COMPARATIVE BIOLOGY OF POTATO PSYLLID, BACTERICERA COCKERELLI
(HEMIPTERA: TRIOZIDAE), HAPLOTYPES

By

TARIQ MUSTAFA

A dissertation submitted in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

WASHINGTON STATE UNIVERSITY
Department of Entomology

DECEMBER 2014

© Copyright by TARIQ MUSTAFA, 2014
All Rights Reserved
To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of TARIQ MUSTAFA find it satisfactory and recommend that it be accepted.

______________________________________
Richard S. Zack, Ph.D., Chair

______________________________________
Laura Corley Lavine, Ph.D.

______________________________________
Joseph E. Munyaneza, Ph.D.

______________________________________
Peter J. Landolt, Ph.D.

______________________________________
David Horton, Ph.D.

______________________________________
Andrew Jensen, Ph.D.
ACKNOWLEDGMENT

I would wish to express sincere gratitude to Dr. Richard S Zack, my adviser and committee chairman, for his counsel, wisdom and optimism throughout the completion of my stage. I want to thank the members of my committee for their efforts in guiding me through the procedure of writing my dissertation. I especially want to thank Dr. Joseph Munyaneza for his energy, enthusiasm, writing advice and supplying me with the opportunity to promote not only my career and but also my knowledge his help and guidance has been invaluable to me. I would also wish to thank Drs. David Horton and William Rodney Cooper for providing me with invaluable suggestions and advice with experimental design, and helping in data interpretation and analysis. In addition, I would wish to express thanks to my committee members, Drs. Laura Corley Lavine, Peter J. Landolt and Andrew Jensen, who provided me with guidance and constructive critique.

I would wish to thank everyone at the USDA-ARS laboratory in Wapato, WA, especially the people in Joe Munyaneza lab: Millie Heidt, Kylie Swisher, Venkat Sengoda, Stacey Pettit, Jennifer Delgado and Francisco de la Rosa., as well as the members of the Dr Horton lab: Deb Bearers' and Eugene Miliczky. None of this work would have been possible without the sustenance of each and every one of you.

I would like to acknowledge the WSU Entomology department, especially Adam Williams and Doris Lohrey-Birch, who assisted me through this journey; it has been a neat experience.
I would also like to acknowledge the University of Agriculture, Faisalabad, Pakistan for giving me an opportunity for my doctoral studies and acknowledge Higher Education Commission for their fiscal backing.

Finally, I would like to thank my parents, brothers, sister, and my wife for their continued support and encouragement. I am very grateful to all of you.
Zebra chip, a new and economically important disease of potato, is threatening potato production in the United States, Mexico, Central America, and New Zealand. The disease is associated with the bacterium ‘Candidatus Liberibacter solanacearum’ (Lso), vectored by potato psyllid, Bactericera cockerelli (Šulc) (Hemiptera: Triozidae). This insect pest feeds on over 40 plant species, including cultivated and wild solanaceous plants. Recent discoveries of four genetic variants (haplotypes) of potato psyllid, coupled with the finding that it overwinters in the Pacific Northwest on bittersweet nightshade (Solanum dulcamara), may complicate management of this psyllid, if differences exist in biological traits among the haplotypes. The present study compared development and reproduction among three psyllid haplotypes (Central, Western, and Northwestern) commonly found on Pacific Northwest potato crops, when reared on potato or bittersweet nightshade. Lso transmission efficiency among the three haplotypes was also assessed. The results showed that development time was longer for psyllids reared on nightshade than potato. The duration of the pre-oviposition period, egg incubation requirements, nymphal development time, and total developmental time was higher on bittersweet nightshade compared
to potato. The largest host effects were found for the Central haplotype, which exhibited an extended 5 d preoviposition period on bittersweet nightshade compared to potato. Fecundity differed significantly among haplotypes, with an average lifetime fecundity of 1050, 877, and 629 eggs for Northwestern, Western, and Central females, respectively. Egg hatch was significantly reduced in psyllids reared on nightshade. Adult psyllids lived longer on nightshade than on potato. Females of the Northwestern haplotype failed to produce viable eggs when mated by males of either the Western or Central haplotypes, suggesting partial interhaplotype incompatibility. The stylet probing behaviors and Lso transmission efficiency of the three haplotypes were similar, suggesting that the psyllids probe and feed in a similar manner. However, Lso transmission rate for each of the psyllid haplotypes significantly increased with inoculation access period. The minimum inoculation time required for successful infection of potato plants with Lso was less than 10 min. Information from this research increases understanding of potato psyllid biology and will help to develop effective management strategies for zebra chip.
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>x</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>xiv</td>
</tr>
<tr>
<td>CHAPTER ONE</td>
<td></td>
</tr>
<tr>
<td>GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1. References</td>
<td>6</td>
</tr>
<tr>
<td>CHAPTER TWO</td>
<td></td>
</tr>
<tr>
<td>EFFECTS OF HOST PLANT ON DEVELOPMENT AND BODY SIZE OF THREE HAPLOTYPES OF BACTERICERA COCKERELLI (HEMIPTERA: TRIOZIDAE)</td>
<td>14</td>
</tr>
<tr>
<td>1. Abstract</td>
<td>14</td>
</tr>
<tr>
<td>2. Introduction</td>
<td>15</td>
</tr>
<tr>
<td>3. Materials and Methods</td>
<td>16</td>
</tr>
<tr>
<td>4. Results</td>
<td>20</td>
</tr>
<tr>
<td>5. Discussion</td>
<td>23</td>
</tr>
<tr>
<td>6. References</td>
<td>26</td>
</tr>
</tbody>
</table>
**CHAPTER THREE**

