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Characterization of the Tolerance against Zebra Chip Disease in Tubers of Advanced Potato Lines from Mexico

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Abstract Potato zebra chip disease (ZC), a threat to potato production in the USA, Mexico, New Zealand, and Central America, is associated with the bacterium "*Candidatus* Liberibacter solanacearum" (Cls) that is vectored by the potato psyllid (*Bactericera cockerelli* Sulc.). ZC control currently depends on insecticide applications, but sustainable control will require development of resistant and/or tolerant varieties. This study characterized four promising potato lines (246, 865, 510 and NAU) exposed to Cls-positive adult psyllids in choice and no-choice assays for ZC resistance. Psyllids preferred to settle on Atlantic over 246 and 865, and oviposit on Atlantic compared to 510. However, tolerance to ZC appeared more dependent on host responses to Cls infection. All four of these potato genotypes exhibited putative ZC tolerance in raw tubers compared to the susceptible commercial variety Atlantic. Expressed tolerance was associated with reduced concentrations of phenolic compounds in Cls-infected raw tubers with corresponding reductions in freshly-cut symptoms. However, these four genotypes exhibited ZC-linked discoloration of fried tuber slices, which was associated with increased sugar content that occurred following Cls-infection. As a result, these four ZC-tolerant experimental potato lines could be useful if the tubers produced are used for fresh, but not processing, markets.

Resumen La zebra chip (ZC) o papa rayada, que es una amenaza para la producción de papa en los Estados Unidos, México, Nueva Zelanda y Centroamérica, esta asociada con la bacteria "*Candidatus Liberibacter solanacearum*" (Cls), que es transmitida por el psílido de la papa (*Bactericera cockerelli* Sulc.). El control de ZC actualmente depende de aplicaciones de insecticidas, pero un control sustentable requerirá del desarrollo de variedades resistentes y/o tolerantes. Este estudio caracterizó cuatro líneas prometedoras (246, 865, 510 y NAU) expuestas a psílidos adultos positivos a Cls en ensayos de selección y no selección para resistencia a ZC. Los psílidos prefirieron posarse en Atlantic que en 246 y 865, y ovipositaron en Atlantic comparados con 510. No obstante, la tolerancia a ZC parecía más dependiente de las respuestas del hospedante a la infección por Cls. Las cuatro líneas exhibieron tolerancia aparente a ZC en tubérculos crudos en comparación a la variedad comercial susceptible Atlantic. La tolerancia expresada estuvo asociada con concentraciones reducidas de compuestos fenólicos en tubérculos crudos infectados con Cls con las correspondientes reducciones en síntomas en cortes frescos. De todas maneras, estas cuatro líneas exhibieron pigmentación asociada a ZC en hojuelas fritas de tubérculo, lo que estuvo asociado con el aumento en el contenido de azúcar que se presentó después de la infección con Cls. Como resultado, estas cuatro líneas experimentales de papa tolerantes a ZC pudieran ser de utilidad si los tubérculos producidos se utilizan para mercado fresco, no de procesamiento.

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Introduction

Potato "Zebra Chip" disease (ZC) is associated with the bacterium "*Candidatus* Liberibacter solanacearum" (syn. Psyllaurous) (Cls), which is transmitted by the potato psyllid *Bactericera cockerelli* Sulc (Munyaneza 2012). This disease is threatening potato production in the USA, Mexico, New Zealand, and Central America (Munyaneza 2012). Plants infected in early growth stages fail to produce tubers and often die. Plants infected after the onset of the tubers can produce tubers with a discoloration that makes potato products unmarketable, thus reducing yield (Buchman et al. 2011; Buchman et al. 2012).

Currently, ZC management involves insecticide use to reduce insect vector populations (Butler and Trumble 2012; Guenthner et al. 2012). In places of high disease incidence in Mexico, farmers usually make two applications of insecticides per week and still must accept tuber yield and quality losses (Rubio-Covarrubias et al. 2011). Insecticide-based control systems increase production costs, intensify the risk of contamination of the environment, and also harm beneficial insects (Butler and Trumble 2012; Guenthner et al. 2012). The use of resistant (or tolerant) varieties is a basic component of integrated pest control and represents a long-term sustainable management strategy for ZC. A number of breeding programs for ZC resistance are underway in ZC-affected countries and are focused on the generation of resistant genotypes (Cadena-Hinojosa et al. 2003; Butler et al. 2011a; Anderson et al. 2012; Diaz-Montano et al. 2014; Rubio-Covarrubias et al. 2015; Cooper and Bamberg 2014; Novy et al. 2013; Munyaneza et al. 2013; Scheuring et al. 2013; Wallis et al. 2015a). While a few potato lines have been found to be tolerant to ZC, no tolerant or resistant varieties have yet been released.

Effective disease resistance can target the vector, the pathogen, or both. Host tolerance to disease that involves reducing vector populations or inoculation success can take the form of antixenosis, where the vector is expected to choose an alternate acceptable host, or it can be exhibited as antibiosis, which involves some performance factor becoming influenced by host plants (Smith 2005). Conversely, a plant may be resistant to a pathogen, in which case, the insect vector will be capable of utilizing the plant as a host but no infection (disease) will occur. An unaffected yield and lower incidence of diseased tubers should be evident regardless the type resistance or tolerance, and is the goal regardless of whether it is due to tolerance or true resistance. Resistance may involve no Cls-infection of the tubers, Cls-infection but failure to exhibit typical symptoms, or the symptoms may not lead to a significant yield loss.

In Mexico, researchers of INIFAP (Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias en México [National Institute for Forestry, Agriculture and Livestock Research in Mexico]) generated potato genotypes that exhibited resistance against ZC (Cadena-Hinojosa et al. 2003; Rubio-Covarrubias et al. 2013; Rubio-Covarrubias et al. 2015). These have been screened under field conditions and results indicate that some advanced lines have lower ZC incidence and less intense internal tuber discoloration. Some of the experimental genotypes, besides having shown resistance to ZC, also possess good agronomic and commercial characteristics. However, it is necessary to determine the mechanisms of resistance that these potato lines possess, whether due to resistance to insect vectors, the pathogen, or both. To achieve this objective, a selected group of four advanced potato lines were examined for both resistance and/or tolerance to either *Bactericera cockerelli* or to the Cls pathogen in the form of antixenosis, antibiosis, or biochemical patterns. Specifically, insect development assays, insect behavioral bioassays, choice assays, transmission assays, examination of trichomes, and biochemical analyses were performed in healthy and diseased plants and tubers.

Materials and Methods

Insects

Bactericera cockerelli (central biotype) used in these experiments were originally collected near Weslaco, Texas, USA, and have been in culture at the University of California, Riverside, CA, USA for over 50 generations. Periodically, fresh material collected from the same location was added to the colony. The colony was maintained under greenhouse conditions at 20–30 °C and 20–40% RH. The host plants were tomatoes (variety 'Yellow Pear') and occasionally supplemented with potatoes (variety 'Atlantic'). Post-teneral adults were used for all the experiments. The presence of Cls in colonies (haplotype B) was confirmed by periodically testing adult psyllids and plants using real-time quantitative (qPCR) using the methods of Butler et al. (2011b).

Plants

Four advanced clones (510, NAU, 865 and 246), generated by researchers at INIFAP, were used in the experiments. A commercial potato variety, Atlantic, was included as a control. In previous field evaluations in Mexico, the four advanced clones have shown tolerance against ZC (Cadena-Hinojosa et al. 2003; Rubio-Covarrubias et al. 2013; Rubio-Covarrubias et al. 2015). In addition, in the same field tests

carried out in the Toluca Valley, which is regarded as the origin center of *Phytophthora infestans* (Alarcón-Rodríguez et al. 2014), the clones 865 and 246 have exhibited tolerance to late blight. This resistance is derived from *Solanum demissum* Lindl. The genotype 510 is suitable for chipping while the others are only suitable for table consumption.

Greenhouse-raised mini-tubers were used in all the experiments. Potato mini-tubers were individually seeded in 4.9-L pots containing the soil substrate UCR Mix 3 (Matkin and Chandler 1957). All the plants were initially kept in a greenhouse maintained at 23–26 °C, 30–50% RH, a photoperiod of 14:10 (L:D) h. and were fertilized once every two weeks with the label rate of Miracle-Gro® nutrient solution (Scotts Company, Marysville, OH, USA).

Plants were used for the choice assay six weeks after emergence, once they reached the 'tuber development' stage (Growth Stage III) (Dwelle 2003). Plants used for no-choice assays were exposed to the psyllids four weeks after emergence. The two tests were performed on intact plants and the inoculated leaves remained attached to the plants after being exposed to the potato psyllids. All plant infestations in the choice assay occurred in an insect rearing room with 23–25 °C, 40–60% RH and artificial light controlled to provide a photoperiod of 14:10 (D:L). The plants remained in the room until they were processed or disposed of. Once at the appropriate stage, plants were haphazardly assigned to conditions and experiments. All the plants were grown under identical greenhouse conditions described previously and the plants were never removed from the greenhouse.

