Investigating behavior of the potato psyllid *Bactericera cockerelli* (Šulc), (Hemiptera: Triozidae) on three potato germplasms with putative resistance to *Candidatus* Liberibacter solanacearum

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# Authorization to Submit Thesis

This thesis of Austin N. Fife, submitted for the degree of Master of Science with a major in Entomology and titled, “Investigating behavior of the potato psyllid *Bactericera cockerelli* (Šulc), (Hemiptera: Triozidae) on three potato germplasms with putative resistance to *Candidatus* Liberibacter solanacearum,” has been reviewed in final form. Permission, as indicated by the signatures and dates given below, is now granted to submit final copies to the College of Graduate Studies for approval.

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# Abstract

Zebra chip disease (ZC) in potato is associated with “*Candidatus* Liberibacter solanacearum” (Lso), which is transmitted by the potato psyllid *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae). ZC can cause large economic losses when disease incidence is high. ZC management is currently focused on managing populations of the psyllid vector with insecticides. Host plant resistance to Lso and ZC has been investigated, but no commercial potato variety has been found resistant to the pathogen or the disease symptoms. Three Lso-resistant breeding clones with reduced ZC symptoms have been derived from a wild potato variety *Solanum chacoense* Bitter. Our study was designed to screen these germplasms for their effects on the psyllid’s host acceptance behavior and fecundity. The breeding clones selected were: ‘A07781-10LB’ (‘10LB’), ‘A07781-3LB’ (‘3LB’) and ‘A07781-4LB’ (‘4LB’). ‘Russet Burbank’ (*Solanum tuberosum* ) L. was used as a Lso-susceptible control. We conducted no-choice assays with intact potato leaflets and observed the following behaviors: probing, walking, cleaning and leaving the leaf. We also compared oviposition and egg fertility for psyllids held on these germplasms. Probing frequency was highest on Russet Burbank. Female psyllids walked more frequently on Russet Burbank than both sexes on 10LB and more than other females on 3LB. Females also walked longer on Russet Burbank. Together, these results suggest greater activity on Russet Burbank than these resistant germplasms. Oviposition and egg fertility were not significantly different among germplasms or the control. Our results found little to no evidence of antixenotic or antibiotic effects on psyllid vector feeding or oviposition for these germplasms.

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Thank you.

# Dedication

For Liz, Violet and Fifes to come

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**Chapter 1: Introduction**

## Research context

The potato/tomato psyllid, *Bactericera cockerelli* (Šulc), (Hemiptera: Triozidae) is a small sternorrhynchan insect pest of solanaceous crops such as potato and tomato (Martin 2008, Knowlton and Thomas 1934, Wallis 1955). First discovered in Colorado (Šulc 1909), potato psyllids have a history closely tied to potato growing regions and plant diseases (Richards 1973). *Bactericera cockerelli’*s geographical distribution ranges from southern Canada to Central America, throughout the western United States (Butler and Trumble 2012, Munyanzea 2007, Rehman 2010) and a recent introduction to New Zealand (Liefting et al. 2008, 2009, Martin 2008, Teulon 2009). Publications regarding the psyllid initially emerged from 1926-1928, due a condition affecting solanaceous plants known as ‘psyllid yellows’ (Eyer and Crawford 1933, Richards 1928, 1973).

Potato psyllids have recently been identified as vectors of the phytoplasma “*Candidatus* Liberibacter solanacearum” (Lso) (Rhizobiaceae; Alphaproteobacteria) (Cicero 2016, Goolsby 2007a, Liefting et al. 2009, Munyaneza 2007). Lso is an uncultured gram-negative α-proteobacterium (Liefting et al 2009) that infects solanaceous plants. Lso is transmitted to the plant’s phloem by the psyllid’s saliva while feeding (Cooper et al. 2014). Symptoms in potato include stunting, swollen axillary buds, aerial tubers, leaf purpling, chlorosis and reduced yield (Munyaneza et al, 2007, 2008). Infection also alters tuber sugars and phenolics, resulting in brown stripes which char and blacken when fried (Alvarado et al 2012, Buchman et al. 2012, Navarre et al. 2009) This condition is known as ‘zebra chip’ disease (ZC) (Crosslin et al 2010, Hansen et al. 2008, Liefting 2009, Lin et al. 2009). ZC-affected tubers are unmarketable, which results in large economic losses for growers (Munyaneza 2007a, Rosson 2006). Yield reduction from Lso infection has ranged from 43% to 93% in some cases (Munyanzea et al. 2008, 2011).

