $P < 0.05$, 0.01 , and 0.001 , respectively.

ception was sesquiterpenoid levels in the upper stem. Some of these results could be considered in contrast to previous results of 'Ca. L. solanacearum'-induced physiological shifts in tuber tissue, which observed increases in the majority of amino acids, sugars, and phenolics in 'Ca. L. solanacearum'-infected plants compared with those noninfected (33,41,44).

Unlike previous studies examining metabolites in potato tubers (33,41,44), the levels of metabolites in this study may have been affected by psyllid feeding in addition to 'Ca. L. solanacearum' infection. However, 'Ca. L. solanacearum' infections would not be possible in field conditions without feeding from psyllids; therefore, such changes in chemistry due to both 'Ca. L. solanacearum' infection and psyllid infestation would be expected in all ZC-diseased plants. In previous studies, psyllid feeding was shown to affect host chemistry, albeit when much greater numbers of psyllids were allowed to feed on tissues (7.5 per versus 20 per plant by Sengoda et al. [37] or even greater insect densities

[22,32]) up to when plant samples were collected (22,32,37). Currently, frequent insecticide application is the only way to limit 'Ca. L. solanacearum' spread (16); therefore, this study tried to simulate a more likely scenario of 'Ca. L. solanacearum' infection, whereby potato plants had psyllids killed by insecticides after a limited feeding period (34). Regardless, based on previous work, psyllids and the psyllid-feeding associated condition known as "psyllid yellows" can cause some foliar symptoms on potato hosts (37). However, such symptoms were never observed to be as severe then when 'Ca. L. solanacearum' infections also were present (37). Examining specifically the effects of psyllid feeding or 'Ca. L. solanacearum' infection decoupled from each other were beyond the scope of this work but could provide interesting data and, therefore, should be the focus of future studies.

Regarding the changes in amino acid levels observed by this study, foliar levels of proline and serine were greater in infected plants than controls, whereas foliar levels of asparagine, aspartic

TABLE 3. Mean ($\pm$ standard error) levels (μ g/g FW) of phenolics in noninfected and infected leaf, upper stem, and lower stem tissue^a