Choice Bioassay of Antibiosis, Antixenosis, and Infection

Suitability and acceptability of a cultivar for use by an insect can vary among cultivars. When provided a choice of a known acceptable host plant and another species or cultivar, insects may choose preferentially oviposit, settle or feed on one of another of the cultivars presented. We examined these patterns via laboratory choice experiments. This experiment was performed using experimental arenas formed by two pieces, each one constructed from a foam ring 20 cm diameter, 3 cm wide and 0.6 mm thick, covered with a sheet of transparent plastic 20 cm diameter and 0.5 mm thick. A terminal leaflet of the uppermost fully-expanded leaf of one of the experimental clones was placed on one side of the arena and a terminal leaflet of the Atlantic variety was placed on the opposite side. The two covers were put together, as a sandwich with the leaflets in the middle, and the edges were sealed with banana hair clips. Once the leaflets were within sealed arenas, one clip was removed and five male: female pairs of adult *B. cockerelli* were introduced in the box. Ten replications were performed for each of the clonal potato genotypes.

Three days after the introduction to experimental arenas, the psyllids and eggs were removed. Plants were then maintained within insect rearing rooms until the foliage was removed at 11 weeks post sowing. Two weeks after cutting the foliage, the tubers from each plant were harvested. Two months after harvest, the tubers were cut to score their internal discoloration using a range of 0 (no symptoms) to 3 (severe symptoms) (Fig. 1). The same tubers were cut in 2 mm thick slices, fried in sunflower oil at 180 °C and the presence or absence of dark color was recorded. Concurrent to discoloration scoring, the two largest tubers from each plant were stored and later processed for biochemical analysis. A slice of tuber from the basal end of the tuber was removed with a fresh razor blade, 3 mm from the edges was eliminated, and the remaining tissue was smashed using a mortar and pestle. Three 0.1-g aliquots of this smashed tissue were placed into separate 1.5-ml micro-centrifuge tubes, immediately placed in ice and later kept at −20 °C until further chemical analyses. A total of 16 tubers per genotype (eight from exposed and eight from unexposed plants) were used for biochemical analysis. After cutting the tuber slices for biochemical analysis, the remaining tissue of each tuber was placed in a Ziploc® bag (S.C. Johnson and Sons, Racine, WI, USA), and stored at 4 °C until testing for Cls via qPCR using methods of Butler et al. (2011b). A single tuber per plant was analyzed, resulting in a total of 8 tubers of each potato genotype (four from exposed and four from non-exposed plants) tested.

No-Choice Bioassays of Antibiosis and Antixenosis

Two distinct no-choice bioassays were performed. The first bioassay was designed to test for antibiosis or similar responses of psyllids to genotype. In particular, the aim was to determine if oviposition or performance (development) differed with respect to potato line. These bioassays compliment choice bioassays in that they force the insects to use plants they would reject (choose against) in choice contexts. The second bioassay was designed to evaluate the effect of psyllid exposure on each of the potato lines.

To conduct the psyllid-focused assays (hereafter "no-choice assays"), five pairs (male: female) of post-teneral *B. cockerelli* were confined to a single terminal leaflet of one fully-expanded potato leaf in a 10 by 8 cm organza mesh bag (Jo-Ann Stores, Inc., Hudson, OH, USA) for a 72-h access period. Following this period, the bag and adult insects were

Fig 1 Examples of color scale used in scoring ZC symptoms in freshly cut tubers

removed and the number of eggs was counted. Plants were then examined daily and the numbers of eggs, small (1st or 2nd instar) nymphs, large (3rd to 5th instar) nymphs, and adults were counted. As soon as the nymphs of 5th instar molted to adults, they were removed. Plants were examined until all nymphs were either adults or died. Plants were maintained in a climate controlled insect rearing room at 23–25 °C and 40–60% relative humidity for the length of the experiment. There were 10 replications for each genotype, with each plant being used once (a single caged leaf). To determine the effect of genotype on psyllid development, growth index (GI) was calculated using the method of Zhang et al. (1993). The GI is defined as the sum of the highest growth stage that individuals would achieve in an ideal controlled population. GI ranges from zero to one, with one indicating most individuals survived to adulthood, while zero indicates no insects survived beyond the first stage.

No-Choice Bioassays of Transmission

To conduct the plant focused assays (hereafter "transmission assays"), three male psyllids were confined to the terminal leaflet of one fully expanded potato leaf of one of the potato clones. Psyllids were confined for 72 h using organza mesh bags, after which they were removed from the leaflet with an insect aspirator. Seven weeks post psyllid exposure, the exposed potato leaves were removed from the plant, placed in a Ziploc® bag and stored at −80 °C until qPCR analysis. The plants were maintained for 10 weeks after potato psyllid exposure. The plants were then cut and one week later the tubers were harvested and their internal tuber discoloration was scored as in the choice assays. The tubers that were not used for qPCR analysis were cut in 2 mm thick slices, fried in oil at 180 °C and the presence or absence of dark color was recorded.

Behavioral Bioassays

This test was performed using the previously described methods of Liu and Trumble (2004). Briefly, the behavior of insects on leaves was observed in arenas made by layering the following components: a Plexiglass® rectangle (9 by 11.5 cm), the test leaflet (psyllid was placed on abaxial surface), foam (0.5 by 8 by 9 cm) with a 2.5 cm^2 hole cut in it, and a 12.5-cm-diameter glass plate that covered the arena. The leaflet was not detached from the plant in order to avoid potential physiological changes associated with leaf excision. An unsexed post-teneral psyllid was placed into the arena and allowed to acclimate for five minutes before initiating behavioral recording. Behavioral observations were performed for 15 min., which is enough to observe all important behaviors (Liu and Trumble 2004). The observations were recorded using the Noldus Observer program (Noldus, Wageningen, The Netherlands). Specific behaviors recorded included cleaning, feeding, probing, off-leaflet, resting, and walking. The behavioral observations were replicated 10 times for each plant genotype, and a naïve psyllid was used for each replication.

Trichome Measurement

Bactericera cockerelli are known to respond to tactile differences in plants (Prager et al. 2013; Yang et al. 2010), which can result from artificial materials, but can also vary due to trichomes on plant leaves. To that end, the length of density of trichomes on the different plant genotypes was examined. When leaves were examined under magnification, it was observed that the length and density of trichomes in the five potato genotypes were lower on the edges of the leaves. Considering that *B. cockerelli* prefers to feed on the midrib of the leaves, this site was selected as a representative spot to count and measure the trichomes. A 2 cm square location was identified and the number of trichomes within that area was counted. Additionally, the length of each trichome was measured with a micrometer. Ten leaflets of each genotype were examined.

Biochemical Analyses

Phenolics, amino acids, and sugars were quantified according to the methods of Rashed et al. (2013) and Wallis et al. (2014). All solvents and standards were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless indicated otherwise. In brief, plant samples were pulverized with a mortar and pestle and liquid nitrogen, with 0.1 g of pulverized tissue weighed and placed in two (for tuber tissue) or three (for foliar tissue) separate microcentrifuge tubes. One of the tissue aliquots in one of these tubes was extracted twice overnight at 4 °C in 1 mL of methanol (0.5 mL each night, with the supernatants of both combined to yield 1 mL). Another aliquot of sample in a separate tube was extracted similarity twice overnight in 1 mL of phosphate buffer saline (PBS) adjusted to pH 6.8, and the last aliquot (for foliar tissue only) was extracted similarly twice overnight in 1 mL of methyl-tert butyl ether (MTBE) containing 100 ppm n-pentadecane.

The methanol extracts were used to analyze phenolic compounds using a Shimadzu (Columbia, MD, USA) LC-20 AD high-performance liquid chromatography (HPLC) system equipped with a Supelco Ascentis RP-18 column (Sigma-Aldrich, St. Louis, MO, USA) and a Shimadzu PDA-20 photodiode array detector, with peaks analyzed at 280 nm. A binary water and methanol (Fisher Scientific, Pittsburgh, PA, USA) gradient was used as described by Rashed et al. (2013). Peak identification and compound quantification was performed using a combination of LC-MS (running the same column and gradient on a Shimadzu LCMS2020 system)

and standards representative of each major phenolic compound class (Rashed et al. 2013).

The PBS extracts were used to analyze sugars using a Shimadzu LC-10 AD HPLC system equipped with a Supelco C-611 ion-exchange column and Shimadzu RID-10 refractive index detector for quantification (Rashed et al. 2013). Additional PBS extract was used to assess amino acids via the EZ-FAAST GC-FID commercially available kit from Phenomenex (Torrance, CA, USA) following manufacturer's protocols and with a Shimadzu GC2010 gas chromatograph (GC) with a flame ionization detector (Rashed et al. 2013).

The MTBE extracts were used to analyze terpenoids in foliar tissue only using a Shimadzu GC-MS2010S mass spectrometer equipped with a SHRXI-5MS column (30 m × 0.25 mm × 0.25 μm) using similar temperature gradient and operating conditions as Wallis et al. (2015b). In addition to the n-pentadecane internal standard, an external standard mixture of 30 different terpenoid compounds obtained from Sigma-Aldrich was run as a serial dilution to assist with compound identification and quantification.