INTERHAPLOTYPE FERTILITY AND EFFECTS OF HOST PLANT ON REPRODUCTIVE TRAITS OF THREE HAPLOTYPES OF *BACTERICERA COCKERELLI* (HEMIPTERA: TRIOZIDAE)

1. Abstract  
   2. Introduction  
   3. Materials and Methods  
   4. Results  
   5. Discussion  
   6. References

**CHAPTER FOUR**

PROBING BEHAVIOR AND TRANSMISSION EFFICIENCY OF “*CANDIDATUS LIBERIBACTER SOLANACEARUM*” BY THREE HAPLOTYPES OF *BACTERICERA COCKERELLI* (HEMIPTERA: TRIOZIDAE)

1. Abstract  
   2. Introduction  
   3. Materials and Methods  
   4. Results  
   5. Discussion  
   6. References

**CHAPTER FIVE**

GENERAL CONCLUSIONS
LIST OF TABLES

1. Eigenvectors for the first two principal component axes 30

2. Proportion of eggs that hatched (± 95% confidence intervals) in each of nine possible intra- and interhaplotype crosses 56
CHAPTER TWO

1. **Figure 1.** Example of small rearing cage used in all assays. Each cage consists of two 5-oz clear Polystyrene 40 Dram capped plastic vials glued together top to top and containing potato or bittersweet nightshade clipping onto which fifth instar nymphs or psyllid adult pairs were placed 31

2. **Figure 2.** Measurements used for examining the effects of bittersweet nightshade and potato on size of male and female psyllids. Measurements include, distance between eyes (vertex), width of mesopraescutum, mesoscutum, 3 wing measures (2 length, 1 width), length of tibia of hind leg, antennal length 32

3. **Figure 3.** Mean (± SEM) preoviposition (A-C) and egg incubation times (D-F) for three haplotypes of potato psyllid reared on two host plant species. Panels show the host x haplotype means (A, D) and the main effect means for host species (B, E) and haplotype (C, F). Asterisks depict significant host plant effects. Capital letters above bars (C, F) show haplotypic differences in mean fecundity (by Tukey’s test) 33

4. **Figure 4.** Mean (± SEM) nymphal development (A-C) and total development times (D-F) for three haplotypes of potato psyllid reared on two host plant species; total development time is the sum of the egg incubation period (Fig. 3 D-F) and nymphal development times. Panels show the host x haplotype means (A, D) and the main effect means for host species (B, E) and haplotype (C, F). Asterisks depict significant host plant effects. Capital letters above bars (C, F) show haplotypic differences in mean fecundity (by Tukey’s test) 34
5. **Figure 5.** Scatter plots showing clustering of haplotypes along first and second principal component axes for male (upper panel) and female (lower panel) potato psyllids. Symbols along top and right axes of the plots are haplotype x host plant means: triangles indicate potato rearing host; circles indicate nightshade rearing host.