Tissue, compound	Noninfected	Infected	F (N = 32)	Symptom ρ (N = 14–16)	Titer ρ (N = 16)
Leaf					
Betuloside	43.9 $\pm$ 6.9	23.7 $\pm$ 14.7	6.622*	0.453	-0.662**
Chichoric acid	25.5 $\pm$ 3.7	18.8 $\pm$ 2.3	2.460	0.032	-0.385
Dichlorogenic acid	2.1 $\pm$ 0.26	7.7 $\pm$ 0.71	54.747***	0.375	-0.088
Diflavone	25.0 $\pm$ 3.4	14.3 $\pm$ 2.8	6.016*	0.199	-0.535*
Epicatechin gallate	12.8 $\pm$ 1.8	25.3 $\pm$ 4.9	5.707*	0.581*	0.059
Ferulic acid derivative	41.9 $\pm$ 4.6	21.5 $\pm$ 2.0	16.690***	0.611*	-0.776***
Flavanol methyl ester	11.1 $\pm$ 3.3	14.2 $\pm$ 3.6	0.413	0.586*	-0.047
Flavonoid glycoside 1	19.2 $\pm$ 2.8	31.0 $\pm$ 5.9	3.236	0.503	0.241
Flavonoid glycoside 2	11.7 $\pm$ 1.8	14.9 $\pm$ 3.4	0.716	0.151	-0.029
Flavonoid glycoside 3	17.9 $\pm$ 4.4	11.3 $\pm$ 4.1	1.203	0.446	-0.594*
Procyanidin A	66.6 $\pm$ 10.6	32.9 $\pm$ 7.5	6.784*	0.014	0.197
Procyanidin B isomer 1	265.0 $\pm$ 38	256.0 $\pm$ 37	0.029	0.636*	0.006
Procyanidin B isomer 2	50.6 $\pm$ 9.5	49.2 $\pm$ 11.3	0.010	0.641*	-0.209
Procyanidin B isomer 3	39.3 $\pm$ 5.7	54.8 $\pm$ 9.5	1.956	0.618*	-0.388
Procyanidin B isomer 4	16.7 $\pm$ 2.5	99.1 $\pm$ 5.9	165.368***	0.357	-0.185
Procyanidin C isomer 1	79.6 $\pm$ 12.0	75.6 $\pm$ 10.6	0.063	0.576*	0.041
Procyanidin C isomer 2	29.0 $\pm$ 4.1	26.7 $\pm$ 3.0	0.221	0.496	-0.368
Procyanidin digalloyl dimer	20.6 $\pm$ 2.0	39.8 $\pm$ 7.0	6.950*	-0.231	0.197
Protocatechuic acid hexoside	23.0 $\pm$ 2.6	22.4 $\pm$ 2.2	0.037	-0.483	-0.032
Quercetin diglycoside 1	15.8 $\pm$ 3.4	12.8 $\pm$ 3.1	0.422	0.126	0.041
Quercetin diglycoside 2	29.7 $\pm$ 4.7	17.8 $\pm$ 3.3	4.194*	0.476	-0.044
Quinic acid	25.0 $\pm$ 2.8	7.5 $\pm$ 1.10	34.093***	-0.387	0.197
Upper stem					
Apigenin glucoside	8.69 $\pm$ 2.48	7.25 $\pm$ 1.51	0.246	0.718**	-0.206
Chichoric acid	3.99 $\pm$ 0.78	2.30 $\pm$ 0.37	3.856	0.263	0.088
Chlorogenic acid	23.1 $\pm$ 3.67	31.9 $\pm$ 4.2	2.469	0.777**	0.082
Cryptochlorogenic acid	96.9 $\pm$ 13.8	90.5 $\pm$ 10.6	0.136	0.082	-0.032
Ellagitannin 1	24.8 $\pm$ 6.7	53.0 $\pm$ 0.7	17.52***	-0.237	-0.347
Ellagitannin 2	13.5 $\pm$ 2.6	22.0 $\pm$ 0.9	9.475**	-0.144	-0.188
Flavonoid diglycoside	3.47 $\pm$ 0.89	1.88 $\pm$ 0.40	2.645	0.295	0.097
Flavonoid glycoside	5.44 $\pm$ 0.83	6.18 $\pm$ 1.26	0.238	0.240	0.153
Flavonoid rhamnoside glycoside	13.70 $\pm$ 4.1	31.8 $\pm$ 0.5	19.443***	-0.100	0.015
Protocatechuic acid hexoside	6.33 $\pm$ 1.94	7.92 $\pm$ 1.23	0.480	-0.225	0.279
Quercetin diglycoside	2.60 $\pm$ 0.64	2.30 $\pm$ 0.42	0.152	0.055	0.350
Quinic acid	4.92 $\pm$ 0.94	8.56 $\pm$ 0.79	8.801**	-0.318	0.224
Rutin	6.93 $\pm$ 2.7	5.09 $\pm$ 0.95	0.430	0.687**	-0.076
Syringic acid derivative	1.51 $\pm$ 0.20	1.16 $\pm$ 0.10	2.534	-0.354	-0.009
Lower stem					
Apigenin glucoside	5.47 $\pm$ 1.02	5.23 $\pm$ 1.19	0.022	0.338	0.091
Chichoric acid	1.60 $\pm$ 0.30	1.09 $\pm$ 0.176	2.196	0.649**	-0.091
Chlorogenic acid	18.4 $\pm$ 1.9	28.2 $\pm$ 3.8	5.259*	0.689**	-0.000
Cryptochlorogenic acid	42.7 $\pm$ 4.5	44.4 $\pm$ 4.6	0.071	0.546*	0.168
Ellagitannin 1	1.50 $\pm$ 0.16	53.8 $\pm$ 0.8	4,075***	0.222	-0.418
Ellagitannin 2	2.80 $\pm$ 0.21	23.7 $\pm$ 0.57	1,187***	0.474	-0.297
Flavonoid diglycoside	1.24 $\pm$ 0.19	0.728 $\pm$ 0.248	2.681	0.312	0.012
Flavonoid glycoside	2.94 $\pm$ 0.46	4.15 $\pm$ 0.36	4.232*	-0.204	0.341
Flavonoid rhamnoside glycoside	1.94 $\pm$ 0.41	34.7 $\pm$ 0.4	1,652***	0.119	0.529*
Protocatechuic acid hexoside	5.69 $\pm$ 1.47	7.40 $\pm$ 0.97	0.943	-0.421	0.309
Quercetin diglycoside	2.07 $\pm$ 0.40	1.71 $\pm$ 0.33	0.502	0.392	-0.035
Quinic acid	11.7 $\pm$ 1.0	10.9 $\pm$ 0.8	0.409	-0.061	0.624**
Rutin	6.51 $\pm$ 1.63	7.94 $\pm$ 1.33	0.463	-0.073	0.276
Syringic acid derivative	0.733 $\pm$ 0.081	0.484 $\pm$ 0.032	8.154**	0.371	-0.162