In total, 10 phenolic compounds in tuber tissues [chlorogenic acid (CGA), five CGA derivatives, cryptochlorogenic acid (CCGA), and a CCGA deriv., a flavonol glucoside, and protocatechuic acid hexoside], 14 phenolic compounds in foliar tissues [catechin, chlorogenic acid (CGA), two CGA derivatives, cryptochlorogenic acid (CCGA), a dichlorogenic acid, and 8 unidentified flavonoids], up to 19 amino acids in both tissues [alanine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine (not quantified in foliar tissues as it was below detection thresholds) phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine], three sugars in both tissues (fructose, glucose, and sucrose), and 14 terpenoids in foliar tissues (bornyl acetate, carvone, linalool, pulgone, α-copaene, α-humulene, α-phellandrene, α-pinene, α-terpinene, β-caryophyllene, β-ionone, β-pinene, Δ-3-carene, and γ-terpinene) were quantified from both zebra chip-exposed and non-exposed plants from the choice test.

Statistical Analyses

In no-choice assays, numbers of eggs was zero inflated and thus non-normally distributed; GLM was performed using negative binomial probability distributions performed in SAS (SAS Institute v. 9.3 2012) PROC GLIMMIX. Hatch proportion met all assumptions of ANOVA. GI was not normally distributed, and was examined using non-parametric Kruskal-Wallis tests. In all instances, genotype was included as the fixed effect in each analysis. When an overall model was significant, subsequent analyses of means was performed using the Tukey-Kramer post-hoc method. Since the behaviors examined in observational bioassays were potentially correlated, they were examined using MANOVA with a model that contained a single fixed term for cultivar. Two-choice assays were examined as individual assay experiments considering each genotype vs. Atlantic. Number of eggs was examined using GLM with a negative binomial probability distribution. Location was examined daily for three days, by counting the number of *B. cockerelli* on all leaflets. Discoloration was examined using a GLM with a Poisson probability distribution and a model that included terms for genotype with nested terms for plant and tuber, since multiple tubers were examined from each plant. In analyzing biochemical data, prior to analysis, all variables were tested for assumptions of normality. Since not all compounds examined were normally distributed, Permutated Multivariate Analyses of Variance (PERMANOVA) was performed using the Adonis function of the R package vegan. Adonis was used to examine overall differences in classes of compounds. In each instance the model contained terms for infection status, clone, and an interaction term. Individual compounds were examined for differences with respect to genotype using Kruskal-Wallis tests. *P*-values were adjusted for multiple comparisons using the Holm-Bonferroni method (Holm 1979). When significant differences were detected in individual compounds, further follow-up analyses were performed using Dunn's Test of Multiple Comparisons Using Rank Sums (Dunn 1964) adjusted using the Holm-Bonferroni method and performed using the package dunn.test.

Results

Choice Assays

When presented a choice of either an experimental genotype or Atlantic, there were no daily trends in settling for any of the variety pairs examined. Thus, the effect of day was pooled in subsequent analyses. When examined as pairs, the experimental lines 246 and 865 were preferred for settling by adult psyllids compared with the susceptible variety Atlantic (Fig. 2a). In examining host-plant choice for oviposition, 510 had significantly fewer eggs deposited than Atlantic, with no other significant differences observed ($P > 0.05$) (Fig. 2b).

The presence of Cls also was examined for the choice bioassays, and confirmed the presence of Cls in 78% of the tubers, while all the qPCR analyzed tubers from unexposed plants were negative for Cls and ZC symptoms. Additionally, tubers were also directly scored for discoloration symptoms of ZC. Cls-infected tubers from the four experimental genotypes (246, 865, 510 and NAU) presented a significantly lower discoloration index score than Atlantic (Fig. 3a) (GLM: $X^2 = 184.300$; 4, 200; $P < 0.001$). Among the clones, 246, 510 and NAU did not show any internal tuber discoloration, and 865 only exhibited a barely perceptible discoloration. However, all

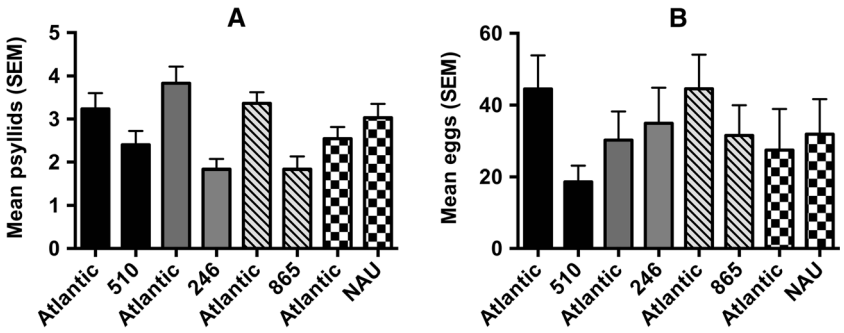

Fig. 2 Preference as indicated by (**a**) mean (SEM) settling and (**b**) mean oviposition (SEM) on four potato experimental lines paired with Atlantic as a control. *Marked pairwise comparisons denote significant differences (GLM: $P < 0.05$) ($N = 10$ per pairing)

five genotypes presented the classical zebra chip symptoms after the tubers of exposed plants were fried.

No-Choice Bioassays of Antibiosis and Antixenosis

When examined in a no-choice context, there were significant differences in oviposition among genotypes (GLM: $X^2 = 5.941$; df = 25, 4, 44; $P < 0.001$), with psyllids ovipositing least on the genotype 246, and most on the genotype NAU (Table 1). There were significantly more eggs laid on 510 than 246 (Table 1). There were no significant differences in either the proportion of hatching (ANOVA: $F = 1.500$; df = 4, 44; $P = 0.230$) or in the growth index (KW: $X^2 = 5.490$; df = 4; $P = 0.200$) (Table 1).

No-Choice Bioassays of Transmission

In the plant focused no-choice bioassyas, the presence of Cls was confirmed in 66% of plants. All the qPCR analyzed tubers from unexposed plants were free of Cls and did not exhibit ZC symptoms after frying.

In addition to testing for Cls via qPCR, tubers were also directly scored for discoloration symptoms of ZC. When tubers from transmission assays were examined (Fig. 3b), there was a significant difference in tuber discoloration score ($X^2 = 14.400$; df = 4, 257; $P < 0.001$). The Cls-infected tubers of the four experimental clones (246, 865, 510 and NAU) all had lower browning intensity scores than Atlantic. These results are similar to those from the choice test. However, in the no-choice test all experimental lines showed a barely noticeable discoloration, that was not observed in the choice assays.

Behavioral Bioassays

All the recorded behaviors were examined collectively as both the total time spent performing the behavior (MANOVA: $F = 1.341$; df = 4, 44; $P = 0.140$) (S1) and as the number of times a behavior was performed (MANOVA: $F = 1.080$; df = 4, 43; $P = 0.450$) (S2). As, there were no significant differences ($P > 0.05$) in either of these measurements due to cultivar, no further analyses were conducted and individual behaviors were not examined.

Trichomes

Both the density of trichomes, measured as total number in the examined area (ANOVA: $F = 29.470$; df = 4,25, $P < 0.001$), and the length of trichomes (ANOVA: $F = 7.000$, df = 4, 25, $P < 0.001$) differed among genotypes (Fig. 4a). Experimental lines 246, 865 and 510 had significantly longer trichomes than Atlantic (Fig. 2). Furthermore, 246, 865 and NAU had significantly greater trichome density than Atlantic (Fig. 4b).

Evaluation of Cls and Zebra Chip

All of the genotypes examined, including Atlantic, proved highly susceptible to infection with Cls based on incidence (S3), with a range between 80 and 100% infection based on a Ct score of less than 32 indicating positive for Cls in the

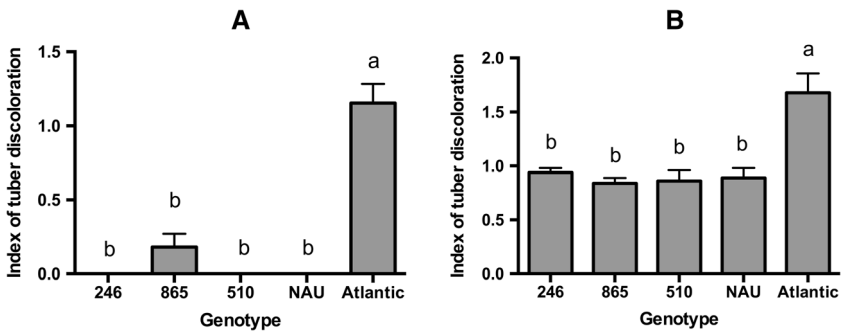

Fig 3 Mean (SEM) tuber color index of raw tubers from five potato genotypes exposed to psyllids in (**a**) choice and (**b**) no-choice transmission specific bioassays. Different letters indicate significant differences (Tukey test $P < 0.05$)

Table 1 Mean (SEM) growth index, number of eggs, and proportion of hatching eggs of *B. cockerelli* reared on five potato genotypes

Genotype	Growth Index	No. Eggs	Prop. Hatching
246	0.57 ± 0.033 a	18.1 ± 6.2 b	59.8 ± 6.6 a
865	0.59 ± 0.032 a	36.9 ± 7.6 ab	67.6 ± 8.5 a
510	0.62 ± 0.019 a	45.0 ± 6.3 a	71.3 ± 5.1 a
NAU	0.61 ± 0.026 a	52.6 ± 11.4 a	70.8 ± 6.7 a
Atlantic	0.65 ± 0.011 a	24.5 ± 5.7 ab	81.0 ± 4.2 a

Means followed by different letters are significantly different (Tukey-Kramer test $P < 0.05$)

qPCR analysis. There were no significant differences among genotypes ($X^2 = 3.700$; df = 4; $P = 0.450$) with respect to incidence.