Lso and ZC symptoms were first described in 1994 in Mexico (Secor and Rivera-Varas 2004, Munyanzea et al. 2009) and was detected in the United States in 2009 (Abad et. al 2009). Lso and ZC were first detected in the Pacific Northwest (PNW) states of Idaho, Washington, and Oregon in 2011 (Murphy et al. 2012, Crosslin et al. 2012). Since 2011, Lso and ZC have remained a continuing threat to potato production in the PNW and contribute substantively to production costs (Greenway, 2014, Greenway and Rondon 2018, Guenther et. al. 2012, Wenninger et al. 2017).

Various pest management practices have been investigated for management of Lso and ZC. Psyllid management traditionally relies on insecticides (Echegaray and Rondon 2017) to manage vector populations, using chemicals such as abamectin, imidacloprid, spiromesifen, thiamethoxam and dinotefuran (Gharalari 2009, Goolsby 2007b, Guenthner et al 2012, Vega-Gutierrez et al, 2008). Psyllid populations have the potential to develop resistance to common insecticides such as neonicotinoids and abamectin (Hernandez-Bautista et al, 2013, Chavez et. al. 2015, Liu and Trumble 2007, Prager et al, 2013,). Multiple pesticide applications also increase production costs (Greenway 2014, Guenthener et al. 2012). Around half of Eastern Idaho growers’ insecticide expenditures was related to ZC control in 2018 (Greenway and Rondon 2018). The difficulty and large expense of psyllid control emphasizes the need for alternative and improved pest management strategies such as host plant resistance.

Host plant resistance is a valuable part of integrated pest management (Butler and Trumble 2012, Diaz-Montano et al. 2013, Kogan 1988, Munyanzea 2012). Even a small amount of resistance or tolerance of a plant to a pathogen or a vector may help reduce damage below action thresholds and reduce pesticide applications (Kennedy et al. 1987). Host plant resistance also increases pesticide efficiency and helps to delay insect’s pesticide resistance (Gharalari 2009). Currently, no commercial potato varieties have been found with acceptable resistance to Lso (Anderson et al. 2012, Munyaneza et al. 2011). Uncultivated and wild solanaceous plants such as *Solanum chacoense* Bitter (Rashidi et al 2017) and *Solanum berthaultii* Hawkes (Butler et al. 2011) have shown less Lso infection and/or ZC symptoms than other cultivars. These plants have special traits that can be bred or cloned into commercial cultivars, conferring them the same resistance to disease (Casteel et al. 2006, 2007, Kaloshian 2004). However, it remains unclear whether these wild plants are resistant or tolerant to Lso or to the psyllid vector (Butler et al. 2011, Putten et al. 2001, Kennedy et al. 1987).

In order to clarify how these plants resist infection and ZC, we examined psyllid probing, walking and cleaning behaviors as well as female fertility on three potato breeding clones: ‘A07781-10LB’ (‘10LB’), ‘A07781-3LB’ (‘3LB’) and ‘A07781-4LB’ (‘4LB’) (Rashidi et al. 2017). These germplasms were derived from the wild potato *Solanum chacoense* and exhibit high tolerance and low susceptibility to Lso (Rashidi et al. 2017). Russet Burbank was used as a susceptible control (Munyaneza et al. 2011). The results will help clarify the mechanisms of resistance found in these germplasms and help inform plant breeders in the development of Lso-resistant potatoes (Kennedy et al. 1987).