^a F test and Spearman's ρ correlation statistics are given; *, **, and *** indicate $P < 0.05$, 0.01, and 0.001, respectively.

acid, and glutamine were less in infected plants compared with controls (Table 1). These findings are similar to those by Wallis et al. (44), who observed greater proline and serine in tubers from plants infected for 4 weeks, yet reduced levels of asparagine, aspartic acid, and glutamic acid in the same tubers. By contrast, stem levels of asparagine and aspartic acid were greater in '*Ca. L. solanacearum*'-infected plants. In foliar tissues, levels of asparagine, aspartic acid, and glutamine being lower in '*Ca. L. solanacearum*'-infected than noninfected plants might be attributed to these amino acids acting as precursors to stress-related metabolites, including proline, arginine, or γ -amino butyric acid (6). These compounds being lowered in foliage of infected plants also might be a result '*Ca. L. solanacearum*' or psyllid consumption, which has been found with another biotrophic pathogen (38), or due to translocation to the stem. The possible translocation to the stem could be a potential explanation of why asparagine and aspartic acid were greater in '*Ca. L. solanacearum*'-infected

plants versus controls in the stem but not in foliage. Increased foliar levels of proline in response to '*Ca. L. solanacearum*' infection might serve to protect diseased plants from reactive oxygen species (3) as well as serve in cell wall strengthening via production of proline- and hydroxyproline-rich proteins (9). Thicker cell walls could, hypothetically, be a host defense response to both '*Ca. L. solanacearum*' and the potato psyllid vector.

Sugar levels in this study were generally greater in noninfected than '*Ca. L. solanacearum*'-infected plants (Fig. 1). Gao et al. (15) also observed greater glucose levels in noninfected plants compared with those likely infected with '*Ca. L. solanacearum*' (i.e., those infested with '*Ca. L. solanacearum*'-positive potato psyllids). This is likely because of decreased photosynthesis because ZC symptoms developed due to necrosis of leaf tissues, the use of carbohydrates by the host to provide energy for a defense response, and '*Ca. L. solanacearum*' consuming sugars

TABLE 4. Mean ($\pm$ standard error) levels (ng/g FW) of monoterpenoids in noninfected and infected leaf, upper stem, and lower stem tissue^a