Tuber Biochemical Analysis

A total of 19 amino acids, three sugars, and 10 phenolic compounds were quantified in tuber extracts from both Cls-exposed and non-exposed plants (Tables 2 and 3). These were initially examined by class using permutated MANOVA and a model that included terms for both infection status and genotype. In these analyses, there was a significant effect of infection on every class of compound examined (Amino Acids: $F = 7.800$; df = 1; $P < 0.004$; Sugars: $F = 30.400$; df = 1; $P < 0.001$; Phenolics: $F = 54.200$; df = 1; $P < 0.001$). There were also significant differences due to genotype for levels of amino acids ($F = 9.900$; df = 4; $P < 0.01$) and phenolic compounds ($F = 10.700$; df = 4; $P < 0.001$), but not for sugars ($F = 1.900$; df = 4; $P = 0.07$). There also were significant genotype by infection interactions for effects on phenolic compounds ($F = 6.700$; df = 4; $P < 0.001$) and sugars levels ($F = 2.200$; df = 4; $P = 0.05$), but no significant interaction for amino acid levels ($F = 2.100$; df = 4; $P = 0.06$).

Despite overall differences with respect to infection status when considering compound classes as a whole, when individual compounds were examined, no statistically significant differences were detected ($P > 0.05$ in all cases) (Tables 2, 3).

Leaf Biochemical Analysis

A total of 18 amino acids, three sugars, 14 phenolic compounds, and 14 terpenoids were quantified in leaf extracts from both Cls-exposed and non-exposed plants (Tables 4 and 5). Similar to the tuber material, compounds were initially examined as classes using permutated MANOVA with fixed terms for genotype and Cls infection status (Tables 4 and 5). These analyses indicated a significant effect of infection on all the compounds examined (Amino Acids: $F = 6.700$, df = 1; $P < 0.0100$; Phenolics: $F = 16.169$; df = 1; $P < 0.001$; Sugars: $F = 1.700$; df = 1; $P < 0.001$; Terpenoids: $F = 42.279$; df = 1; $P < 0.001$). Additionally, there were significant differences among genotypes (Amino Acids: $F = 8.800$, df = 4, $P < 0.001$; Phenolics: $F = 12.362$; df = 4; $P < 0.001$; Sugars: $F = 3.400$; df = 4; $P < 0.001$; and Terpenoids: $F = 8.808$; df = 4, $P < 0.001$). For amino acids ($F = 2.800$; df = 4; $P < 0.01$) and sugars ($F = 2.900$; df = 4; $P < 0.05$) there were significant interactions of infection and genotype, but not so for phenolics or terpenoids ($P > 0.05$).

When healthy (unexposed) and Cls infected tubers were examined individually, the amino acids glutamic acid, serine, and threonine differed among cultivars (Tables 2 and 3). The greatest levels of glutamic acid and threonine were found in Atlantic, while serine was noticeably less in 865. There were also significant differences among unexposed cultivars in fructose and glucose with the least fructose in 865 and the most in 510 (Tables 2 and 3). When tubers were exposed to Cls, the cultivars 865 and NAU had the least fructose. For the terpenoids β-caryophyllene and α-copaene, there also were significant differences in levels among the examined cultivars with the least β-caryophyllene in uninfected 246 and the least α-copaene in uninfected Atlantic (Tables 3 and 4). When compounds were examined with respect to Atlantic, it was found that nearly all of the significant differences were between either 246 or 865 and Atlantic (Tables 2 and 3). This pattern was also detected in both infected and uninfected leaves, but was most obvious in amino acid levels of infected plants.

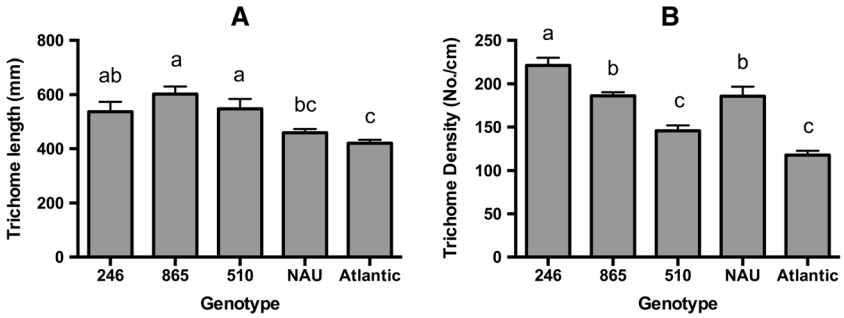

Fig. 4 (**a**) Mean (SEM) trichome density and (**b**) mean (SEM) trichome length when measured on the main rib of the abaxial side of five potato genotypes. Different letters indicate significant differences (Tukey test $P < 0.05$)

Table 2 Mean (SEM) concentrations of amino acids, sugars, and phenolic compounds in potato tubers infected with Cls

	246	865	510	NAU	Atlantic
Amino acids					
Alanine	0.25 ± 0.04	0.49 ± 0.03	0.25 ± 0.02	0.34 ± 0.05	0.54 ± 0.06
Asparagine	19.85 ± 9.87	46.73 ± 6.19	20.64 ± 2.23	28.26 ± 4.26	33.12 ± 2.54
Aspartic acid	1.66 ± 0.28	2.32 ± 0.33	1.58 ± 0.08	1.52 ± 0.14	1.94 ± 0.13
Glutamic acid	1.26 ± 0.3	1.73 ± 0.42	1.22 ± 0.25	1.05 ± 0.25	1.09 ± 0.09
Glutamine	6.91 ± 3.52	25.39 ± 3.71	12.64 ± 1.85	10.51 ± 1.92	15.21 ± 2.06
Glycine	0.19 ± 0.07	0.41 ± 0.08	0.21 ± 0.02	0.18 ± 0.02	0.29 ± 0.05
Histidine	0.54 ± 0.19	1.09 ± 0.17	0.62 ± 0.06	0.66 ± 0.13	0.62 ± 0.06
Isoleucine	0.69 ± 0.24	1.78 ± 0.28	1.02 ± 0.14	0.87 ± 0.17	1.43 ± 0.13
Leucine	0.26 ± 0.09	0.77 ± 0.17	0.42 ± 0.09	0.27 ± 0.05	0.42 ± 0.05
Lysine	0.82 ± 0.16	1.5 ± 0.22	1.38 ± 0.15	1.13 ± 0.16	1.31 ± 0.10
Methionine	0.3 ± 0.11	0.76 ± 0.07	0.51 ± 0.06	0.37 ± 0.05	0.72 ± 0.08
Ornithine	0.1 ± 0.03	0.12 ± 0.02	0.05 ± 0.01	0.11 ± 0.04	0.06 ± 0.01
Phenylalanine	0.46 ± 0.14	1.04 ± 0.15	0.69 ± 0.11	0.55 ± 0.13	0.74 ± 0.09
Proline	1.26 ± 0.46	2.58 ± 0.75	1.0 ± 0.14	1.31 ± 0.28	1.79 ± 0.37
Serine	4.45 ± 0.99	7.96 ± 1.29	6.23 ± 0.56	5.45 ± 0.24	7.86 ± 0.4
Threonine	0.47 ± 0.11	0.85 ± 0.13	0.78 ± 0.08	0.51 ± 0.04	0.73 ± 0.04
Tryptophan	0.32 ± 0.1	0.59 ± 0.18	0.61 ± 0.12	0.46 ± 0.09	0.44 ± 0.08
Tyrosine	0.1 ± 0.05	0.04 ± 0.02	0.03 ± 0.01	0.20 ± 0.05	0.01 ± 0.00
Valine	1.74 ± 0.68	4.16 ± 0.62	2.84 ± 0.29	2.24 ± 0.47	3.41 ± 0.31
Sugars					
Fructose	0.37 ± 0.11	1.14 ± 0.46	1.2 ± 0.33	0.58 ± 0.19	1.54 ± 0.35
Glucose	2.32 ± 0.6	3.55 ± 1.01	3.37 ± 0.68	3.60 ± 0.78	4.75 ± 0.73
Sucrose	2.68 ± 0.36	4.23 ± 1.49	8.26 ± 2.33	3.32 ± 0.46	4.11 ± 0.86
Phenolics					
Chlorogenic acid	31.85 ± 7.12	35.28 ± 5.00	8.22 ± 0.99	65.35 ± 9.61	108.79 ± 35.47
Chlorogenic acid derivative 1	14.59 ± 1.91	18.3 ± 3.74	5.62 ± 0.62	15.86 ± 1.77	36.7 ± 7.91
Chlorogenic acid derivative 2	24.49 ± 6.27	15.54 ± 2.65	27.54 ± 3.67	16.05 ± 1.12	23.58 ± 4.70
Chlorogenic acid derivative 3	15.4 ± 2.10	18.56 ± 2.83	7.66 ± 2.22	12.03 ± 1.46	7.1 ± 1.69
Chlorogenic acid derivative 4	13.93 ± 2.41	7.76 ± 2.06	5.7 ± 2.07	27.14 ± 4.52	49.46 ± 13.3
Chlorogenic acid derivative 5	35.24 ± 3.03	24.93 ± 5.72	24.64 ± 2.65	33.41 ± 6.11	92.57 ± 22.64
Cryptochlorogenic acid	4.6 ± 0.67	5.82 ± 1.87	2.39 ± 0.44	14.74 ± 1.74	21.92 ± 6.80
Cryptochlorogenic acid derivative 2	8.47 ± 0.50	4.37 ± 1.03	5.47 ± 0.73	7.79 ± 1.6	4.98 ± 1.54
Flavonoid glycoside 1	2.9 ± 1.05	4.59 ± 1.72	3.83 ± 1.05	8.18 ± 1.18	21.17 ± 6.41
Protocatechuic acid	60.96 ± 9.75	98.22 ± 16.98	27.1 ± 1.67	63.86 ± 8.77	73.67 ± 13.65