# Chapter 2: Materials and methods

## Plant characteristics and living conditions

## Potato clones were provided by the USDA-ARS, Small Grains and Potato Germplasm Research Unit, Aberdeen, ID, USA. We used three sibling clones derived from *Solanum chacoense* Bitter with resistance to Lso: A07781-3LB, A07781-4LB, and A07781-10LB (Rashidi et al. 2017). ‘Russet Burbank’ was used because it is susceptible to Lso (Munyaneza et al. 2011) and because of its large impact on potato production in the Pacific Northwest (NASS 2017).

## The selected potatoes were grown in a greenhouse maintained between 25-32°C, 32% RH, with a photoperiod of 16:8 (L:D). Plants were grown in pots of approximately 8.5 cm length × 8.5 cm width × 9.5 cm height, with a soil mixture of 4:4:4:1 peat moss: compost: coconut coir: perlite. Fertilizer was not used on experimental plants to avoid nitrogen increases which may affect insect feeding behaviors (Pfeiffer and Burts 1983, 1984). We used plants in their vegetative growth stage (growth stage II) (Dwelle et al. 2003).

## Insect characteristics and living conditions

A Lso-positive potato psyllid colony was reared in the same greenhouse conditions as described above to avoid phenological asynchrony (Hodkinson et al. 2015). Psyllids were allowed free access to both Russet Burbank potatoes and ‘Yellow Pear’ tomatoes (*Solanum lycopersicum*) L. Colony plants were fertilized once weekly with approximately 17 g of 24:8:16 NPK fertilizer per gallon of water (MiracleGro® All Purpose Plant Food, Scotts Company, Marysville, OH). Plants were replaced as needed.

**Lso confirmation**

Idaho harbors four haplotypes of the potato psyllid: Northwestern, Western, Central and Southwestern and two haplotypes of Lso: A and B (Dahan et al. 2017, Wenninger et al. 2017). The ‘Central’ psyllid haplotype was confirmed in our laboratory colony via the methods described in Swisher and Crosslin (2014) and analyzed with ethidium bromide-stained agarose gels.

A sample of forty psyllids taken from the colony were transferred to individual microcentrifuge tubes filled with 70% ethanol. Lso incidence was tested according to established protocols at the Aberdeen Research and Extension Center (Aberdeen, ID, USA) (Dahan et al. 2017). DNA extraction was based on the methods described by Marzachi et al. (1998). Individual psyllids were ground by a homogenizer (Omni International Inc., Kennesaw, GA), macerating each psyllid for 1 minute at high speed and an additional minute at medium speed in 500 µl of CTAB 2% % (Alpha Teknova, Inc., Hollister, CA, Cat. No. C2190) (Composition: 2% CTAB, 100mM Tris-HCl, pH 8.0, 20mM EDTA, pH 8.0, 1.4M Sodium Chloride (NaCl). Microcentrifuge tubes were then incubated at 60°C for 30 minutes and gently mixed by inversion every 10 minutes while incubating. Tubes were then spun in a centrifuge at 14,000 rpm for 5 minutes and then the supernatant was transferred to clean 2 ml tubes. The supernatant was vortexed for approximately 20 seconds with 500 ml of chloroform:isoamyl alcohol (24:1 v:v) (Sigma-Aldrich, Inc., Atlanta, GA; Catalogue number C0549), then centrifuged at 14,000 rpm for 5-10 minutes at 4℃. The clean supernatant was transferred to a new tube, then refrigerated isopropanol (Sigma-Aldrich, Inc., Atlanta, GA; Catalogue number I9516) was added at a rate of 2/3 of the volume of the supernatant. The mixture was then refrigerated at ˗20°C for 20-30 minutes. DNA was precipitated by centrifuging the mixture for 20 minutes at 14,000 rpm at 4℃, gently pouring off the supernatant and keeping the precipitated DNA pellet. The pellet was washed in 300 µl of 70% ethanol and centrifuged for 5 minutes at 10,000 rpm. The pellet was then dried overnight in a fume hood. Once dry, 30 µl of nuclease-free water was added. DNA was stored at ˗20°C.