Tissue	Retention time	Compound	Noninfected	Infected	<i>F</i> (<i>N</i> = 28–32)	Symptom ρ (<i>N</i> = 12–16)	Titer ρ (<i>N</i> = 14–16)
Leaf	12.11	Camphene ^b	8.98 $\pm$ 0.26	10.7 $\pm$ 0.2	24.811***	-0.463	-0.107
	12.28	Δ -2-Carene ^b	9.14 $\pm$ 0.33	10.9 $\pm$ 0.3	16.937***	-0.444	-0.284
	12.93	(+)- β -Pinene	5.78 $\pm$ 0.74	4.78 $\pm$ 0.78	0.859	0.275	0.075
	13.02	(-)- β -Pinene	1.98 $\pm$ 0.15	2.04 $\pm$ 0.17	0.064	-0.108	0.031
	13.54	α -Phellandrene	9.92 $\pm$ 0.19	11.2 $\pm$ 0.2	18.500***	-0.400	-0.226
	13.77	α -Terpinene	3.17 $\pm$ 0.12	3.80 $\pm$ 0.23	5.931*	-0.437	0.042
	14.67	β -Phellandrene ^b	18.1 $\pm$ 0.5	22.2 $\pm$ 0.5	34.643***	-0.444	-0.248
	14.82	Para-cymene	17.1 $\pm$ 0.4	20.6 $\pm$ 0.5	29.345***	-0.393	-0.323
	14.95	β -Ocimene	8.91 $\pm$ 0.21	10.3 $\pm$ 0.2	21.628***	-0.451	-0.196
	15.22	γ -Terpinene	4.40 $\pm$ 0.13	5.38 $\pm$ 0.11	32.767***	-0.429	-0.218
	15.34	1,8-Cineole	3.09 $\pm$ 0.10	3.63 $\pm$ 0.11	13.526**	0.051	-0.262
	15.98	α -Terpinolene	1.56 $\pm$ 0.06	1.67 $\pm$ 0.05	1.987	-0.160	0.033
	18.00	Allo-ocimene	2.11 $\pm$ 0.35	3.28 $\pm$ 0.60	2.880	-0.240	-0.244
	19.01	β -Linalool	8.47 $\pm$ 0.51	10.4 $\pm$ 0.7	4.813*	-0.036	-0.081
	19.99	Bornyl acetate	11.2 $\pm$ 0.3	12.8 $\pm$ 0.2	19.180***	-0.058	-0.358
	21.23	Terpin-4-ol	24.7 $\pm$ 0.8	30.9 $\pm$ 0.6	37.334***	-0.466	-0.020
	25.01	Neryl acetate	7.25 $\pm$ 0.57	8.60 $\pm$ 0.32	4.224*	-0.298	-0.468
Upper stem	12.11	Camphene ^b	8.63 $\pm$ 0.22	11.5 $\pm$ 0.2	79.548***	0.055	0.222
	12.28	Δ -2-Carene ^b	8.46 $\pm$ 0.24	12.0 $\pm$ 0.3	101.485***	0.075	0.218
	12.93	(+)- β -Pinene	2.58 $\pm$ 0.18	3.11 $\pm$ 0.10	6.469*	0.259	0.137
	13.02	(-)- β -Pinene	2.01 $\pm$ 0.10	2.65 $\pm$ 0.12	17.316***	0.070	0.116
	13.54	α -Phellandrene	9.49 $\pm$ 0.21	12.1 $\pm$ 0.2	71.796***	0.049	0.494
	13.77	α -Terpinene	3.18 $\pm$ 0.8	4.63 $\pm$ 0.21	42.090***	-0.336	0.565*
	14.67	β -Phellandrene ^b	17.4 $\pm$ 0.4	24.2 $\pm$ 0.4	122.005***	-0.017	0.450
	14.82	Para-cymene	16.4 $\pm$ 0.4	22.4 $\pm$ 4.1	114.829***	0.075	0.358