6. **Figure 1.** Example of small rearing cage used in all assays. Each cage consists of two 5-oz clear Polystyrene 40 Dram capped plastic vials glued together top to top and containing potato or bittersweet nightshade clipping onto which fifth instar nymphs or psyllid adult pairs were placed.

7. **Figure 2.** Filled and open circles show mean (± SEM) number of eggs deposited per 4-d monitoring interval and surviving female for three haplotypes of potato psyllid reared on two host plant species, from the onset of egg laying until female death. Solid lines and dashed lines show percentage (of the original 12 females per haplotype and host plant) alive. Note that the fecundity scale (left axis) changes among the three panels.

8. **Figure 3.** Mean (± SEM) lifetime fecundity (A-C) and percentage egg hatch (D-F) for three haplotypes of potato psyllid reared on two host plant species. Panels show the host x haplotype means (A, D) and the main effect means for host species (B, E) and haplotype (C, F). Asterisks depict significant host plant effects. Capital letters above bars (C) show haplotypic differences in mean fecundity (by Tukey’s test).

9. **Figure 4.** Mean (± SEM) longevity of adult female (A-C) and adult male (D-F) potato psyllids for three haplotypes of psyllid reared on two host plant species. Panels show the host x haplotype means (A, D) and the main effect means for host species (B, E) and
haplotype (C, F). Asterisks depict significant host plant effects. Capital letters above bars (F) show haplotypic differences in male longevity (by Tukey’s test)

10. **Figure 5.** Box plot showing numbers of consecutive egg-free intervals (each of 4 d in length) preceding female death. Box boundaries depict 25th and 75th percentiles, horizontal line within box shows median, error bars depict 90th and 10th percentiles, and circles show outlying points

**CHAPTER FOUR**

11. **Figure 1.** EPG waveforms produced by psyllids of the three potato psyllid (*Bactericera cockerelli*) haplotypes during the Intracellular Stylet Penetration/Pathway Phase (C) and Initial Contact with Phloem Tissue (D). The waveforms are compared to characteristic EPG waveforms identified and described by Pearson et al. (2014)

12. **Figure 2.** EPG waveforms produced by psyllids of the three potato psyllid (*Bactericera cockerelli*) haplotypes during the ingestion of xylem sap (G) variants (Gv1 and Gv2). The waveforms are compared to characteristic EPG waveforms identified and described by Pearson et al. (2014)

13. **Figure 3.** EPG waveforms produced by psyllids of the three potato psyllid (*Bactericera cockerelli*) haplotypes during the salivation into phloem sieve elements (E1), and ingestion of phloem sap (E2). The waveforms are compared to characteristic EPG waveforms identified and described by Pearson et al. (2014)

14. **Figure 4.** Average number of different stylet probing behavior events and average time spent in each probing behavior event in 24 h by individual psyllids of each of the three potato psyllid (*Bactericera cockerelli*) haplotypes
15. **Figure 5.** Average time it took individual potato psyllids (*Bactericera cockerelli*) of each of the three haplotypes to make the first Probe (C), first Phloem Salivation (E1), and first Phloem Ingestion (E2).

16. **Figure 6.** Average ‘*Candidatus Liberibacter solanacrum*’ (Lso) transmission rate among potato psyllids (*Bactericera cockerelli*) of each of three psyllid haplotypes (Central, Western, and Northwestern). The psyllids were given inoculation access period of 1, 2, 3, 4, 5, 6, 12, and 24h.

