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## Insecticide Resistance and Resistance Management

# Examining the Potential Role of Foliar Chemistry in Imparting Potato Germplasm Tolerance to Potato Psyllid, Green Peach Aphid, and Zebra Chip Disease

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## Abstract

Long-term, sustainable management of zebra chip disease of potato, caused by ‘*Candidatus Liberibacter solanacearum*’ (Lso) and vectored by potato psyllids (*Bactericera cockerelli* Sulc [Hemiptera: Trioziidae]), requires development of cultivars resistant or tolerant to infection or capable of reducing spread or both. We examined the influence that five experimental breeding clones of potato had on potato psyllids and their ability to vector Lso. The ability of these potato clones to resist aphids (green peach aphids, *Myzus persicae* Sulzer [Hemiptera: Aphididae]) also was examined. Due to the importance of host chemistry on plant–insect interactions, levels of primary metabolites of amino acids and sugars, as well as secondary metabolites including polyphenolics, terpenoids, and alkaloids were compared between breeding clones and a commercial cultivar. Findings for compound levels then were associated with observed changes in host susceptibility to psyllids or aphids. Psyllids oviposited less on three breeding clones than Atlantic, but no significant effects of breeding clones on psyllid feeding or choice were observed. Aphid reproduction was reduced on two clones relative to Atlantic. A05379-211 had greater sugar levels and postpsyllid amino acid levels than Atlantic. Total alkaloid and phenolic levels were greater in all breeding clones than Atlantic. Total terpenoid levels were greater in PALB03016-3 and PALB03016-6 than Atlantic, which might explain, in part, the observed resistance to psyllid oviposition and aphid reproduction. Overall, these results suggest that increased levels of certain metabolites in breeding clones could affect psyllid and aphid reproduction.

**Key words:** plant defense response, amino acid, carbohydrate, phenolic, terpenoid

*Bactericera cockerelli* Sulc (Hemiptera: Trioziidae), the potato psyllid, is a piercing sucking insect known to be a pest of various solanaceous crop species (Butler and Trumble 2012). Known host plants include tomato and potato both of which are subject to a disease known as ‘psyllid yellows.’ Potato psyllids also pose a serious threat to commercial potato (*Solanum tuberosum* L.) production not only due to direct damage but also their ability to vector ‘*Candidatus Liberibacter solanacearum*’ (Lso) (syn. ‘*Candidatus Liberibacter psyllaurosum*’), which is the causal agent of zebra chip (ZC) disease present in Central America, Western North America, and New Zealand (Secor and Rivera-Varas 2004; Munyaneza et al. 2007; Liefting et al. 2008; Wen et al. 2009; Crosslin et al. 2012a,b; Munyaneza 2012). Symptoms of ZC disease include chlorotic leaves, stunted growth, aerial tubers, reduced tuber quality, and occasionally plant death (Butler and Trumble 2012). ZC infection results in

a characteristic striped ‘zebra’ pattern when tubers are fried (Butler and Trumble 2012). In Texas alone, ZC has resulted in millions of dollars in crop losses, and ZC-related yield losses can exceed 80% (Munyaneza et al. 2007, 2009).

Current potato psyllid management involves costly (~\$700/hectare) chemical control programs (Guenther et al. 2012, Greenway 2014). Moreover, many insecticide treatments will reduce psyllid numbers but fields will still show substantial levels of ZC infection (Prager et al. 2013). Compounding this, in some locations, *B. cockerelli* is developing resistance to commonly used insecticides (Prager et al. 2013). Other growers, such as those who farm organically, need psyllid control options other than the commonly used commercial pesticides.

As a result of these factors, sustained management of ZC disease will require a robust IPM plan that extends beyond insecticides. One

element of these plans is the development of resistant or tolerant potato varieties and genotypes that display either reduced susceptibility to Lso or that induce either antixenosis or antibiosis or both in the insect vector, *B. cockerelli*. The development of such varieties is an ongoing effort. Butler et al. (2011) examined 22 potato genotypes and documented differential *B. cockerelli* behavioral responses that resulted in reduced Lso transmission. Diaz-Montano et al. (2014) subsequently examined four of the most promising genotypes for antixenosis or antibiosis. More recently, Wallis et al. (2015a) examined 29 breeding clones for changes in host chemistry and/or Lso infection and identified tolerant clones.

Plants possess multiple defenses to combat and resist insects. These include mechanical defenses such as thickened tissues like bark or structures like trichomes, as well as chemical defenses such as certain proteins, phenolics, toxins, and volatile compounds. Chemical defenses can directly harm insects, affect feeding behaviors, or both. Such defensive compounds vary between species and varieties in quantity and type. In addition, many compounds can increase in response to insect feeding as part of a coordinated defense. When a disease is transmitted, other changes in host physiology and quantities of defense-related compounds may occur as a coordinated defense response, such as ones observed in potato to Lso infection (Alvarado et al. 2012; Wallis et al. 2012, 2015b) and against other pathogens (Henriquez et al. 2012, Ngadze et al. 2012). Therefore, in response to a pathogen or vector, a combination of host responses may be triggered, with both positive and negative crosstalk of certain defensive pathways resulting in a final physiological state that could interrupt ongoing insect feeding.

This study examines the ability of five breeding clones and one commercial cultivar ('Atlantic') of potato plants to impact psyllid feeding that is responsible for Lso transmission and subsequent ZC in potato. In addition, it reports on choice assays of psyllids and no-choice development bioassays using both psyllids and a second sucking insect, the green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). It further examines the differences in baseline and induced levels of many compounds that could affect psyllid feeding. In particular, levels are examined of both host compounds that are used by insects for nutrition, i.e., amino acids and sugars, as well as levels of defense-associated compounds, i.e., alkaloids, phenolics, and volatiles. By examining biotic effects on multiple insects, insight can be gained into the effects of plant response to infection, insect feeding, and biochemistry as they influence host-plant choices. Resistant breeding clones, or those with similar chemical profiles, could then be selected and through backcrossing insect and pathogen resistance could be pyramided resulting in sustainable, commercial-quality potato plants that do not acquire or better tolerate ZC, as well as possibly other insect-vector diseases.

## Materials and Methods

### Plants and Insects

All psyllid bioassays were conducted using *B. cockerelli* maintained in colonies at the University of California (UC), Riverside for over 7 yr. Original material was collected in potato fields in Weslaco (Hidalgo County), Texas. Insects were tested using the methods of Swisher et al. (2012), and confirmed as the 'central haplotype.' Periodically, insects were removed from colonies and tested to confirm infection with Lso using qPCR (after Butler et al. 2011). Lso was previously confirmed as Lso-B. Insects were maintained on tomato (*Solanum lycopersicum*, variety 'Yellow Pear') in L60 × W60 × H60 cm mesh tents (BugDorm, Bioquip, Rancho Dominguez, CA), and plants were changed as necessary.