	14.95	β -Ocimene	8.38 $\pm$ 0.19	10.8 $\pm$ 0.19	80.406***	0.054	0.440
	15.22	γ -Terpinene	4.30 $\pm$ 0.13	5.83 $\pm$ 0.13	69.736***	0.024	0.396
	15.34	1,8-Cineole	2.74 $\pm$ 0.10	3.65 $\pm$ 0.08	53.549***	-0.235	0.462
	15.98	α -Terpinolene	1.32 $\pm$ 0.05	1.90 $\pm$ 0.05	72.937***	0.199	0.178
	18.00	Allo-ocimene	1.56 $\pm$ 0.42	2.16 $\pm$ 0.58	0.731	0.075	0.444
	19.01	β -Linalool	6.00 $\pm$ 0.35	7.36 $\pm$ 0.29	8.759**	-0.240	0.544*
	19.99	Bornyl acetate	12.0 $\pm$ 0.4	12.3 $\pm$ 0.3	0.482	-0.064	0.588*
	21.23	Terpin-4-ol	29.2 $\pm$ 1.1	31.0 $\pm$ 0.7	1.733	-0.230	0.638**
	25.01	Neryl acetate	6.49 $\pm$ 0.31	6.24 $\pm$ 0.24	0.387	-0.327	0.538*
Lower stem	12.11	Camphene ^b	8.04 $\pm$ 0.19	10.6 $\pm$ 0.3	62.271***	0.073	-0.103
	12.28	Δ -2-Carene ^b	8.06 $\pm$ 0.17	10.9 $\pm$ 0.25	88.823***	0.162	0.043
	12.93	(+)- β -Pinene	2.39 $\pm$ 0.11	2.82 $\pm$ 0.10	8.451**	-0.379	0.024
	13.02	(-)- β -Pinene	1.88 $\pm$ 0.11	2.56 $\pm$ 0.09	23.794***	0.074	-0.296
	13.54	α -Phellandrene	9.12 $\pm$ 0.83	11.3 $\pm$ 0.18	60.718***	-0.017	0.029
	13.77	α -Terpinene	2.96 $\pm$ 0.10	4.55 $\pm$ 0.19	50.764***	0.142	-0.317
	14.67	β -Phellandrene ^b	16.3 $\pm$ 0.4	21.7 $\pm$ 0.5	75.295***	0.096	-0.138
	14.82	Para-cymene	15.2 $\pm$ 0.4	20.4 $\pm$ 0.4	85.378***	0.082	-0.138
	14.95	β -Ocimene	7.67 $\pm$ 0.20	9.78 $\pm$ 1.67	65.959***	0.017	-0.205
	15.22	γ -Terpinene	3.86 $\pm$ 0.11	5.23 $\pm$ 0.10	90.078***	-0.017	-0.327
	15.34	1,8-Cineole	2.60 $\pm$ 0.09	3.26 $\pm$ 0.10	23.328***	0.126	-0.178
	15.98	α -Terpinolene	1.39 $\pm$ 0.04	1.77 $\pm$ 0.04	43.954***	-0.062	-0.371
	18.00	Allo-ocimene	1.31 $\pm$ 0.10	1.92 $\pm$ 0.50	1.360	-0.336	0.374
	19.01	β -Linalool	4.25 $\pm$ 0.22	6.48 $\pm$ 0.18	59.900***	-0.113	0.122
	19.99	Bornyl acetate	10.1 $\pm$ 0.3	11.7 $\pm$ 0.2	19.551***	-0.333	-0.185
	21.23	Terpin-4-ol	22.6 $\pm$ 1.0	28.3 $\pm$ 0.6	24.033***	-0.175	0.029
	25.01	Neryl acetate	3.91 $\pm$ 0.29	5.57 $\pm$ 0.25	18.863***	-0.425	0.035