17. **Figure 7.** Minimum inoculation time required for successful inoculation of ‘*Candidatus Liberibacter solanacearum*’ (Lso) by a single potato psyllid (*Bactericera cockerelli*), estimated using two selected Lso-infected psyllids for each of the three haplotypes that had access to potato plants for 3 h or less, exhibited a single event of phloem salivation (E1), whose exposed potato plants developed zebra chip symptoms and/or tested positive for the bacterium, and had the lowest inoculation time.
DEDICATION

This dissertation is dedicated to the memory of my late brother (Faisal Mustafa), my parents, and my wife. I hope this accomplishment will complete the dream that you all had for me.
CHAPTER ONE

GENERAL INTRODUCTION

Potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is an economically important pest of solanaceous crops. This insect feeds, reproduces and completes its development on over 40 host species, including cultivated and wild Solanaceae species such as potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), tobacco (*Nicotiana tabacum* L.), nightshade (*Solanum* spp.), ground cherry (*Physalis* spp.) and matrimony vine (*Lycium* spp.) (Essig 1917; Knowlton and Thomas 1934; Pletsch 1947; Jensen 1954; Wallis 1955; Butler and Trumble 2012; Munyaneza et al. 2013). The potato psyllid has also been reported on other plant families, including Brassicaceae, Asteraceae, Lamiaceae, Poaceae, Pinaceae, Salicaceae, Polygonaceae, Chenopodiaceae, Malvaceae, Fabaceae, Amaranthaceae, Menthaceae and Convolvulaceae, but there is no indication whether these plants are true or suitable hosts for potato psyllid (Pletsch 1947; Wallis 1955; Cranshaw 1993).

Potato psyllid is believed to be native to North America and is distributed throughout western and central United States (Pletsch 1947; Wallis 1955; Capinera 2001; Butler and Trumble 2012; Munyaneza et al. 2009; Crosslin et al. 2012 a, b); Mexico, Central America (Pletsch 1947; Wallis 1955; Rubio-Covarrubias et al. 2006, Trumble 2008, 2009; Crosslin et al. 2010; Munyaneza et al. 2010; Rehman et al. 2010); Canada (Ferguson and Shipp 2002), and New Zealand (Gill 2006; Liefting et al. 2008, 2009; Teulon et al. 2009; Thomas et al. 2011). It
is reported that the potato psyllid overwinters and breeds in Western Texas, Southern New Mexico, Arizona, California and Northern Mexico (Pletch 1947; Wallis 1955; Cranshaw 1994; Capinera 2001). It has also been suggested that the potato psyllid disappears from southern breeding regions during hot summers and reappears during the autumn with prevailing cooler conditions, mostly on non-cultivated solanaceous species (Romney 1939). Thus, these movements have resulted in potato psyllid outbreaks in Southern California, Baja California, and the Pacific Northwest which led to the discovery of genetically distinct populations (or haplotypes) of the psyllid (Liu et al. 2006; Liu and Trumble 2007; Jackson et al. 2009; Swisher et al. 2012, 2013b, 2014a). Thus far, four genetically distinct haplotypes of potato psyllid have been described in the Americas (Swisher et al. 2012, 2013a, b, c; 2014a, b). Based on their perceived centers of geographic distribution, these haplotypes are referred to as “Central” (Texas and central U.S., eastern Mexico, and Central America), “Western” (southern California, Baja California, Mexico), “Northwestern” (Washington, Idaho, Oregon) and “Southwestern” (New Mexico, southern Colorado, north eastern Colorado). In addition to the discovery of these haplotypes and contrary to previous beliefs, it is now known that potato psyllid overwinters in the Pacific Northwest on bittersweet nightshade (Solanum dulcamara) (Jensen et al. 2012, Murphy et al. 2013, Swisher et al. 2013c). However, little is known concerning the overwintering biology of this psyllid in this major potato growing region of the United States (Horton et al. 2014).