Discussion

In all the bioassays conducted (choice and no-choice), the raw tubers of the four experimental lines showed lower ZC intensity scores than the Atlantic variety, although the presence of Cls in tubers was confirmed by qPCR analyses. These tests, performed under controlled conditions, confirm what was observed under field conditions in the Toluca Valley, Mexico, although the variety Fianna was used as a control (Rubio-Covarrubias et al. 2013; Rubio-Covarrubias et al. 2015). Interestingly, the tubers from the choice test showed lower discoloration index than those from the no-choice. This was probably associated with the time that elapsed between the infection date and the vine clipping. In the choice test the plants were cut one month after they were infected. However, in the no-choice test the plants were cut two months after they were exposed to *B. cockerelli*. It is, though, also possible that this reflects a difference in the number of insects feeding per plant between the two types of bioassay. Regardless, these results are consistent with the work of other researchers who have shown an association between intensity of ZC symptoms and time since infections in the tubers (Gao et al. 2009; Rashed et al. 2013).

Table 3 Mean (SEM) concentrations of amino acids, sugars, and phenolic compounds in healthy potato tubers unexposed to Cls

	246	865	510	NAU	Atlantic
Amino acids					
Alanine	0.21 ± 0.01	0.31 ± 0.06	0.23 ± 0.02	0.27 ± 0.03	0.73 ± 0.12
Asparagine	15.16 ± 2.02	30.56 ± 4.75	22.91 ± 2.88	27.49 ± 2.9	39.65 ± 6.22
Aspartic acid	1.13 ± 0.04	1.45 ± 0.13	1.26 ± 0.04	1.21 ± 0.13	1.41 ± 0.07
Gutamic acid	0.88 ± 0.03	2.88 ± 0.4	2.55 ± 0.17	2.15 ± 0.29	2.45 ± 0.27
Glutamine	7.10 ± 0.88	11.82 ± 1.28	16.09 ± 2.77	12.55 ± 3.06	20.29 ± 3.57
Glycine	0.11 ± 0.00	0.16 ± 0.01	0.17 ± 0.02	0.12 ± 0.01	0.25 ± 0.03
Histidine	0.23 ± 0.01	0.47 ± 0.04	0.31 ± 0.02	0.33 ± 0.04	0.56 ± 0.11
Isoleucine	0.24 ± 0.01	0.54 ± 0.05	0.38 ± 0.04	0.29 ± 0.04	0.91 ± 0.15
Leucine	0.10 ± 0.01	0.21 ± 0.01	0.16 ± 0.02	0.11 ± 0.03	0.29 ± 0.06
Lysine	0.45 ± 0.03	0.74 ± 0.03	0.77 ± 0.08	0.51 ± 0.07	0.92 ± 0.16
Methionine	0.24 ± 0.02	0.51 ± 0.05	0.48 ± 0.06	0.23 ± 0.02	0.69 ± 0.1
Ornithine	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.06 ± 0.02
Phenylalanine	0.12 ± 0.01	0.32 ± 0.04	0.23 ± 0.03	0.17 ± 0.03	0.57 ± 0.14
Proline	0.39 ± 0.04	0.63 ± 0.04	0.51 ± 0.03	0.54 ± 0.05	0.77 ± 0.05
Serine	2.57 ± 0.24	3.49 ± 0.46	3.46 ± 0.22	3.95 ± 0.31	5.7 ± 0.42
Threonine	0.36 ± 0.03	0.48 ± 0.03	0.57 ± 0.06	0.42 ± 0.04	0.67 ± 0.06
Tryptophan	0.13 ± 0.03	0.32 ± 0.04	0.29 ± 0.06	0.16 ± 0.04	0.42 ± 0.09
Tyrosine	0.04 ± 0.01	0.02 ± 0	0.04 ± 0.01	0.05 ± 0.01	0.02 ± 0.01
Valine	0.77 ± 0.05	1.41 ± 0.21	1.55 ± 0.19	0.87 ± 0.07	2.59 ± 0.38
Sugars					
fructose	0.25 ± 0.03	0.05 ± 0.01	0.15 ± 0.02	0.14 ± 0.02	0.27 ± 0.12
Glucose	1.25 ± 0.13	0.63 ± 0.05	0.67 ± 0.10	1.6 ± 0.54	0.94 ± 0.39
Sucrose	2.05 ± 0.30	2.76 ± 0.36	1.78 ± 0.28	1.86 ± 0.17	2.07 ± 0.36
Phenolics					
Chlorogenic acid	3.15 ± 0.67	6.31 ± 0.82	8.27 ± 1.12	2.8 ± 0.99	5.06 ± 0.51
Chlorogenic acid derivative 1	9.01 ± 1.12	0.42 ± 0.04	5.83 ± 2.10	7.87 ± 1.71	7.34 ± 0.91
Chlorogenic acid derivative 2	9.98 ± 2.13	3.72 ± 0.23	15.76 ± 2.79	19.95 ± 2.19	1.1 ± 0.1
Chlorogenic acid derivative 3	10.66 ± 1.56	8.44 ± 0.71	11.29 ± 2.23	15.61 ± 2.08	6.15 ± 0.96
Chlorogenic acid derivative 4	0.27 ± 0.10	0.67 ± 0.07	0.44 ± 0.08	2.04 ± 1.21	0.36 ± 0.08
Chlorogenic acid derivative 5	12.7 ± 1.10	3.41 ± 0.46	9.06 ± 0.74	13.38 ± 1.99	4.88 ± 0.37
Cryptochlorogenic acid	4.52 ± 0.34	3.1 ± 0.41	5.93 ± 1.44	10.18 ± 1.48	2.27 ± 0.27
Cryptochlorogenic acid derivative 2	9.19 ± 0.65	0.25 ± 0.04	3.78 ± 0.97	3.95 ± 0.37	3.94 ± 0.51
Flavonoid glycoside 1	0.26 ± 0.06	7.44 ± 0.94	0.28 ± 0.08	0.04 ± 0.01	0.08 ± 0.01
Protocatechuic acid	17.04 ± 4.79	40.66 ± 3.86	16.35 ± 3.65	50.05 ± 8.29	29.5 ± 5.84

Most of the raw tubers of the four experimental lines did not show discoloration (choice test) or displayed barely perceptible ZC symptoms (no-choice test), but tubers of all genotypes showed clear ZC symptoms after frying. On the other hand, it is important to note that in Mexico 56% of the total potato production intended for consumption is fresh and only 29% for the production of chips (CNSPP 2013). This situation favors the selection of potato genotypes that do not show ZC symptoms or present, to a lesser extent, internal discoloration in raw tubers. NAU and 510 have already been commercially planted by potato growers in Mexico and have been widely accepted because, besides their low susceptibility to internal discoloration of the tubers, they have also shown good agronomic and commercial characteristics (Rubio-Covarrubias et al. 2013; Rubio-Covarrubias et al. 2015).