^a *F* test and Spearman's ρ correlation statistics are given; *, **, and *** indicate *P* < 0.05, 0.01, and 0.001, respectively.

^b Compounds putatively identified based on relative retention times and mass spectra.

for energy. Glucose levels also might have been converted to starch in foliage (15), although this study did not measure starch levels to confirm this. These findings are different than observations of '*Ca. L. solanacearum*' infected tubers (33,44). However, in tubers, '*Ca. L. solanacearum*' infection likely causes tuber physiological changes that contribute to the conversion of starch to reducing sugars. This would result in greater levels of individual disaccharides and monosaccharides in tubers of '*Ca. L. solanacearum*'-infected plants (44).

Depending on tissue, phenolics involved in cell wall strengthening—in particular, ellagitannins (which are hydrolysable tannins) in stems—were greater in '*Ca. L. solanacearum*'-infected than noninfected plants. Therefore, it might be hypothesized that the primary production of phenolics in response to '*Ca. L. solanacearum*' infection was targeted around making thicker cell walls to limit pathogen movement and access to resources. Likewise, because the '*Ca. L. solanacearum*' bacterial infection process involved psyllid feeding, the thicker cell walls also could be a hypothesized host defense response against the vectors. However, direct cell wall measurements were not made and, therefore, should be verified in future studies and subsequently correlated with pathogen titers and insect behaviors. This formation of thicker cell walls likely does not occur in tubers in response to '*Ca. L. solanacearum*' infection, which is why, in tubers, no

decreases in phenolic levels were observed (44). However, levels of most phenolics were not significantly different between '*Ca. L. solanacearum*'-infected and noninfected plants. Certain flavonoid and other phenolics, which either increased in response to infection (e.g., flavonoid rhamnoside glycoside in stems) or were positively correlated with symptoms (e.g., certain procyanidins in leaves and chlorogenic acid in stems) (Table 3), could have been increased by the host in an attempt to ameliorate disease stress or resist '*Ca. L. solanacearum*' and its vector (16,19,31). Greater flavonoid and chlorogenic acid levels also were observed in potato tubers in response to '*Ca. L. solanacearum*' infection, likely for similar reasons (44).

Levels of most monoterpenoids were much greater in '*Ca. L. solanacearum*'-infected than noninfected plants, although few significant correlations were observed between monoterpenoid levels with ZC symptoms or '*Ca. L. solanacearum*' titers. Sesquiterpenoid levels in leaves and lower stem tissue also were increased in '*Ca. L. solanacearum*'-infected plants, although upper stem sesquiterpenoid levels did not differ. It is difficult to explain why upper stem sesquiterpenoid levels did not differ between noninfected and infected hosts when the other tissues differed, although some reports have observed differential changes between tissues and parts of the plant before (5) and this observation warrants consideration in future studies. Most previous

TABLE 5. Mean ($\pm$ standard error) levels (ng/g FW) of sesquiterpenoids in noninfected and infected leaf, upper stem, and lower stem tissue^a