Bioassays on aphids were conducted using green peach aphids (*M. persicae*) maintained in colony on a combination of pepper (*Capsicum annuum* variety 'Cal Wonder') and tomato (*S. lycopersicum* variety 'Yellow Pear'). Starting materials for the colonies were provided from long-term colonies maintained at UC Davis. All colonies were periodically tested for the presence of PVY using qPCR (at UC Davis) or ImmunoStrips (Agdia Inc., Elkhart, IN; at UC Riverside).

Potato entries in this study included breeding clones P2-4, Etb 6-21-3, and A05379-211 which represent Backcross 1 (BC<sub>1</sub>), BC<sub>2</sub>, and BC<sub>4</sub> progeny of somatic hybrids having the potato species *Solanum tuberosum* and *Solanum berthaultii* represented in their genetic background (Novy and Helgeson 1994). Two additional breeding clones, PALB03016-3 and -6, represent full siblings from a cross made by Dr Chuck Brown, USDA-ARS, Prosser, WA, with the potato species *Solanum guerreroense* represented in their pedigrees. All plants of these five breeding clones and the ZC-susceptible cultivar 'Atlantic' were grown in 15 cm diameter pots with UC soil mix no. 3 (Matkin and Chandler 1957). Potato plants were watered and fertilized with the label rate of Miracle Gro nutrient solution (Scotts Company, Marysville, OH) ad libitum. While every effort was made to maintain equivalent sample sizes for all experiments, some entries failed to emerge following planting, failed to grow to sufficient size, or the number of seed pieces was limiting. This resulted in different sample sizes among experiments and entries. Similarly, as some of these plants are quite small relative to other entries at the same age, every effort was made to standardize by both size and age. However, it was not possible to choose one leaf number or size. All plants were used at least 3 wk after emergence and prior to flowering. In particular, experiments with aphids were limited and the entry PALB0316-3 was not available for these experiments.

### Choice Bioassays of Psyllids

To determine the acceptability of the breeding clones as hosts by psyllids, relative to ZC-susceptible Atlantic, choice bioassays were performed. Each bioassay paired an experimental breeding clone with 'Atlantic.' These experiments were conducted in experimental arenas consisting of two foam rings 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. One terminal leaflet from a fully expanded leaf of an intact plant of 'Atlantic' and one from an experimental clone were sandwiched abaxial side up between the foam pieces. The foam pieces were secured with spring loaded metal hair clips. Ten post-teneral *B. cockerelli*, five males and five females, were introduced into the areas using an aspirator. The number of psyllids on each plant and on the sides of the cage was counted every 24 h for 96 h. Following the 96 h, insects were removed and the eggs on each leaf were counted. Choice bioassays were replicated 10 times per entry.

### No-choice Bioassays of Psyllids

No-choice bioassays were conducted by restricting six *B. cockerelli* (three males and three females) onto a single leaflet using 4 × 6 cm mesh sachet party favor bags (Joanna Fabric and Craft Stores) for 48 h. Prior to placement in cages, psyllids were aspirated from mesh tents and placed into size 1 gelatin pill capsules sex (CapsluCN International Co., China) to facilitate sorting by sex. Only postteneral individuals were used in bioassays. Two mesh cages were placed onto each plant and five plants were examined per entry (total = 10 replications). These experiments were conducted twice. For those experiments intended to examine infection rate of Lso, following the 72-h period, psyllids were removed and the numbers of eggs on each leaflet were counted and then removed. Each leaflet that had been

exposed to insects was then marked and plants were maintained for 21 d after which marked leaflets, tubers, and other plant tissue were collected for qPCR. In the second version of the experiment, intended to measure fertility of eggs and development, eggs were counted but retained on plants. The number of nymphs of each stage was then recorded daily until they died or were removed as adults.

### No-choice Bioassays of Aphid Development

To determine whether the host-plant experimental cultivars had antibiotic effects on aphids, no-choice bioassays were also conducted. Twenty green peach aphids were transferred from colonies maintained on pepper and tomato onto a terminal leaf of one of the potato breeding clones or the susceptible control 'Atlantic.' Aphids were allowed to remain on the plants for 21 d and the number of aphids was counted daily. No aphid alates were observed and plants were physically separated, within cages and, therefore, individual leaves did not need to be caged to keep aphids on leaves. In addition to growth rates of aphids, all the potato cultivars were monitored daily for visual evidence of disease such as chlorosis, necrotic spots, or a degradation of plant health. Following aphid removal, plants were tested using ImmunoStrips for the presence of PVY which would indicate transmission of PVY by aphids. While aphids were not tested directly for PVY, all host plants used in rearing colonies were tested for PVY and insects were used to inoculate uninfected plants as a test for infection status.

### Biochemical Analyses

A maximum of eight plants of each of the plant entries in this study were grown for either exposure to psyllid feeding, or to be left uninfested as controls. Exposed plants had psyllids feeding on leaves within an insect cage for a period of 48 h. After the 48-h period, psyllids were physically removed and leaves were harvested. Control plants were left unexposed within cages as well, with leaf samples harvested at the same time as those of the psyllid-exposed plants. All samples were immediately placed in liquid nitrogen for flash-freezing and kept frozen at -80°C until chemical analyses were conducted.

Extractions for chemical analyses were prepared according to the methods of Wallis et al. (2008, 2014). In brief, leaves were pulverized with a mortar and pestle in liquid nitrogen, and 0.10 g of tissue was weighed into three aliquots in separate microcentrifuge tubes. Each of the aliquots was twice-extracted overnight at 4°C with 0.5 ml of one of these solvents: phosphate buffered saline (PBS) buffer (pH 7.8), methanol, or methyl tert-butyl ether with 100 ppm *n*-pentadecane as an internal standard. This resulted in 1 ml of extract for each of these solvents.