It is interesting to note that in the field evaluations performed in Mexico (Rubio-Covarrubias et al. 2013; Rubio-Covarrubias et al. 2015), the tubers of the four experimental lines have shown greater internal discoloration intensity than that observed in the tests performed under controlled conditions in this study. These differences may be due to the interaction of many factors. In the field tests, the plants are exposed to inclement weather and to the attack of other insects and diseases that may stimulate plant defense reactions, such as

Table 4 Mean (SEM) concentrations of various compounds in leaf tissue infected with Cls

	246	865	510	NAU	Atlantic
Amino Acids					
Alanine	**0.46 ± 0.06**	**0.66 ± 0.08***	**0.64 ± 0.04**	**0.60 ± 0.05**	**0.60 ± 0.05**
Asparagine	**0.4 ± 0.15**	**0.19 ± 0.04***	**0.35 ± 0.06**	**1.16 ± 0.52**	**0.67 ± 0.36**
Aspartic acid	**0.58 ± 0.04**	**0.83 ± 0.05***	**0.73 ± 0.06**	**0.70 ± 0.06**	**0.71 ± 0.06**
Glutamic acid	**0.71 ± 0.07***	**1.13 ± 0.06**	**0.76 ± 0.04**	**0.76 ± 0.06**	**1.03 ± 0.05**
Glutamine	**0.13 ± 0.02**	**0.14 ± 0.02***	**0.15 ± 0.01**	**0.36 ± 0.13**	**0.34 ± 0.12**
Glycine	**0.12 ± 0.01**	**0.12 ± 0.01***	**0.1 ± 0.01**	**0.1 ± 0.01**	**0.14 ± 0.01**
Histidine	**0.06 ± 0.01**	**0.05 ± 0.00***	**0.08 ± 0.01**	**0.11 ± 0.04**	**0.08 ± 0.03**
Isoleucine	**0.12 ± 0.01**	**0.11 ± 0.01***	**0.17 ± 0.01**	**0.16 ± 0.03**	**0.15 ± 0.03**
Leucine	**0.18 ± 0.02**	**0.21 ± 0.03***	**0.28 ± 0.02**	**0.22 ± 0.02**	**0.24 ± 0.03**
Lysine	**0.18 ± 0.02**	**0.17 ± 0.02***	**0.26 ± 0.02**	**0.18 ± 0.02**	**0.21 ± 0.02**
Methionine	**0.03 ± 0.00**	**0.03 ± 0.00***	**0.03 ± 0.00**	**0.03 ± 0**	**0.03 ± 0.00**
Phenylalanine	**0.12 ± 0.01**	**0.11 ± 0.01***	**0.16 ± 0.01**	**0.16 ± 0.03**	**0.14 ± 0.02**
Proline	**0.22 ± 0.03**	**0.17 ± 0.02***	**0.25 ± 0.02**	**0.25 ± 0.04**	**0.23 ± 0.02**
Serine	**1.04 ± 0.11**	**0.77 ± 0.06***	**1.41 ± 0.12**	**1.13 ± 0.08**	**0.87 ± 0.05**
Threonine	**0.06 ± 0.01***	**0.05 ± 0.01***	**0.05 ± 0.00***	**0.08 ± 0.01**	**0.16 ± 0.01**
Typtophan	**0.13 ± 0.02**	**0.08 ± 0.01***	**0.15 ± 0.02**	**0.24 ± 0.07**	**0.15 ± 0.03**
Tyrosine	**0.01 ± 0.00***	**0.00 ± 0.00***	**0.02 ± 0.01**	**0.01 ± 0**	**0.10 ± 0.02**
Valine	**0.21 ± 0.02**	**0.21 ± 0.03***	**0.25 ± 0.01**	**0.28 ± 0.06**	**0.20 ± 0.02**
Sugars					
Fructose	**0.95 ± 0.09**	**0.49 ± 0.12**	**1.11 ± 0.14**	**0.25 ± 0.1**	**0.65 ± 0.05**
Glucose	0.96 ± 0.10	0.51 ± 0.14	1.06 ± 0.13	0.24 ± 0.08	0.63 ± 0.09
Sucrose	**0.23 ± 0.01**	**0.25 ± 0.02***	**0.37 ± 0.06**	**0.21 ± 0.03**	**0.29 ± 0.04**
Phenolics					
Catechin	**36.34 ± 4.69**	**73.53 ± 8.43**	**39.13 ± 4.75**	**95.92 ± 14.55**	**72.57 ± 5.64**
Chlorogenic acid	**15.67 ± 2.45**	**15.25 ± 3.03**	**32.05 ± 5.27**	**11.67 ± 2.04**	**25.15 ± 4.25**
Chlorogenic acid deriv. 1	**16.35 ± 3.16**	**20.4 ± 1.75**	**33.84 ± 4.39**	**22.23 ± 3.46**	**35.29 ± 3.82**
Chlorogenic acid deriv. 2	**65.11 ± 12.58**	**143.2 ± 11.59**	**110.43 ± 17.01**	**180.38 ± 24.18**	**129.42 ± 33.23**
Cryptochlorogenic acid	**33.92 ± 5.72**	**27.65 ± 3.23**	**97.19 ± 13.61**	**37.24 ± 5.54**	**33.14 ± 5.11**
Dichlorogenic acid	**47.03 ± 6.54**	**75.76 ± 12.09**	**84.78 ± 11.79**	**72.86 ± 13.35**	**113.21 ± 31.96**
Flavonoid 1	**7.59 ± 0.69**	**16.9 ± 4.05**	**17.06 ± 1.74**	**19.08 ± 2.25**	**20.38 ± 3.88**
Flavonoid 2	**20.64 ± 2.8**	**20.24 ± 3.12**	**33.68 ± 3.88**	**29.53 ± 4.46**	**38.71 ± 4.82**
Flavonoid 3	**35.12 ± 3.18**	**54.37 ± 4.94**	**47.45 ± 7.15**	**61.51 ± 8.41***	**45.38 ± 8.2**
Flavonoid 4	**17.19 ± 1.35**	**29.54 ± 2.94**	**27.42 ± 4.11**	**31.43 ± 4.08**	**26.02 ± 3.34**
Flavonoid 5	**15.67 ± 1.37**	**14.7 ± 2.46***	**20.07 ± 3.15**	**17.08 ± 2.91***	**12.58 ± 2.17**
Flavonoid 6	**8.04 ± 0.98**	**13.31 ± 1.1**	**10.87 ± 1.35**	**18.64 ± 2.85**	**12.62 ± 1.85**
Flavonoid 7	**7.94 ± 1.34**	**21.63 ± 2.42**	**12.2 ± 1.74**	**26.64 ± 3.47**	**23.94 ± 3.49**
Flavonoid 8	**21.33 ± 1.25**	**29.13 ± 3.09**	**24.37 ± 1.66**	**34.26 ± 3.23***	**19.38 ± 1.68**
Protocatechuic acid	**23.55 ± 3.77**	**11.53 ± 1.81**	**24.39 ± 5.13**	**37.8 ± 10.9**	**21.44 ± 3.71**
Terpenoids					
Bornyl acetate	**2.11 ± 0.10**	**2.43 ± 0.1**	**2.27 ± 0.13**	**2.46 ± 0.11**	**2.11 ± 0.14**
Carvone	**1.79 ± 0.10**	**1.95 ± 0.06***	**1.77 ± 0.08**	**2.05 ± 0.07**	**1.8 ± 0.06**
Linalool	1.86 ± 0.09	1.82 ± 0.09	1.69 ± 0.07	1.95 ± 0.09	1.82 ± 0.07
Pulegone	0.77 ± 0.05	0.77 ± 0.03	0.71 ± 0.05	0.78 ± 0.06	0.71 ± 0.05
α-copaene	**2.67 ± 0.15**	**4.34 ± 0.36**	**2.97 ± 0.25**	**3.48 ± 0.35**	**2.59 ± 0.14**
α-humulene	0.41 ± 0.02	0.42 ± 0.02	0.38 ± 0.02	0.42 ± 0.02	0.46 ± 0.02
α-phellandrene	2.81 ± 0.10	3.04 ± 0.12	2.78 ± 0.08	3.04 ± 0.12	2.77 ± 0.06
α-pinene	0.88 ± 0.08	0.8 ± 0.04	0.79 ± 0.07	0.89 ± 0.04	0.86 ± 0.07
α-terpinene	**1.98 ± 0.51**	**1.15 ± 0.09**	**1.16 ± 0.14**	**1.49 ± 0.23**	**0.92 ± 0.07**

Table 4 (continued)

	246	865	510	NAU	Atlantic
β-caryophyllene	**0.93 ± 0.05***	**2.65 ± 0.21**	**1.9 ± 0.19***	**2.81 ± 0.2**	**3.1 ± 0.25**
β-ionone	**0.69 ± 0.03**	**0.75 ± 0.05***	**0.65 ± 0.07**	**0.81 ± 0.06**	**0.69 ± 0.08**
β-pinene	1.67 ± 0.08	1.86 ± 0.09	1.46 ± 0.11	1.83 ± 0.06	1.54 ± 0.1
γ-terpinene	1.85 ± 0.04	1.91 ± 0.08	1.78 ± 0.12	2.01 ± 0.09	1.69 ± 0.06
Δ-3-carene	2.41 ± 0.10	2.56 ± 0.1	2.29 ± 0.09	2.59 ± 0.07	2.3 ± 0.05

Bolded lines indicate a significant difference among cultivars using Kruskal-Wallis test (based on *p*-values adjusted with Holms method)

* Indicates a significiant difference from Atlantic using Dunn's Test adjusted with Holm's method

production of phenolic compounds, and therefore increase ZC symptoms. Further, we do not specifically know the haplotype of Cls plants in Mexico are exposed to as both haplotypes exist, while we specifically used insects collected in Texas with the B haplotype. In contrast, in the tests carried out under controlled conditions in the present work, the plants were only exposed to the transmission of Cls by *B. cockerelli* while other factors that could have influenced host physiology were kept to a minimum.