Like many other members of the Psylloidea, the potato psyllid is monomorphic and reproduces sexually with an oviparous life cycle (Hodkinson 1974). Being hemimetabolous, the potato psyllid life cycle consists of an egg, nymphal instars, and adult stages. The females of
reaches reproductive maturity within 24 h after eclosion and males become reproductively mature 1 day after eclosion (Guédot et al. 2012). After successful mating, dark yellows to orange colored stalked eggs are deposited singly, preferably along the leaf edges and eclose within 3-7 days (Pletsch 1947; Wallis 1955; Abdullah 2008; Yang and Liu 2009; Yang et al. 2010; Butler and Trumble 2012; Munyaneza 2012). The nymphs are dorso-ventrally flattened, and scale-like in appearance. Nymphs develop through five instars (all of which are similar in appearance other than size), and are primarily found on the lower surface of leaves where they typically remain sedentary throughout their developmental period. Total nymphal development time has been reported to be between 12 and 24 days, and the adult life span ranges from 16-97 days depending upon temperature and host plant (Knowlton and Janes 1931; Pletsch 1947; Wallis 1955; Liu and Trumble 2007; Abdullah 2008; Yang and Liu 2009; Yang et al. 2010). Morphologically, the potato psyllid adult is characterized by its whitish dorsal bands, with a broad transverse white band on the first abdominal segment and an inverted V-shaped white mark on the last abdominal segment (Pletsch 1947; Wallis 1955). On average, potato psyllid fecundity ranges from 300-500 eggs per female over her lifetime (Knowlton and Janes 1931; Pletsch 1947; Abdullah 2008; Yang and Liu 2009; Yang et al. 2010). The females usually live two to three times longer than males, depending on host plant (Pletsch 1947; Abdullah 2008; Yang and Liu 2009; Yang et al. 2010). High fecundity and a relatively long lifespan allow potato psyllids to reach economically injurious levels in a relatively short period of time (Munyaneza 2012).

Historically, the potato psyllid has been associated with psyllids yellows disease of potato and tomato. It is thought that the psyllid yellows disease is associated with the feeding of potato psyllid nymphs, which inject toxins into the plant (List 1925; Carter 1939; Wallis 1955; Arslan et
Psyllid yellows are characterized by the yellowing or/and purpling of potato leaves and shoots, shortened and thickened internodes, and formation of aerial tubers (Richards 1929, 1931; Eyer and Crawford 1933; Richards and Blood 1933; Eyer 1937; Wallis 1955; Arslan et al. 1985; Cranshaw 1994, 2001; Sengoda et al. 2010). Substantial yield losses due to the production of numerous small and unmarketable tubers have been reported (Schall 1938). In the early 2000s, the potato psyllid first became a major problem when zebra chip, a new disease of potato was reported in Mexico and Texas (Secor et al. 2004). Zebra chip is caused by the new bacterium “Candidatus Liberibacter solanacearum” (also known as “Ca. L. psyllaurous), which is vectored by potato psyllid (Hansen et al. 2008, Liefting et al. 2009; Munyaneza et al. 2007; Munyaneza 2012). This bacterium is phloem limited and belongs to order Rhizobiales of the α2-subdivision of the Gram-negative Proteobacteria (Lin et al. 2009; Liefting et al. 2009; Thompson et al. 2013). Above-ground symptoms of zebra chip are similar to those of psyllid yellows and include retarded growth, erectness of new foliage, chlorosis and purpling of foliage with basal cupping and upward rolling of leaves, shortened and thickened internodes, enlarged nodes, and aerial tubers. (Munyaneza et al. 2007a, b; Secor et al. 2009; Munyaneza 2012). Unlike psyllid yellows, the below-ground symptoms of zebra chip include collapsed stolon’s, browning of vascular tissue and streaking of the medullary ray tissues. Upon frying, these symptoms become more prominent and chips and fries processed from affected potato tubers show dark blotches and stripes, making the tubers unmarketable (Munyaneza et al. 2007a, b, 2008; Secor et al. 2009; Crosslin et al. 2010; Miles et al. 2010; Munyaneza 2012; Munyaneza and Henne, 2012).
Currently, the only means to effectively manage zebra chip is by targeting potato psyllid for control (Munyaneza 2012). The presence and overwintering of different haplotypes of potato psyllid in Pacific Northwest may complicate management if differences in biological traits that determine their respective contributions to zebra chip epidemiology exist. These traits may include development, reproduction, and vectoring efficiency. Thus, the objectives of the present dissertation research are to: 1) compare developmental traits (preoviposition period, egg incubation, nymphal and total development) and reproductive performance among the three potato psyllid haplotypes (Central, Western, and Northwestern) predominantly found in the Pacific Northwest on bittersweet nightshade and potato, and 2) determine feeding behavior and liberibacter vectoring efficiency by the three haplotypes. The results of this research will increase our understanding of potato psyllid haplotypes biology as it relates to zebra chip epidemiology and spread, leading to development of effective management strategies for this important potato disease.