Extracted DNA samples were then processed using a Sybgreen method. SsoAdvanced™ Universal SYBR® Green Supermix (Biorad, Hercules, CA) was mixed in a CFX Connect Real-Time PCR Detection System (Biorad, Hercules, CA). HLBr (5’-GCG TTA TCC CGT AGA AAA AGG TAG-3’) and LsoF (5’-GTC GAG CGC TTA TTT TTA ATA GGA-3’) were used as primers (Li et al. 2006; Li et al. 2009) and 10 µl of Sybgreen supermix was added to 150 nM of each primer with 1 µl of DNA template. The program cycle was as follows: one cycle at 98°C for 2 mins followed by 40 cycles of 95°C for 10 sec and 62°C for 20 sec. The melt curve was 65°C to 95°C, with increments of 0.5°C sec-1. DNA of a healthy tuber was used as a negative control. DNA of a Lso-infected tuber was used as a positive control and water was used as a no-template control in all tests. pIDTSmart Kan (Synthetic Genomics, SGI-DNA, CA) with a 250 bp region was amplified with the primer HLBr. The plasmid was diluted 10-fold and used with the following dilutions: 1 × 10-2, 1 × 10-3, 1 × 10-4, 1 × 10-6, 1 × 10-7, and 1 × 10-8 ng. Pathogen quantity was reported as copy number of Lso; copy numbers were determined using the methods of Levy et al. (2011).

Each psyllid tested positive for Lso, suggesting a 100% rate of infection for the colony.

## No-choice behavior assays

No-choice assays were conducted in a climate-controlled room maintained at 26°C. Assays were conducted on a wire shelving unit, which allowed the testing arena to be lit both from above and below. Three Smith-Victor Digilight fixtures (Smith-Victor Corporation, Bartlett, IL) were used with three Azlo (Akces Media LLC dba ALZO Digital, Bethel, CT) full-spectrum CFL bulbs per light fixture (100-240 volts, 60 Hz, color temp 5500K CRI 91, 750 lumens, 15 watts). Two lights were placed with their light sources 35 cm above the testing arena and the light was softened with a diffusion material. The remaining light fixture was placed so that its light source was 45 cm below the testing arena and was softened with diffusion material as well. Illuminance was 3600 lx at the surface of the arena (Sekonic L-308DC-U Light Meter, Sekonic Corporation, Tokyo, Japan).

The observation arena (Fig. 2.1) was modeled after the design described by Liu and Trumble (2004), but modified to use leaflets of intact, potted plants. This permitted us to observe the psyllids with minimal interference to plant physiology and avoided altering plant volatiles or chemical defenses activated by damaging plant tissues (Klingler 2005). A recording arena was formed by sandwiching a panel of glass, a wetted filter paper, a leaf, and a piece of Plastazote® polyethylene foam (Zotefoams Inc., Croydon, UK), with a circular opening in the center (28 mm diameter). The arena was held together with two clips. This arena was then suspended by a suction cup held by an adjustable burette clamp. We used leaves from the upper canopy of the plants. The filter paper was discarded between observations. The glass pane and foam were replaced with each new plant and washed and dried at 90°C before reuse to remove potential volatile accumulation. Recordings were done with a L3CMOS C-mount USB camera and ToupView recording software (L3CMOS14000KPA, Hangzhou ToupTek Photonics Co., Ltd, Hangzhou, Zhejiang, China).

We collected psyllids from the colony by aspiration and transferred them to 8 **×** 35 mm glass shell vials. All psyllids were used within 90 minutes from the time of collection. Psyllids were introduced to the arena and recorded for five minutes. Psyllid sex was identified and psyllids were preserved in 95% ethanol for later testing for Lso by PCR. We recorded similar categories as Butler et al. (2011): probing, walking, cleaning, and whether the psyllid was on or off the leaf. Probing behaviors have putative significance with disease transmission and host selection (Prager et al. 2014a, Prager et al. 2014b). Behavior was scored using CowLog3 (Hänninen and Pastell 2009), which recorded incidence and timestamps for the behaviors observed.

## Fecundity assays

## Fecundity assays were conducted with greenhouse conditions, plants, and insects as previously described (see ‘*Plant characteristics and living conditions*’ and ‘*Insect characteristics and living conditions*’, above). A female/male pair of teneral psyllids (identified by their green body color) was introduced to a plant covered with an insect rearing sleeve (MegaView Science Co., Ltd., Taiwan). Rearing sleeves were supported over the plant using two lengths of galvanized steel wire with a diameter of 1.63 mm. Each wire was curved into a parabolic shape and each end of the wire was inserted into the soil on opposite corners of the plant pot (Fig. 2.2).