In the choice bioassays, significant differences were found with respect to the cultivars 246 and 865 that were preferred for settling relative to Atlantic. The cultivar 510 was less preferred for oviposition in both choice and no-choice contexts, suggesting possible antixenosis in 510 (Cooper and Bamberg 2014; Butler et al. 2011a; Diaz-Montano et al. 2014; Novy et al. 2013). In no-choice bioassays, psyllids exhibited reduced oviposition on the genotype 246 and most on the genotype NAU. However, none of these differences were associated with differences in either the proportion of hatching or in the growth index. This suggests limited or no antibiosis in any of the examined genotypes. Finally, there were no differences in the behavioral bioassays with respect to any behavior or cultivar. This, again, indicates very limited to no antixenosis. In particular, it suggests any effect is too subtle to result in the psyllids leaving the plant or to refrain from feeding. Moreover, even though there were some significant differences in these bioassays that suggest that lines 246, 865 and 510 might have resistance or tolerance mechanisms against *B. cockerelli*, the mechanisms of the clones 246 and 865 were not enough to change the behavior and the development of the insects, or to prevent the transmission of Cls by the potato psyllid. Notably, none of these bioassays examined feeding, which is necessary to truly examine transmission of Cls, but they generally indicated that the insects spend enough time on a plant to both feed and transmit the Cls bacteria.

In examining the length and density of trichomes in the leaves, it was observed that the trichomes of the clones 246, 865 and 510 were larger than in Atlantic, and the trichome density was greater in 246, 865 and NAU than in the control. These findings may partially explain the preference of *B. cockerelli* for Atlantic instead of the lines 246 and 865, but do not elucidate the absence of preference between NAU and Atlantic. These results suggest that, most likely, there are other physical and chemical barriers that plants may have to avoid the penetration of the insect stylets (Will and van Bel 2006). Furthermore, Butler et al. (2011a) tested the resistance of various potato genotypes against *B. cockerelli*, including two with resistance based on the repellent effect of the trichomes, and observed that this effect was not enough to prevent the Cls infection of the plants. It has also been demonstrated that particle film materials, which alter tactile qualities, will influence *B. cockerelli* behavior (Prager et al. 2013; Yang et al. 2010). Everything considered, these results indicate that reduced ZC symptoms are less likely to have resulted from resistance to the insect than to the relationship between the plant and Cls.

Regarding plant chemical profile differences, both before (which would include constitutive defensive compounds) and after (which would include induced defensive compounds) infestation, very few differences were observed that could explain why 246, 865, or 510 were different than Atlantic. Prior to infestation, Atlantic foliar tissue had greater levels of glutamic acid, serine, and threonine that could make plants more nutritious for psyllids, but, with the exception of threonine, such differences in amino acids were not as apparent in infested plants. Pre- and post-infestation α-copaene levels and post-infestation α-terpinene levels were lower in Atlantic than the other cultivars, suggesting that these compounds might have led to any potential antixenosis observed. However, greater study is needed to confirm that these compounds may work as anti-attractants for psyllids, as previous studies with ambrosia beetles and olive fruit flies observed that α-copaene was an attractant (de Alfonso et al. 2014; Kendra et al. 2016).

The analyses of individual compounds in raw tubers indicated that asparagine was the most abundant amino acid in the five genotypes. However, this amino acid did not change with Cls infection. Asparagine has been considered the main precursor of acrylamide, which is a product of the Millard reaction between amino acids and reducing sugars, and it has been associated with the dark color of potato chips (Mottram et al. 2002; Parker et al. 2012). Even though asparagine did not

Table 5 Mean (SEM) concentration of various compounds in leaf tissue unexposed to Cls

	246	865	510	NAU	Atlantic
Amino acids					
Alanine	0.60 ± 0.03	0.48 ± 0.05	1.01 ± 0.16	0.56 ± 0.06	0.88 ± 0.07
Asparagine	2.02 ± 0.96	0.13 ± 0.03	1.94 ± 0.98	0.85 ± 0.51	1.98 ± 0.70
Aspartic acid	0.73 ± 0.10	0.47 ± 0.03	0.74 ± 0.07	0.84 ± 0.18	1.06 ± 0.21
Glutamic acid	**0.56 ± 0.05***	**0.90 ± 0.06**	**0.79 ± 0.10***	**0.93 ± 0.08**	**1.09 ± 0.12**
Glutamine	0.66 ± 0.29	0.09 ± 0.01	0.70 ± 0.30	0.76 ± 0.42	1.25 ± 0.43
Glycine	0.09 ± 0.01	0.06 ± 0.00	0.13 ± 0.02	0.07 ± 0.01	0.13 ± 0.01
Histidine	0.14 ± 0.05	0.03 ± 0.00	0.25 ± 0.10	0.15 ± 0.07	0.30 ± 0.09
Isoleucine	0.27 ± 0.08	0.09 ± 0.01	0.46 ± 0.16	0.18 ± 0.05	0.45 ± 0.10
Leucine	0.38 ± 0.08	0.17 ± 0.01	0.65 ± 0.18	0.28 ± 0.04	0.71 ± 0.12
Lysine	0.26 ± 0.03	0.12 ± 0.01	0.40 ± 0.07	0.22 ± 0.03	0.42 ± 0.05
Methionine	0.03 ± 0.00	0.02 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.06 ± 0.01
Phenylalanine	0.24 ± 0.06	0.10 ± 0.00	0.40 ± 0.14	0.21 ± 0.05	0.43 ± 0.09
Proline	0.27 ± 0.04	0.16 ± 0.01	0.33 ± 0.02	0.38 ± 0.08	0.42 ± 0.04
Serine	**1.27 ± 0.10**	**0.59 ± 0.07**	**1.60 ± 0.21***	**1.22 ± 0.14**	**1.64 ± 0.15**
Threonine	**0.06 ± 0.01***	**0.07 ± 0.01***	**0.05 ± 0.01***	**0.13 ± 0.02**	**0.28 ± 0.03**
Tryptophan	0.18 ± 0.06	0.05 ± 0.01	0.29 ± 0.10	0.24 ± 0.10	0.33 ± 0.08
Tyrosine	0.03 ± 0.01	0.00 ± 0.00	0.12 ± 0.05	0.02 ± 0.00	0.30 ± 0.06
Valine	0.40 ± 0.10	0.17 ± 0.01	0.69 ± 0.24	0.34 ± 0.08	0.58 ± 0.11
Sugars					
Fructose	**0.10 ± 0.04**	**0.05 ± 0.02**	**0.11 ± 0.03**	**0.05 ± 0.02**	**0.06 ± 0.02**
Glucose	**0.23 ± 0.03**	**0.20 ± 0.04**	**0.24 ± 0.03**	**0.23 ± 0.04**	**0.20 ± 0.02**
Sucrose	0.13 ± 0.03	0.07 ± 0.02	0.17 ± 0.03	0.15 ± 0.03	0.18 ± 0.02
Phenolics					
Catechin	**21.65 ± 2.30***	**48.6 ± 4.73**	**27.10 ± 1.74***	**84.61 ± 6.38**	**39.94 ± 2.62**
Chlorogenic acid	**17.23 ± 2.35**	**28.45 ± 6.78**	**12.86 ± 1.98**	**94.31 ± 37.40**	**39.8 ± 9.78**
Chlorogenic acid deriv. 1	**19.85 ± 3.22***	**29.61 ± 3.98**	**40.21 ± 3.61**	**77.69 ± 28.36**	**28.50 ± 5.65**
Chlorogenic acid deriv. 2	**135.64 ± 21.85**	**210.89 ± 11.95**	**180.45 ± 16.98**	**281.92 ± 30.96**	**347.30 ± 24.02**
Cryptochlorogenic acid	**42.9 ± 4.96**	**16.64 ± 3.08**	**96.61 ± 12.85**	**30.80 ± 5.80**	**33.13 ± 5.0**
Dichlorogenic acid	44.61 ± 6.43	80.17 ± 10.156	80.80 ± 12.90	103.54 ± 9.60	60.10 ± 5.43
Flavonoid 1	**9.14 ± 0.66***	**17.57 ± 2.95**	**14.7859 ± 0.90**	**43.19 ± 9.69**	**20.60 ± 6.45**
Flavonoid 2	**21.34 ± 2.18***	**23.11 ± 2.22***	**31.77 ± 1.97**	**40.39 ± 3.15**	**27.51 ± 2.91**
Flavonoid 3	**45.23 ± 6.66**	**70.23 ± 6.25**	**54.96 ± 4.77**	**79.12 ± 9.06**	**44.26 ± 3.54**
Flavonoid 4	20.86 ± 2.80	33.39 ± 4.17	27.08 ± 2.12	39.55 ± 3.86	25.71 ± 3.16
Flavonoid 5	**17.80 ± 2.51**	**19.40 ± 2.27**	**20.52 ± 1.41**	**25.46 ± 3.01**	**8.82 ± 1.17**
Flavonoid 6	**10.25 ± 1.39**	**21.28 ± 1.73**	**14.92 ± 1.68**	**30.41 ± 2.65**	**17.86 ± 2.90**
Flavonoid 7	**9.74 ± 1.51***	**27.54 ± 4.20**	**11.47 ± 1.61**	**34.09 ± 6.42**	**15.27 ± 1.31**
Flavonoid 8	**28.66 ± 2.03**	**36.90 ± 4.64**	**32.97 ± 1.83**	**47.09 ± 6.52**	**22.64 ± 2.27**
Protocatechuic acid	27.35 ± 8.75	10.47 ± 3.34	43.54 ± 11.75	39.41 ± 15.62	51.77 ± 12.07
Terpenoids					
Bornyl acetate	3.0 ± 0.10	3.14 ± 0.16	2.84 ± 0.12	2.59 ± 0.12	2.76 ± 0.12
Carvone	2.40 ± 0.16	2.85 ± 0.135	2.46 ± 0.14	2.30 ± 0.15	2.30 ± 0.09
Linalool	2.16 ± 0.13	2.36 ± 0.13	2.24 ± 0.11	2.02 ± 0.10	2.16 ± 0.06
Pulegone	0.94 ± 0.07	1.04 ± 0.10	0.89 ± 0.04	0.87 ± 0.07	0.87 ± 0.03
α-copaene	**4.29 ± 0.40**	**5.94 ± 0.76***	**4.23 ± 0.47**	**4.73 ± 0.22**	**3.75 ± 0.41**
α-humulene	0.51 ± 0.03	0.57 ± 0.04	0.46 ± 0.04	0.49 ± 0.05	0.46 ± 0.02
α-phellandrene	3.44 ± 0.23	3.84 ± 0.15	3.46 ± 0.13	3.20 ± 0.15	3.34 ± 0.11
α-pinene	0.87 ± 0.057	1.02 ± 0.07	0.98 ± 0.04	0.89 ± 0.06	0.87 ± 0.06
α-terpinene	1.67 ± 0.18	1.81 ± 0.29	2.12 ± 0.27	1.44 ± 0.32	1.32 ± 0.16