Tissue	Retention time	Compound	Noninfected	Infected	<i>F</i> (<i>N</i> = 28–32)	Symptom <i>p</i> (<i>N</i> = 12–16)	Titer <i>p</i> (<i>N</i> = 14–16)
Leaf	24.80	α -Copanene	2.83 $\pm$ 0.22	3.75 $\pm$ 0.09	15.276**	–0.240	0.059
	25.28	α -Gurjunene ^b	3.79 $\pm$ 0.24	5.03 $\pm$ 0.09	23.906***	–0.393	0.007
	25.56	β -Caryophellene	7.18 $\pm$ 0.31	8.47 $\pm$ 0.16	13.605**	–0.473	–0.191
	25.67	β -Gurjunene ^b	6.45 $\pm$ 1.26	4.87 $\pm$ 0.67	1.226	–0.036	0.011
	26.60	(<i>Z,E</i>)- α -farnesene	4.85 $\pm$ 0.26	5.95 $\pm$ 0.15	13.373**	–0.467	0.396
	26.70	α -Humulene	8.53 $\pm$ 0.34	9.43 $\pm$ 0.18	5.567*	–0.378	–0.314
	26.85	(<i>E,E</i>)- α -farnesene	5.17 $\pm$ 0.25	6.42 $\pm$ 0.13	19.835***	–0.393	–0.037
	27.31	(<i>E,E</i>)- β -farnesene	11.3 $\pm$ 1.3	11.0 $\pm$ 0.6	0.068	–0.400	0.231
	27.56	α -Murolene ^b	7.37 $\pm$ 0.48	8.48 $\pm$ 0.18	4.673*	–0.168	–0.040
	28.64	Δ -Cadinene ^b	3.36 $\pm$ 0.22	3.89 $\pm$ 0.12	4.159	0.211	–0.235
	30.53	Δ -Cadinol ^b	5.94 $\pm$ 0.24	6.69 $\pm$ 0.14	7.455*	–0.590	0.086
	30.69	Germacrene D ^b	15.9 $\pm$ 0.7	17.7 $\pm$ 0.3	5.617*	–0.306	–0.275
	31.51	Germacrene D-4-ol ^b	10.4 $\pm$ 0.4	12.0 $\pm$ 0.3	9.598**	–0.480	–0.262
	33.02	Citronellyl isobutyrate ^b	21.0 $\pm$ 0.7	25.8 $\pm$ 0.5	26.879***	–0.546	–0.099
Upper stem	24.80	α -Copanene	3.44 $\pm$ 0.14	3.28 $\pm$ 0.11	0.791	–0.204	0.721**
	25.28	α -Gurjunene ^b	5.54 $\pm$ 0.26	5.22 $\pm$ 0.19	1.008	–0.230	0.594*
	25.56	β -Caryophellene	9.41 $\pm$ 0.42	8.55 $\pm$ 0.28	2.867	0.038	0.556*
	25.67	β -Gurjunene ^b	0.754 $\pm$ 0.034	0.782 $\pm$ 0.044	0.244	–0.415	0.292
	26.60	(<i>Z,E</i>)- α -farnesene	5.52 $\pm$ 0.20	5.12 $\pm$ 0.13	2.770	–0.156	0.662**
	26.70	α -Humulene	9.52 $\pm$ 0.34	8.88 $\pm$ 0.21	2.579	–0.131	0.634**
	26.85	(<i>E,E</i>)- α -farnesene	6.33 $\pm$ 0.25	5.94 $\pm$ 0.17	1.689	–0.102	0.726**
	27.31	(<i>E,E</i>)- β -farnesene	8.35 $\pm$ 0.33	7.80 $\pm$ 0.23	1.869	–0.073	0.597*
	27.56	α -Murolene ^b	7.87 $\pm$ 0.34	7.59 $\pm$ 0.23	0.473	–0.283	0.647**
	28.64	Δ -Cadinene ^b	3.54 $\pm$ 0.14	3.57 $\pm$ 0.07	0.021	–0.234	0.552*
	30.53	Δ -Cadinol ^b	6.24 $\pm$ 0.24	4.92 $\pm$ 0.12	23.092***	0.138	0.594*
	30.69	Germacrene D ^b	19.1 $\pm$ 0.8	18.7 $\pm$ 0.4	0.190	–0.362	0.629**
	31.51	Germacrene D-4-ol ^b	10.9 $\pm$ 0.3	9.89 $\pm$ 0.24	5.860*	0.078	0.630**
	33.02	Citronellyl isobutyrate ^b	23.8 $\pm$ 0.8	23.6 $\pm$ 0.6	0.022	0.005	0.606*
Lower stem	24.80	α -Copanene	2.17 $\pm$ 0.17	3.06 $\pm$ 0.10	19.731***	–0.456	–0.088
	25.28	α -Gurjunene ^b	3.68 $\pm$ 0.25	4.71 $\pm$ 0.14	13.727**	–0.339	0.003
	25.56	β -Caryophellene	6.80 $\pm$ 0.31	7.72 $\pm$ 0.20	6.430*	0.017	0.091
	25.67	β -Gurjunene ^b	0.604 $\pm$ 0.035	0.705 $\pm$ 0.046	2.978	–0.724**	0.191
	26.60	(<i>Z,E</i>)- α -farnesene	3.83 $\pm$ 0.23	4.88 $\pm$ 0.11	18.044***	–0.627**	0.187
	26.70	α -Humulene	7.35 $\pm$ 0.33	8.22 $\pm$ 0.18	5.695*	–0.539*	–0.102
	26.85	(<i>E,E</i>)- α -farnesene	4.43 $\pm$ 0.28	5.59 $\pm$ 0.14	14.037**	–0.535*	0.065
	27.31	(<i>E,E</i>)- β -farnesene	5.84 $\pm$ 0.34	7.09 $\pm$ 0.20	10.341**	–0.470	0.091
	27.56	α -Murolene ^b	5.64 $\pm$ 0.41	7.21 $\pm$ 0.22	11.658**	–0.748**	0.109
	28.64	Δ -Cadinene ^b	2.50 $\pm$ 0.15	3.09 $\pm$ 0.06	14.363**	–0.387	–0.102
	30.53	Δ -Cadinol ^b	4.04 $\pm$ 0.17	4.51 $\pm$ 0.11	5.659*	–0.717**	–0.057
	30.69	Germacrene D ^b	13.3 $\pm$ 0.6	16.0 $\pm$ 0.4	16.734***	–0.304	–0.243
	31.51	Germacrene D-4-ol ^b	8.44 $\pm$ 0.41	9.65 $\pm$ 0.26	6.575*	–0.658**	–0.026
	33.02	Citronellyl isobutyrate ^b	17.8 $\pm$ 0.8	21.7 $\pm$ 0.5	16.843***	–0.522*	–0.015