## Plants were blocked by germplasm in rows of four and placed inside 60 cm length x 60 cm width x 60 cm height mesh-covered PVC-framed cages. Plants were watered on alternating days by soaking pots in 56 cm length × 28 cm width × 6 cm height plastic trays until the soil became saturated (approximately 45 minutes).

## After a period of six to eight days the male was removed from plants and the female transferred to a new plant of the same germplasm. The female psyllid was then transferred to a new plant every four days at three intervals. Eggs were counted on each plant after the female was removed. Nymphs were counted four days, eight days and twelve days later to allow time for hatching. Each nymph was removed as it was counted. The number of nymphs that hatched was considered an indicator of egg fertility. Fertility percentages were calculated as the ratio of nymphs divided by egg counts for each sample.

## Statistical analysis

## Statistical analysis was preformed using R Version 3.5.1 (RCT 2013). Assumptions of normality were investigated with qqplots and Cullen and Frey graphs from the R package {fitdistrplus} (Delignette-Muller 2015). No-choice experiments and egg count data were analyzed using Generalized linear mixed modeling techniques (GLMM) (Stroup 2015) from the {glmer} function (Bates, 2015). A Poisson link was selected to model count data. Egg fertility was modeled with a binomial link to account for ratios. Behavioral models had fixed factors of germplasm, sex and the interaction of germplasm × sex. Psyllid replicate was treated as a random factor. The interaction of germplasm × sex was excluded from the off-leaf model due to low occurrences (n = 20 out of 182 observations), which did not allow an interaction to be estimated by the model. Fecundity models had fixed factors of germplasm, period and germplasm x period. Psyllid replicate was considered the random factor. Egg fertility was modeled with germplasm and period as fixed factors and individual psyllids as the random factor. All data were tested with Wald's χ2 tests. All data were tested with Wald's χ2tests, followed by least-squares means with Tukey's adjustments to test for multiple comparisons. Statistical significance was considered at α = 0.05.

## Chapter 3: Results

## No-choice assays

The number of probing events observed was significantly different among germplasms (Table 3.1). Psyllids probed more frequently on Russet Burbank than on A07781-10LB, and A07781-3LB, which did not differ among each other (Table 3.2). This effect appeared to reflect the trend of more probing by females on Russet Burbank (Table 3.2); however the germplasm x sex interaction was not significant (Table 3.1). Probing frequency did not differ by sex (Table 3.1). Overall, psyllids spent more time engaged in probing behavior than in the other activities recorded. (Figure 3.2), (Table 3.1, 3.2).

The number of walking events differed significantly among germplasms as well as by the interaction of germplasm x sex (Table 3.1). Psyllids walked more on Russet Burbank than on 10LB (Table 3.3). Female psyllids on Russet Burbank walked significantly more than males and females on 10LB and females on 3LB (Figure 3.3). The other means did not differ among each other. Walking duration did not differ among germplasms or between sexes, but the interaction term was significant (Table 3.1). Female psyllids walked significantly longer on Russet Burbank than for all other germplasm x sex combinations (Table 3.3). (Figure 3.4)

Cleaning and leaf-leaving behaviors generally were uncommon and of short duration. The interaction between germplasm and sex could not be analyzed for off-leaf behavior due to the low number psyllids observed leaving the leaf (n = 20 out of 181). The frequencies and durations of these two behaviors were not significantly different among germplasms, between sexes, or (in the case of cleaning behavior) in their interaction terms (Table 3.1).

## Fecundity assays

Neither the number of eggs laid nor percent egg fertility differed significantly among germplasm (Table 3.4). However, both factors differed significantly among observation periods as well as with respect to the interaction term (Table 3.4). This interaction effect was an artifact of calculating multiple comparisons of different germplasms across observation periods. When multiple comparisons were grouped by period there were no significant differences among germplasms within any given observation period.