Total alkaloids were estimated using a modified high-performance liquid chromatography (HPLC) procedure of Sotelo and Serrano (2000). An aliquot of 50 µl of PBS buffer was injected by a Shimadzu (Columbia, MD) SIL-20A auto-injector into an HPLC system using two Shimadzu LC-10AD pumps. The solvent program was isocratic with one pump set at 0.3 ml/min with distilled water, and the other set at 0.7 ml/min of acetonitrile (Sigma, St. Louis, MO) for 6 min. After 6 min, the conditions were set to 100% acetonitrile to flush the column until 9.5 min since the sample run start, at which time conditions were returned to 30% acetonitrile until 12.5 min after the sample run was started to prepare for the next sample. Separation was performed using a monolithic RP18 Onyx column (Phenomenex, Torrance, CA) kept at room temperature. Detection was made using a Shimadzu SPD-20A UV/Vis detector set at 200 nm. Peak identification was made using equivalent runs with commercially available chaconine and solanine from Sigma.

In these conditions, chaconine and solanine peaks had substantial overlap within the samples, and thus were integrated together as one summed feature (i.e., a total alkaloid peak). Conversion from peak area to mg/g FW amounts was made using a standard curve of solanine (Sigma-Aldrich).

For amino acid analyses, an aliquot of 100 µl of PBS buffer extract was used in the commercially available EZ: FFAST GC amino acid quantification kit by Phenomenex, whereby derivatization and analysis was performed via kit instructions. A Shimadzu GC-2010 gas chromatograph with a flame ionization detector was used to quantify amino acids using the kit-included Zebron AAA column and following kit oven gradient methods.

Sugars (fructose and glucose) were also measured using the PBS buffer in HPLC analysis, according to the methods of Wallis et al. (2014). Using the same HPLC system as the alkaloids, an isocratic run on 50 µl of injected extract was performed using one pump moving 0.1M phosphoric acid in water at 0.1 ml/min through a Supelco C-610H ion-exchange column (Sigma) kept at 30°C. Quantification was performed using a Shimadzu RID-10 refractive index detector. The total run time per sample was 60 min. Commercial fructose and glucose standards (both from Sigma) were used to identify peaks and make standard curves to convert peak areas to mg/g FW amounts.

Phenolics were measured by injecting 50 µl of methanol extract with a Shimadzu SIL-20AHT auto-injector into a Shimadzu LC-20AD-based HPLC system. A binary gradient was used with solvent A being 0.2% acetic acid in water, and solvent B being 0.2% acetic acid in methanol. A RP-C18 Ascentis column (Sigma) was used for separation, with a binary gradient proceeding from 5% solvent B to 100% solvent B over 35 min, with five additional minutes to return to 5% solvent B for the following sample run. Quantification was performed using a Shimadzu photodiode array detector, with peaks measured at 280 nm. Peak identities were confirmed with commercial standards (from Sigma-Aldrich) if possible, otherwise putative identifications were made based on UV/Vis spectra maxima and corresponding molecular weights determined by LC-MS run on the same conditions and HPLC system, with a flow splitter delivering the compounds to a Shimadzu LC-MS2020 detector (Wallis et al. 2014). Compounds were converted to gram amounts using standard curves of other commercially available compounds (all from Sigma-Aldrich), i.e., chlorogenic acid for all chlorogenic acids, procyanidin B2 for all procyanidin compounds, and quercetin glucoside for all flavonoid glycosides.

Terpenoid compounds were analyzed by injecting 2 µl of methyl tert-butyl extract in a Shimadzu 2010 gas chromatograph equipped with a Shimadzu GC-MS2010S mass spectrometer for peak identity matching and quantification, according to the condition based on Wallis et al. (2015). A 30 m Shimadzu SHRXI-5MS column was used (Sigma-Aldrich) for separations. In brief, analyses proceeded with an oven gradient from 60 to 230°C over 45 min. Compound identification and quantification was performed using a standard mix of 30 different terpenoid compounds obtained commercially from Sigma, as described in Wallis et al. (2014). For unidentified compounds,  $\beta$ -pinene was used for standard curve conversions for putative monoterpenoids, and  $\beta$ -caryophyllene used for standard curve conversions for putative sesquiterpenoids.

### Statistical Analyses

All analyses were performed using R 3.3.3 (R Core Team, 2017). Choice bioassays of location were examined individually for each Atlantic versus breeding clone pairing. Permutated Multivariate Analyses of Variance (MANOVA) was implemented using the *adonis* functions in the *vegan* package (Oksanen et al. 2017) with the fixed

factor of entry and number of psyllids on each entry daily as the response. Number of eggs oviposited on each entry was also examined individually using Mann–Whitney–Wilcoxon tests. Oviposition in no-choice bioassays was examined using GLM with a negative binomial probability distribution and model with cultivar as a fixed factor and number of eggs as the response (Venables and Ripley 2002). Fertility, measured as hatch percentage (calculated as number of first instar larva divided by the number of eggs deposited) was examined using a Kruskal–Wallis rank-sum test, and posthoc analyses were performed using Dunn's test of multiple comparisons using ranked-sums (package FSA; Ogle 2017). Relative growth index was calculated using the methods of Zhang et al. (1993). These data were square-root transformed to meet assumptions of ANOVA and were tested with squared relative growth index as a response and entry as a fixed effect.

For analyses of potato foliar chemistry, IBM-SPSS software version 22.0 (IBM, Armonk, NY) was used with  $\alpha = 0.05$ . Total amino acids, phenolics, and terpenoids were derived by summing together compound levels. ANOVAs were performed on total alkaloids, total amino acids, fructose, glucose, total phenolics, and total terpenoids with infection status and potato breeding clone/cultivar as factors. When a significant interaction was present, ANOVAs were performed with potato breeding clone as the main factor for controls and infected plants separately. When the effects of potato breeding clones were significant, follow-up least significant difference (LSD) tests were used to determine pairwise comparisons.

In addition, MANOVA of amino acids, phenolics, or terpenoids were conducted with breeding clone and infection and the interaction of breeding clone and infection as factors. Additional MANOVAs were performed if the interactions were significant on controls and infected plants separately. If the MANOVA Pillai's trace statistic was significant for either breeding lines or infection status, differences in individual compounds were examined using ANOVAs and, if those were significant for breeding clones, then LSD tests were performed.

## Results

### Choice Bioassays With Psyllids

During choice bioassays in which psyllids were provided a choice of either an experimental breeding clone or the susceptible commercial cultivar 'Atlantic,' psyllids numbers did not differ between plants (Table 1). There were no instances in which the psyllids demonstrated a preference for Atlantic or a breeding clone. In pairwise comparisons with Atlantic, none of the experimental entries examined differed from Atlantic in the number of eggs laid (Table 1). Following the choice bioassays, leaves were collected from a minimum of four plants for each cultivar and tested for Lso via qPCR. At a  $ct$ -value of 35, all entries tested with the proportion of infected plants ranging from 25 (A05379-211) to 75% (P2-4) (Fig. 1).