^a *F* test and Spearman's ρ correlation statistics are given; *, **, and *** indicate *P* < 0.05, 0.01, and 0.001, respectively.

^b Compounds putatively identified based on relative retention times and mass spectra.

studies observing induction of terpenoid production following infection, or the ability of terpenoids to limit pathogen growth, have focused on necrotrophic fungi (5,16,20,43). However, it was observed that *Pseudomonas syringae* infection can induce terpenoids in *Arabidopsis* (2), and likely '*Ca. L. solanacearum*' infection of potato plants may increase terpenoid levels as well (13).

Although the role terpenoids have in defense against pathogens remains poorly understood, terpenoids can greatly affect insect behaviors and physiology (20), and levels in the host may have shifted in response to psyllid feeding in combination with '*Ca. L. solanacearum*' infection. In fact, in many plants, including potato, it should be expected that psyllid feeding could affect terpenoid levels, perhaps as a specific response to herbivory (22,32). Because '*Ca. L. solanacearum*' is vectored by psyllids, the increase in terpenoids as a host response to infection or even prior feeding may alter efficiency of pathogen spread. There is some evidence that increased terpenoids in plant hosts will act as feeding deterrents and increase insect pest movement (4,12). This might increase '*Ca. L. solanacearum*' spread by psyllids as long as the bacteria could be acquired prior to insect movement. However, Davis et al. (13) observed that psyllids settled more on plants possibly inoculated with '*Ca. L. solanacearum*' 7 days previously than noninoculated controls, even though psyllid-infested plants had greater terpenoid levels. Greater time spent on a plant may reduce spread of '*Ca. L. solanacearum*' by lowering plant-to-plant vector movement but it also increases likelihood of '*Ca. L. solanacearum*' acquisition by psyllids. Therefore, studies to examine plant-psyllid interactions, especially the influence that terpenoids have on insect behaviors and how that may affect pathogen spread, are warranted because our current knowledge remains limited.

In conclusion, this study observed potential changes in potato physiology in response to '*Ca. L. solanacearum*' infection and associated psyllid feeding that could be directly or indirectly associated with the expression of aboveground ZC symptoms in potato plants. These changes in physiology could affect the formation of tubers, because disruptions in metabolism overall should affect resources needed for proper tuber development. Shifts in metabolism in the aboveground portions of potato plants are hypothesized to affect both '*Ca. L. solanacearum*' and its psyllid vector. This, in turn, would affect both spread and progression of ZC disease in its potato hosts. These results also highlight the need to conduct further studies to examine what occurs when potato plants are attacked by both '*Ca. L. solanacearum*' and its psyllid vectors. In particular, future studies are warranted to examine how potato host chemistry is affected by '*Ca. L. solanacearum*'-negative psyllid feeding. Conversely, it would be important to examine how potato host chemistry can be affected by '*Ca. L. solanacearum*' infections that began without psyllid feeding, such as those by graft inoculations. Finally, analyzing changes in defense-related protein levels and regulations of host genes in addition to shifts in host physiology over the course of an infection would aid in developing a very comprehensive picture of potato host responses to '*Ca. L. solanacearum*' or psyllids.

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