## Chapter 4: Discussion

## No-choice assays

Other investigations with putatively resistant potato germplasms have found significant differences in psyllid fecundity and psyllid settling behaviors (Butler et al. 2001, Cooper and Bamberg 2014, Diaz-Montano et al. 2014, Prager et al. 2014, 2018 Thinakaran et al. 2015), but there does not appear to be a general trend in predicting resistant germplasms. Furthermore, it is difficult to determine what consists of true resistant mechanisms which are biologically meaningful.

A possible explanation for the higher probing and walking frequencies could be that phytoplasmas (including Lso) can alter psyllid preferences (Mayer et al. 2008) and settling behavior (Mas et al. 2014). The psyllids used in our experiment were taken from a Lso-positive colony with a high percentage of infected psyllids. Lso-infected psyllid prefer undamaged, uninfected hosts for oviposition and settling (Davis et al 2014) – a behavior which has been seen in other insect-plant-vector relationships (Cao et al 2016). The Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is a vector of Candidatus Liberibacter americanus, a pathogen which is like Lso (Teixeira et al 2005). Studies of D. citri have examined how host plant volatiles can alter psyllid behavior (Davidson et al. 2014, Wenninger et al. 2009), including increased probing in response to visual and chemical cues from their host plant (Patt et al. 2011). This is a possible explanation for the minor trend we saw with female psyllids probing more often than male psyllids (Table 3.2). It may be that the volatiles from Russet Burbank are more stimulating as a natal plant host for female psyllids, which may be more influenced by familiar cues while selecting host plants for oviposition or feeding (Prager et al 2014a). Further studies into potato psyllid volatile attractiveness would help to determine how these results correlate with host plant acceptance.

Although leaf-leaving duration was statistically significant from our model, the duration of leaf-leaving behaviors was very small and not biologically significant (Table 3.1). It is also important to note that leaf-leaving was defined in the context of our observation arena. On a real plant there is a much larger surface area for a psyllid to explore, so the leaf-leaving events likely represent questing behavior rather than host rejection. It also is possible that the duration between a psyllid’s initial encounter, settling behaviors and eventual plant rejection is longer than the time we allotted for recording. Recording times may also explain some of the differences in walking behaviors, which may have been different if allowed enough time for the psyllid to feed and explore the plant more.

## Fecundity assays

The significant effects for fecundity data were not biologically significant. The statistical differences were created from comparisons between different periods, which we would expect to be different. But there were no significant differences between germplasms in the same period or between consecutive periods when the comparisons were investigated. Generally, more than 50% of psyllid eggs hatched regardless of germplasm for the first four periods (14-20 days). We selected our observation period to correlate with the periods of maximum oviposition reported in the life history tables of Abdullah N. M. M. (2008) and Yang et al. (2010). It was surprising to see the large reduction of egg fertility for some psyllids in period four (18-24 days) (Table 3.7). We only permitted male access to the female psyllids during the initial period to prevent possible harassment of the female psyllids (Arnqvist 2013). It may be that potato psyllids require multiple mates to maintain egg fertility (Arnqvist 2013).

## Conclusion

It is difficult to separate the mechanisms of host plant resistance or tolerance and how they to correlate with psyllid host acceptance (Diaz-Montano et al. 2006, Butler et al. 2011). We were unable to find biologically significant differences on psyllid behavior or fecundity between the germplasms examined.

Although Russet Burbanks received more probes than two other germplasms, the psyllids still probed for long periods on each germplasm and on average each germplasm received at least one probe during the time recorded (Table 3.2). A single psyllid is enough to transmit Lso and the disease progresses independently of bacterial titer (Buchman et al. 2011, Rashed et al. 2012). Disease incubation decreases with greater psyllid feeding and transmission can occur in a little as five minutes (Rashed et al. 2012, Mustafa et al. 2015b). Combined, these studies suggest the low tolerance of potatoes to probing or feeding if they are to resist the disease by dissuading psyllid feeding or settling behavior.

These results suggest that the modality of resistance for the A07781 germplasms is may be to the pathogen itself, not the psyllid vector.