### No-choice Bioassays With Psyllids

Oviposition differed significantly among entries ( $\chi^2 = 15.411$ ;  $df = 5,60$ ;  $P < 0.001$ ). While pairwise comparisons showed no significant differences among entries, when comparisons were made relative to the control 'Atlantic,' the breeding clones PALB03016-3, PALB03016-6, and A05379-211 all had significantly fewer eggs than Atlantic (Fig. 2). When the percentage of eggs that hatched was examined, there again was an overall significant difference ( $\chi^2 = 286.63$ ;  $df = 5$ ;  $P < 0.001$ ). Pairwise comparisons of entries demonstrated significant differences with P2-4 and PALB03016-6 exhibiting greater hatching than Atlantic, with the remaining entries being statistically similar to Atlantic (Fig. 3). There were no significant differences among entries with respect to relative growth index ( $F = 1.71$ ;  $df = 5,48$ ;  $P = 0.34$ ). Following no-choice bioassays, a minimum of five plants from each entry were tested for Lso using qPCR. Based on a  $ct$ -value of 35 as positive, three entries (P2-4, A05379-211, PALB03016-6) had no leaves testing positive for infection. The remaining entries (Etb-6-21-3, PALB03016-3, and the Atlantic) tested positive in 25, 50, and 50% of samples, respectively.

### No-choice Bioassays With Aphids

Aphid population growth was examined as the change in the number of aphids after 20 d postinfestation. The total change in the number of aphids varied significantly among entries (Kruskal–Wallis:  $\chi^2 = 11.906$ ;  $df = 4$ ;  $P < 0.05$ ; Fig. 4). Similar to the no-choice bioassays with psyllids, entries group together with some being more suitable for aphid growth than others. Specifically, Atlantic and

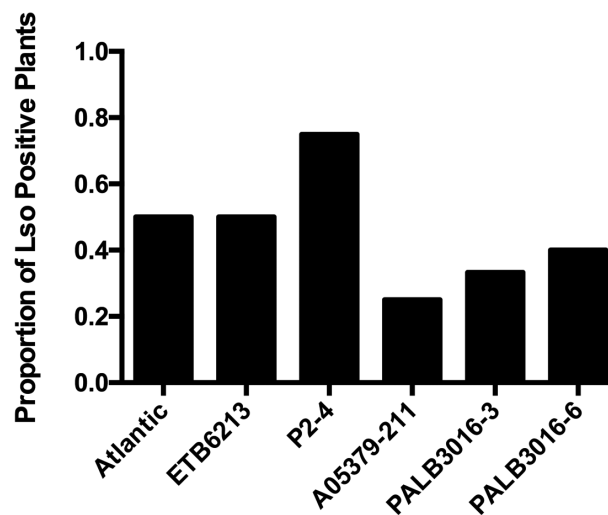


Fig. 1. The proportion of plants from two-choice bioassays that tested positive for Lso. Positive is defined as a  $ct$ -value of  $>35$ .

Table 1. Results from permutated MANOVA of location and Mann–Whitney–Wilcoxon pairwise comparisons of numbers of oviposition (number of eggs) on experimental entries versus Atlantic

Entry	Test statistic	P-value	Test statistic	P-value
	Settling		Eggs	
PALB03016-6	$F_{1,16} = 0.08$	0.90	7	0.89
PALB03016-3	$F_{1,12} = 0.41$	0.90	18.5	0.48
P2-4	$F_{1,14} = 2.57$	0.60	12	0.39
Etb 6-21-3	$F_{1,14} = 1.6$	0.20	36	0.67
A05379-211	$F_{1,14} = 0.63$	0.70	7	0.89



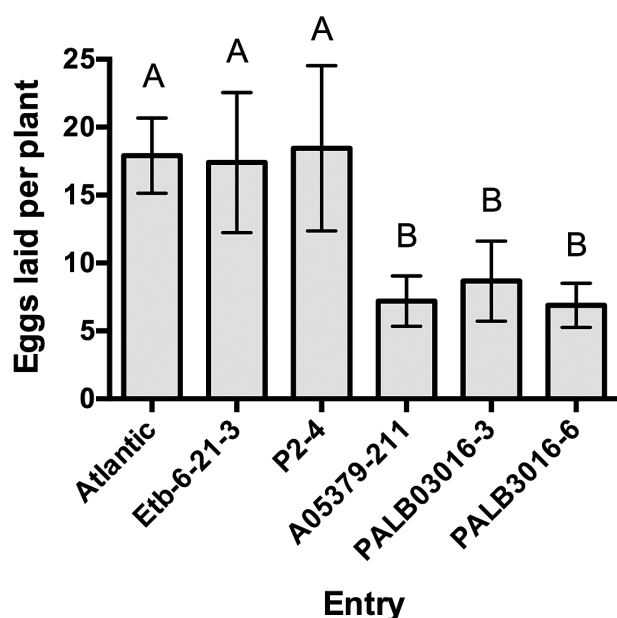


Fig. 2. The mean number of eggs and standard error of mean (SEM) oviposited on each entry in no-choice bioassays. Letters indicate significant difference at  $P = 0.05$ .

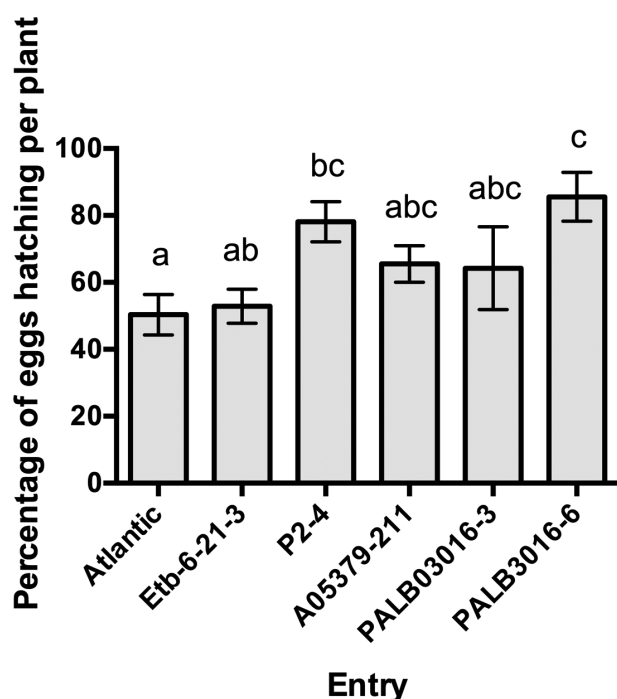


Fig. 3. The mean percentage of potato psyllid eggs and standard error of mean (SEM) that hatched after being oviposited on each entry in no-choice bioassays. Letters indicate significant difference at  $P = 0.05$ .