Table 5 (continued)

	246	865	510	NAU	Atlantic
β-caryophyllene	**1.45 ± 0.15***	**2.95 ± 0.40**	**1.98 ± 0.13**	**3.68 ± 0.34**	**4.33 ± 0.36**
β-ionone	0.97 ± 0.03	1.15 ± 0.06	0.93 ± 0.07	1.0 ± 0.11	0.85 ± 0.06
β-pinene	1.86 ± 0.10	1.97 ± 0.09	1.69 ± 0.08	1.70 ± 0.11	1.84 ± 0.12
γ-terpinene	2.10 ± 0.10	2.35 ± 0.08	2.26 ± 0.06	2.15 ± 0.08	2.09 ± 0.05
Δ-3-carene	2.92 ± 0.18	3.31 ± 0.12	3.00 ± 0.10	2.83 ± 0.13	2.95 ± 0.10

Bolded lines indicate a significant difference among cultivars using Kruskal-Wallis test (based on *p*-values adjusted with Holms's method)

* Indicates a significiant difference from Atlantic using Dunn's Test adjusted with Holms's method

increase in ZC-infected tubers of any genotype, all five genotypes showed ZC symptoms in their fried tubers. This suggests that there are other amino acids that are important in acrylamide production. This is in agreement with a previous work that reported increasing ZC symptoms in spite of decreasing concentrations of asparagine with time after Cls-infection (Wallis et al. 2014). The role of asparagine in ZC symptoms as well as glutamic acid, which decreased with Cls infection in four of five genotypes, is unclear and requires further investigation.

The analyses of individual sugars indicated that the contents of sucrose, glucose, and fructose increased in Cls-infected tubers of four potato genotypes (865, 510, NAU, and Atlantic). The clone 246 was the exception since no significant changes were observed in any sugar. Reduced sugar is a characteristic that could be desirable in potato genotypes used for chip production because the high sugar content has been associated with dark color in chips (Navarre et al. 2009; Wallis et al. 2014). The average content of sucrose, glucose, and fructose in the five potato genotypes comprised 53.9. 36.9 and 9.2%, respectively, of the total analyzed sugars. Sucrose, a non-reducing sugar, is the most abundant sugar in potatoes and could also participate in the Millard reaction in a sugar-limited system (Leszkowiat et al. 1990). Glucose and fructose are reducing sugars that participate in the Millard reaction and can predict the acrylamide content in fries, which is the main product associated with chips browning (Mottram et al. 2002; Parker et al. 2012). Then, it seems that the three analyzed sugars intervene in the browning of chips.

The analyses of individual phenolic compounds indicated that the most abundant were protocatechuic acid and the different isomers of chlorogenic acid, most of them increased in the Cls-infected tubers of the five potato genotypes. The specific role of each phenolic as a response of Cls infection, is unknown. However, it is well known that the enzymatic oxidation of phenolic compounds can be the result of defensive reactions against Cls and that the enzymatic browning of freshly-cut potato tubers may occur because polyphenol oxidases convert phenolics into brown-colored compounds (Navarre et al. 2009; Wallis et al. 2012; Wallis et al. 2014). Analyzing the total phenolic content, it was evident that the concentration was higher in diseased than in healthy tubers and comparing the different potato genotypes it was shown that the four advanced potato lines had lower concentrations of phenolics than Atlantic. On the other hand, the raw tubers of the four clones showed lower tuber discoloration than Atlantic. These results confirm that the brown color of fresh-cut tubers is mainly produced by the enzymatic oxidation of phenolic compounds. Therefore, the low discoloration observed in raw infected tubers from the four experimental lines, which was associated with low phenolic content, indicate that the defense mechanism of these potato lines against Cls does not depend entirely on the production of phenolic compounds and that there may be other mechanisms of tolerance. Munyaneza et al. (2013) suggest that the absence or low intensity of dark staining observed in tubers from nine experimental lines, which were infected by Cls, can be considered as a type of resistance against ZC. Further studies characterized this kind of tolerance as being related to lower increases in some amino acids, sugars, and phenolics upon Cls infection (Wallis et al. 2015a).

When the biochemistry of diseased tubers was examined, results indicated that 865 was the only genotype in which the concentration of total amino acids increased compared with healthy tubers. The five genotypes all increased their sugar content in diseased tubers but there were no significant differences among them. Since the fried tubers of the five genotypes presented dark coloration after they were fried, this suggests that chip color depends more on sugar than on amino acids concentrations. This is in agreement with the work of Leszkowiat et al. (1990) that indicated relatively higher concentration of amino acids compared with sugars in potato tubers. It is thought that sugar content is the limiting factor in non-enzymatic browning of potato chips. Even though amino acids did not increase in four of the five genotypes, their concentrations were enough to react with the sugars that increased in the diseased tubers and consequently produced chips with a burnt appearance. However, there may be other factors that intervene in the chips' color because, in spite of relatively low content of sugars and amino acids, the Cls-infected tubers of the clone 246 showed the typical ZC symptoms. This suggests that the increased phenolics observed in diseased tubers of 246 could contribute to chips' darkening. The mechanism is not clear, but could be associated with the

production of polymeric polyphenolic pigmented species, such as melanin, that can contribute to the ZC symptoms when the tubers are fried (Miles et al. 2010).

In conclusion, the five potato genotypes (246, 865, 510, NAU and Atlantic) appeared equally susceptible to the transmission of Cls by *B. cockerelli*. It is a predictable result given that, in all assays, psyllids were willing to oviposit and were able to develop on all the genotypes. Since Cls is rapidly transmitted in as little as two hours (Mustafa et al. 2015), even limited feeding can be expected to result in Cls infection. Choice bioassays did reveal that the psyllids prefer some genotypes to Atlantic, possibly due to the density or length of trichomes, yet this response was clearly not strong enough to prevent the psyllids from settling and feeding. The four experimental lines showed tolerance to ZC symptoms in the tubers, which was associated with the lower content of phenolic compounds compared with Atlantic. This form of tolerance against ZC can be valuable in Mexico and in some American markets, where most of the potato production is used for table consumption. Although, they will need to be further evaluated in boiling and baking conditions. The four promising potato lines can be used as parents in the breeding programs or three of them (865, NAU and 510) can be released as varieties, since they have good agronomic and commercial characteristics to be accepted by growers, merchants, and consumers. However, the CLs-infected tubers of the five potato genotypes showed the typical ZC symptoms in their fried tubers, and therefore they are unsuitable for potato chip production. The burnt appearance of the chips was associated with the concentrations of sugars and phenolic compounds in the ZC symptomatic tubers.

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