A05379-211 grouped together as most suitable for aphid growth. P2-4 and PALB3016-6 formed an intermediate group, and Etb-6-21-3 proved particularly unsuitable for aphid population growth (Fig. 3). In fact, after 20 d, Etb-6-21-3 had no surviving aphids on any plant while Atlantic and A05379-211 both had replications with over 100 aphids.

In visual inspection for the symptoms of PVY, Atlantic, which was used as a susceptible control, showed symptoms of PVY (Supp

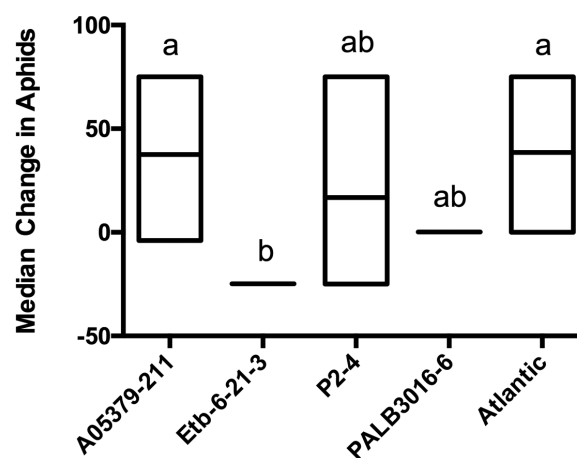


Fig. 4. The median (maximum and minimum) change in aphids over a 20-d period on different potato entries. Letters indicate significant difference at  $P = 0.05$ .

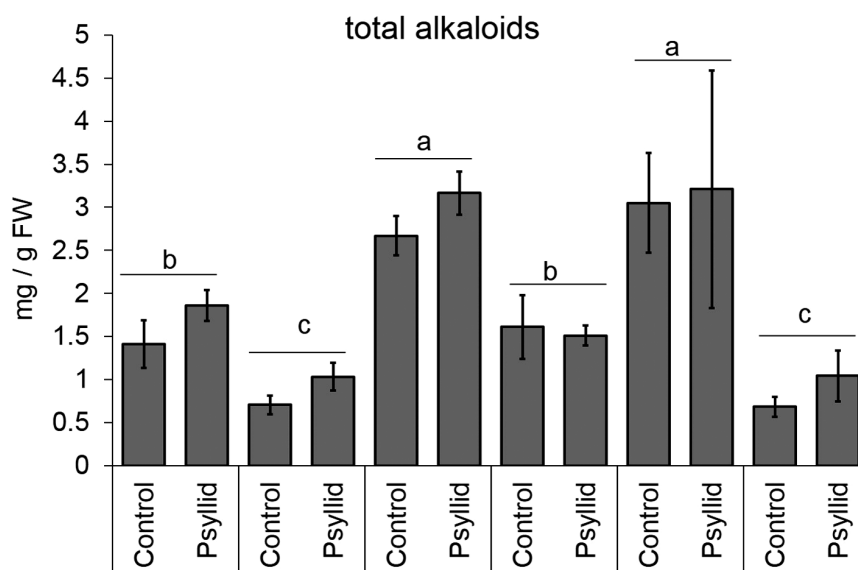
1 [online only]). There were noticeable portions of these plants that had chlorosis and eventually died. Atlantic plants that were not exposed to aphids remained vibrant and healthy. Clone P2-4 showed similar symptoms to Atlantic. Visual evidence of chlorosis and necrotic spots were seen on the leaves of all four replicates. The PALB 3016-6 plants remained healthy and vibrant and were not symptomatic of PVY. Entry Etb 6-21-3 did not support the development of the aphid population and no PVY disease symptoms were observed. Clone A05379-211 showed some chlorosis in the leaves, which would indicate that the PVY was having an effect on the plants. Despite visual symptoms, none of the plants tested positive for PVY infection with the test strips.

#### Compound-Level Differences Among Breeding Clones and Entries

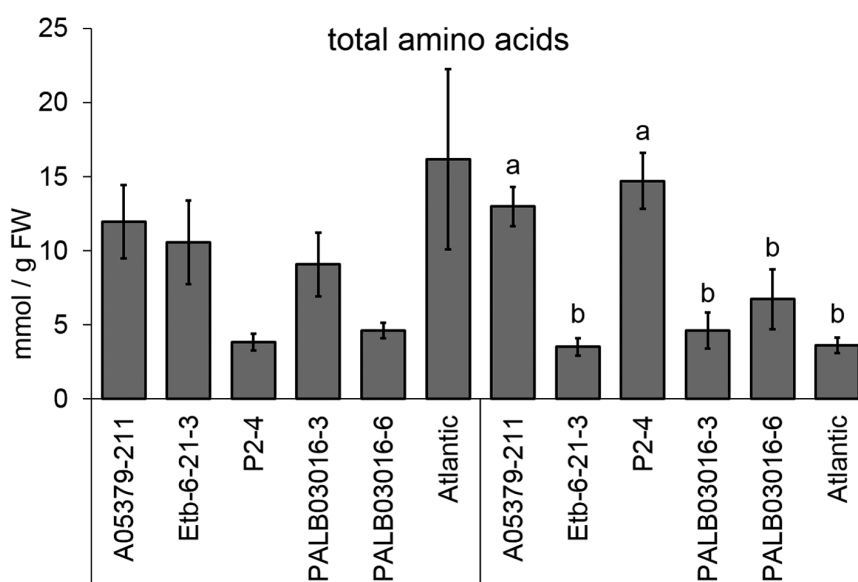
Total alkaloid levels were significantly greater ( $F = 13.231$ ;  $P < 0.001$ ;  $N = 56$ ) in P2-4 and PALB03016-6 than other entries, with Etb6-21-3 and Atlantic displaying the lowest levels (Fig. 5). Psyllid feeding did not affect total alkaloid levels ( $F = 1.727$ ;  $P = 0.196$ ;  $N = 56$ ), nor was there a significant breeding clone by psyllid feeding interaction ( $F = 0.190$ ;  $P = 0.965$ ;  $N = 56$ ).

In contrast, a significant interaction was observed between psyllid feeding and breeding clone effects on total amino acid levels ( $F = 3.095$ ;  $P = 0.018$ ;  $N = 56$ ). Therefore, plants that were exposed to psyllids and those that were unexposed were analyzed separately. For plants unexposed to psyllids, there were no significant differences in total amino acid levels ( $F = 1.284$ ;  $P = 0.301$ ;  $N = 32$ ) (Fig. 6). However, for plants exposed to psyllids, amino acid levels were greatest in A05379-211 and P2-4 than the other breeding clones and entries ( $F = 9.842$ ;  $P < 0.001$ ;  $N = 24$ ) (Fig. 6).

MANOVA revealed a significant interaction on individual amino acid concentrations between breeding clone and psyllid exposure effects (Pillai's trace = 2.715;  $F = 2.0477$ ;  $P < 0.001$ ;  $N = 56$ ). Therefore, unexposed and exposed plants were assessed separately. MANOVA detected significant differences in amino acid levels among breeding clones in unexposed (Pillai's trace = 3.767;  $F = 2.206$ ;  $P < 0.001$ ;  $N = 32$ ) plants. Aspartic acid (greater in Atlantic than all others), glutamic acid (greater in Atlantic than all others), isoleucine (greater in A05379-211 than all others except Etb-6-21-3), leucine (greater in A05379-211 than all others except Etb-6-21-3 and PALB03016-6), phenylalanine (greater in A05379-211 than all



**Fig. 5.** Mean total alkaloid levels for each entry either unexposed or exposed to psyllids. Bars represent standard errors. Different letters represent overall means that differ significantly among breeding lines by LSD tests.

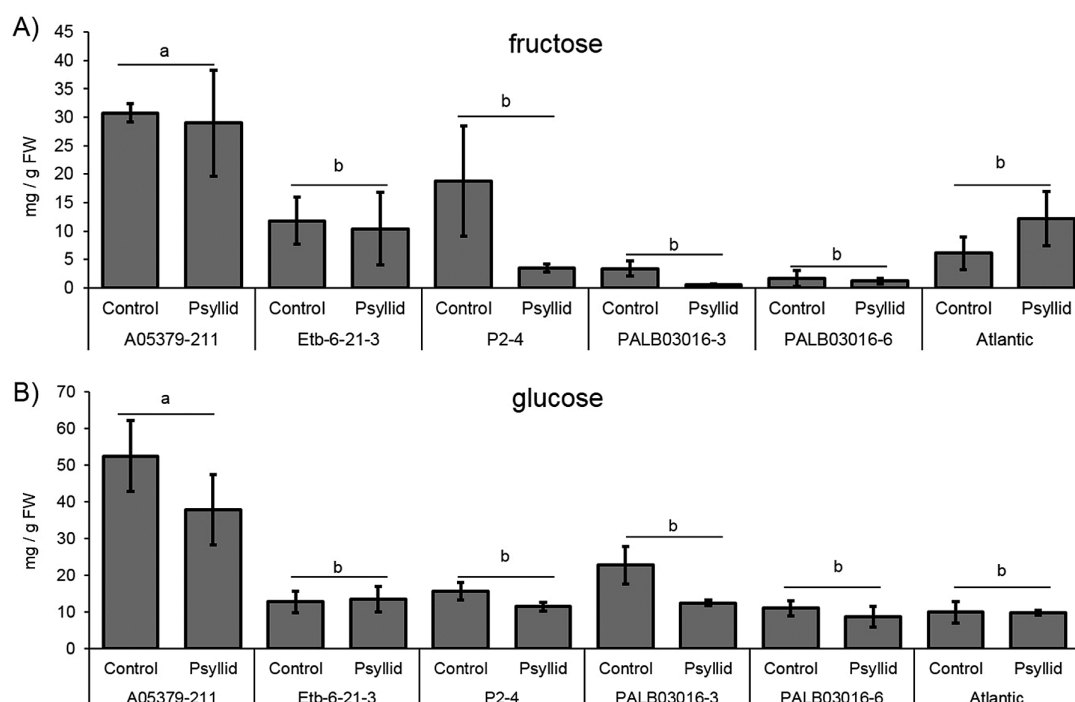


**Fig. 6.** Mean total amino acid levels for each entry either unexposed or exposed to psyllids. Bars represent standard errors. Different letters represent overall means that differ significantly among entries by LSD tests for exposed plants only, as no significant differences were determined among breeding entries for unexposed plants.

others), and serine (greater in A05379-211 than all others except Etb-6-21-3 and PALB03016-3) levels differed ( $P < 0.05$ ) among breeding clones in unexposed plants. MANOVA did not find significant effects of entry on amino acid levels when considering all individual compounds (Pillai's trace = 4.163;  $F = 1.382$ ;  $P = 0.180$ ;  $N = 24$ ). However, subsequent ANOVA performed as follow-up ( $P < 0.05$ ) for the entry effects on the levels of alanine (greater in A05379-211 and P2-4 than all others), aspartic acid (greater in A05379-211 than all others), glycine (greater in A05379-211 than all others except PALB03016-6), isoleucine (greater in A05379-211 than all others), leucine (greater in A05379-211 than all others), lysine (greater in A05379-211 than all others), methionine (greater in A05379-211 than all others), phenylalanine (greater in A05379-211 than all others), serine (greater in P2-4 than all others except A05379-211),

threonine (greater in P2-4 than all others except PALB03016-3), tyrosine (greater in P2-4 than A05379-211), and valcine (greater in A05379-211 than all others except PALB03016-3) levels differed ( $P < 0.05$ ).

Fructose levels in A05379-211 were significantly greater than all other breeding clones ( $F = 8.323$ ;  $P < 0.001$ ;  $N = 56$ ) (Fig. 7A). Psyllid exposure did not significantly affect fructose levels ( $F = 0.649$ ;  $P = 0.425$ ;  $N = 56$ ), and there was no significant breeding clone by psyllid exposure interaction ( $F = 0.879$ ;  $P = 0.503$ ;  $N = 56$ ). Likewise, glucose levels in A05379-211 were significantly greater than all other breeding clones ( $F = 11.703$ ;  $P < 0.001$ ;  $N = 56$ ) (Fig. 7B). Psyllid exposure did not significantly affect glucose levels ( $F = 1.886$ ;  $P = 0.177$ ;  $N = 56$ ), and there was no significant breeding clone by psyllid exposure interaction ( $F = 0.542$ ;  $P = 0.743$ ;  $N = 56$ ).



**Fig. 7.** Mean (A) fructose and (B) glucose levels for each breeding entry either unexposed or exposed to psyllids. Bars represent standard errors. Different letters represent overall means that differ significantly among breeding entries by LSD tests.

Total phenolic levels were variable in the different breeding clones, with levels greater in P2-4 and PALB03016-3 than A05379-211, Etb-6-21-2, and Atlantic; levels greater in PALB03016-6 than Etb-6-21-3 and Atlantic; and levels lower in Atlantic than all the other breeding clones tested ( $F = 8.092$ ;  $P < 0.001$ ;  $N = 56$ ) (Fig. 8). Total phenolic levels also were greater in plants exposed to psyllids than those unexposed ( $F = 9.156$ ;  $P = 0.004$ ;  $N = 56$ ) (Fig. 8). There was not a significant interaction between breeding clone and psyllid exposure ( $F = 0.571$ ;  $P = 0.722$ ;  $N = 56$ ).

MANOVA revealed a significant interaction on phenolic levels between breeding clone and psyllid exposure effects (Pillai's trace = 2.489;  $F = 1.707$ ;  $P = 0.002$ ;  $N = 56$ ). Therefore, unexposed and exposed plants were assessed separately. MANOVA detected significant differences in phenolic levels among breeding clones in unexposed (Pillai's trace = 4.521;  $F = 6.821$ ;  $P < 0.001$ ;  $N = 32$ ) as well as exposed plants (Pillai's trace = 4.885;  $F = 11.755$ ;  $P < 0.001$ ;  $N = 24$ ). For unexposed plants, breeding clone significantly affected ( $P < 0.05$ ) levels of all individual phenolics, including four chlorogenic acid derivatives and 12 flavonoids, except two procyanidins. In all cases, Atlantic possessed less phenolics than at least one, at usually multiple, of the other breeding clones. For psyllid exposed plants, breeding clone significantly affected ( $P < 0.05$ ) levels of three chlorogenic acid derivatives of four, eight of 12 flavonoids, and one of the two procyanidins. For all individual compounds except the significantly affected procyanidin, Atlantic had lowered amounts than at least one other breeding clone. For the procyanidin, P2-4 had lowered levels than A05379-211.

Because of a significant breeding clone by psyllid exposure interaction ( $F = 2.565$ ;  $P = 0.040$ ;  $N = 56$ ), total terpenoid levels were assessed separately for unexposed and psyllid exposed plants. For unexposed plants, PALB03016-3 and PALB03016-6 possessed greater total terpenoids than all other breeding clones ( $F = 13.607$ ;  $P < 0.001$ ;  $N = 32$ ) (Fig. 9). For exposed plants, A05379-211,

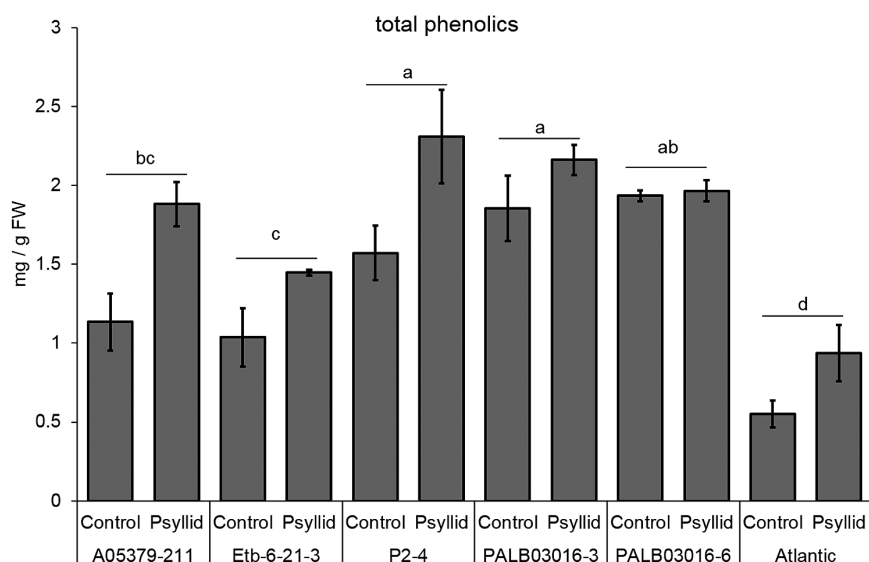
PALB03016-3, and PALB03016-6 possessed greater total terpenoids than all other breeding clones ( $F = 7.650$ ;  $P = 0.001$ ;  $N = 24$ ) (Fig. 9).

For individual terpenoids, MANOVA determined a significant effect of the breeding clone on compound levels (Pillai's trace = 3.769;  $F = 2.496$ ;  $P < 0.001$ ;  $N = 56$ ), but no significant effect of psyllid exposure (Pillai's trace = 0.715;  $F = 1.673$ ;  $P = 0.130$ ;  $N = 56$ ). There was no significant interaction between the breeding clone and psyllid exposure (Pillai's trace = 2.982;  $F = 1.181$ ;  $P = 0.156$ ;  $N = 56$ ). Out of 20 quantified monoterpenoids, only  $\beta$ -myrcene (PALB03016-3 and PALB03016-6 had greater levels than all other breeding clones), bornyl acetate (A05379-211 had lower levels than all other breeding lines), damascone (PALB03016-6 had greater levels than all other breeding clones), geraniol (A05379-211 had lower levels than all other breeding clones except Etb-6-21-3), and nerol (A05379-211 had lower levels than all other breeding lines except Atlantic and Etb-6-21-3) were significantly different ( $P < 0.05$ ) among the breeding clones. However, all seven quantified sesquiterpenoids were significantly ( $P < 0.05$ ) affected by the breeding clones. For all sesquiterpenoids except  $\alpha$ -humulene, PALB03016-3 and PALB03016-6 were greater than other breeding clones. For  $\alpha$ -humulene, A05379-211 was present in greater levels than all other breeding clones. The monoterpenoids identified and quantified were  $\alpha$ -phellandrene,  $\alpha$ -terpinene,  $\alpha$ -terpinolene,  $\beta$ -myrcene,  $\beta$ -pinene, bornyl acetate, *cis*- $\beta$ -ocimene,  $\Delta$ -3-carene, damascone,  $\gamma$ -terpinene, geraniol, linalool, nerol, neryl acetate, pulgone, and four unidentified putative monoterpenoids. The sesquiterpenoids identified and quantified were  $\alpha$ -humulene,  $\beta$ -caryophyllene,  $\beta$ -ionone, and four putative sesquiterpenoids (likely farnesene isomers).

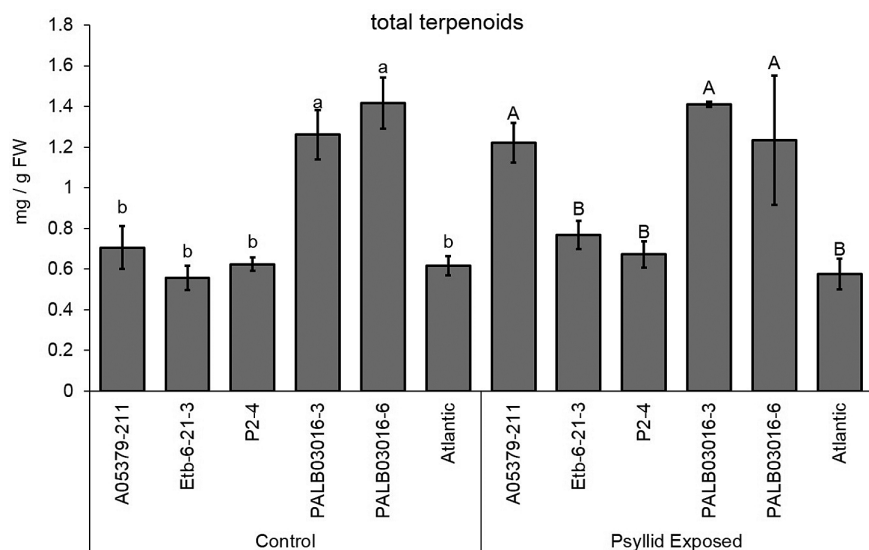
## Discussion

In choice bioassays, there were no significant differences for any pairing. This suggests that when given a choice of any of the breeding clones or the known susceptible commercial cultivar Atlantic,





**Fig. 8.** Mean total phenolic levels for each entry either unexposed or exposed to psyllids. Bars represent standard errors. Different letters represent overall means that differ significantly among entries by LSD tests.



**Fig. 9.** Mean total terpenoid levels for each entry either unexposed or exposed to psyllids. Bars represent standard errors. Different letters represent overall means that differ significantly among entries by LSD tests.

psyllids have no preference either with respect to settling or to oviposition. These results agree with those of [Diaz-Montano et al. \(2014\)](#) who also found no preference when potato psyllids were provided a choice of P2-4 or A05379-211 and Atlantic. The findings for the entries in this study can be contrasted with previous studies ([Rubio-Covarrubias et al. 2017](#)) which applied the same bioassay method to Mexican developed cultivars and did find significant differences in choice, with two entries preferred for settling relative to Atlantic. The authors of that study suggested that trichome length and density may have contributed to the psyllid's preferences. Unfortunately, such data are not available for these entries. It was also found that every entry demonstrated some, and relatively equivalent, susceptibility to Lso. This would correspond to location and oviposition behavior. If psyllids were more common on one entry, it might be expected that they would feed more on that entry and thus would have been more likely to transmit the bacteria. However, it should

be noted that the detection of Lso in potato leaf material is often difficult and can be inconsistent and variable depending on factors such as sampling location on the plant ([Levy et al. 2011](#)).

Contrasting with those results found from choice bioassays, there were significant differences with respect to oviposition in no-choice bioassays. Specifically, three of the entries examined in this study (A05379-211, PALB03016-3, PALB03016-6) demonstrated reduced oviposition compared with Atlantic. When the development of these eggs was examined, all of the entries with reduced oviposition (P2-4, A05379-211, PALB03016-6) two (A05379-211 and PALB03016-6) had greater hatch rates than Atlantic, while Etb-6-21-3 did not. No significant differences were found with respect to relative growth index, indicating that hatched eggs develop to adult with similar success. [Diaz-Montano et al. \(2014\)](#) also examined antibiosis in A05379-211 and P2-4 and found no difference in oviposition or development relative to Atlantic. In contrast to the findings for development in

psyllids, aphids were seemingly affected by entry. Specifically, the entries Etb-6-21-3 and PALB03016-6 proved poor for aphid development, indicating antibiosis with respect to aphids. These studies, however, indicate limited to no antibiosis with respect to psyllids.

Despite generally minimal effects on insect preferences, with the exception being oviposition, there were significant differences in host biochemistry that would be expected to alter feeding and other behaviors. For instance, alkaloids were significantly greater in all breeding clones except Etb-6-21-3 than in Atlantic, suggesting that the experimental breeding clones would be less preferred over the commercial cultivar. However, this was not observed. It is possible that oviposition in psyllids reflects the relative nutritional values of the plants, since psyllids are synovigenic (S. M. Prager, unpublished data) and therefore may develop more eggs on some host plants than others. This effect would resemble the differences in population growth observed in the aphids.

Likewise, Atlantic possess significantly less total and individual phenolic compounds than the breeding lines, and the presence of some or the sum of all phenolics in greater amounts could be expected to clones feeding and insect survival. However, this was not the case with psyllids, yet it could be that greater phenolics could help potato plants tolerate feeding, so increased levels may still be a desirable trait in future entries. Further, aphids may have been affected in part by phenolics, at least because Etb-6-21-3 and PALB03016-6 had greater levels than Atlantic.

Lastly, greater terpenoid levels were present in PALB03016-3 and PALB03016-6 than Atlantic regardless of insect induction, which, as volatile compounds, should predictably affect host choice. While this was observed for aphids, psyllids did not respond as expected. We suspect the specific methodology of the choice bioassays with closed arenas and relatively small space may have made it impossible for the psyllids to perceive distinct volatile signals. Nonetheless, aphids appear to have been affected by greater volatiles seen in lines investigated in this study. The presence of greater terpenoids once again could be a desirable trait to maintain, as other, non-assessed insect pests also could be affected such as Colorado potato beetle (Szczepanik et al. 2005).

Regarding primary metabolites, trends remained unclear linking changes in levels of amino acids or sugars to observed effects on psyllids. A05379-211 had increased sugar levels, and generally higher amino acid levels, than all other breeding clones and Atlantic, but, much like Diaz-Montano et al. (2014), this did not affect observed antibiosis.

In conclusion, this study attempted to link differences in host chemistry before and after insect (psyllid) feeding to observed differences in psyllid and aphid behaviors on potato plants with different genetic backgrounds. For the most part, only psyllid oviposition was affected by potato breeding clone, although primary metabolites may not have directly caused these results. Increased total phenolic and terpenoid levels in breeding lines compared with Atlantic may have affected psyllid and aphid behaviors or success, such as the significant reductions in population growth that were observed for two of the experimental clones. Thus, because these breeding clones exhibited rather substantial differences in the levels of putative defense compounds, they should be evaluated for resistance against other major pests of potato.

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## Supplementary Data

Supplementary data is available at *Journal of Economic Entomology* online.

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