The dissertation is organize into five chapters: 1) General introduction, 2) manuscript #1 “Effects of host plant on development and body size of three haplotypes of Bactericera cockerelli (Hemiptera: Triozidae)”, 3) manuscript #2 “Interhaplotype fertility and effects of host plant on reproductive traits of three haplotypes of Bactericera cockerelli (Hemiptera: Triozidae)”, 4) manuscript #3 “Probing behavior and transmission efficiency of ‘Candidatus Liberibacter solanacearum’ by three haplotypes of Bactericera cockerelli (Hemiptera: Triozidae)”, and 5) General conclusions.
REFERENCES


Horton, D. R., E. Miliczky, J. E. Munyaneza, K. D. Swisher, and A. S. Jensen. 2014. Absence of


Munyaneza, J. E., J. L. Buchman, J. E. Upton, J. A. Goolsby, J. M. Crosslin, G. Bester, G. P. 
Miles, and V. G. Sengoda. 2008. Impact of different potato psyllid populations on zebra 

Journal for Potato Research 86, 513–518.

Southwest. Ent. 35: 471-477.


Munyaneza, J. E., D. C. Henne. 2012. Leafhopper and psyllid pests of potato. In: Giordanengo, 
P., Vincent, P., Alyokhin, A., editors. Insect Pests of Potato: Global Perspectives on 


Murphy, A. F., S. I. Rondon, and A. S. Jensen. 2013. First report of potato psyllid, Bactericera 

Pletsch, D. J. 1947. The potato psyllid Paratrioza cockerelli (Šulc) its biology and control. 

with severe foliar chlorosis, curling, and necrosis and tuber discoloration of potato plants in Honduras. Plant Disease 94: 376.


Richards, B. L. 1931. Further studies with psyllid yellows of the potato. Phytopathology 21: 103.


CHAPTER TWO

EFFECTS OF HOST PLANT ON DEVELOPMENT AND BODY SIZE OF THREE HAPLOTYPES OF BACTERICERA COCKERELLI (HEMIPTERA: TRIOZIDAE) (JOURNAL: ENVIRONMENTAL ENTOMOLOGY)

ABSTRACT

Potato psyllid, Bactericera cockerelli (Šulc) (Hemiptera: Triozidae), is an economic pest of solanaceous crops in North and Central America, and (as an introduction) in New Zealand. Four genetic haplotypes of the psyllid have been identified in North America. Three of these haplotypes (Central, Western, and Northwestern) are common on potato crops within the major potato growing regions of Idaho, Oregon, and Washington. Within this growing region, a weedy perennial nightshade, Solanum dulcamara (bittersweet nightshade), has been identified to be an important overwintering host and spring or summer source of psyllids colonizing potato fields. It is unclear whether bittersweet nightshade is a highly suitable host plant for all three haplotypes known to occur in the Pacific Northwest. The objective of the present study was to examine developmental traits and adult body size of all three haplotypes of psyllids reared on potato and bittersweet nightshade. Averaged over haplotype, development times were longer for psyllids reared on nightshade than potato. Duration of the pre-oviposition period, egg incubation requirements, nymphal development time, and total developmental time averaged 7.4, 5.9, 23.5, and 29.5 d on nightshade and 4.9, 5.5, 22.3, and 27.9 d on potato, respectively. The largest host effects were found for the Central haplotype, which exhibited a substantially extended (by over 5
d) preoviposition period on nightshade compared to potato. Averaged over host plant, nymphal and total development times of the Northwestern haplotype were longer (25.5 and 31.1 d, respectively) than those of the Western and Central haplotypes. The Northwestern haplotype was largest in overall body size, while the Central haplotype had the smallest overall body size, irrespective of host plant. Both sexes exhibited this trend.

**INTRODUCTION**

Potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is an economically important phloem-feeding insect that damages solanaceous crops, including potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), and tobacco (*Nicotiana tabacum* L.) (Knowlton and Thomas 1934, Pletsch 1947, Wallis 1955, Munyaneza 2010, 2012; Munyaneza et al. 2013). The psyllid causes damage to solanaceous species by its direct feeding (Pletsch 1947, Wallis 1955) and by its vectoring of the bacterium “*Candidatus Liberibacter solanacearum*”, the putative causal agent of zebra chip disease of potato (Munyaneza et al. 2007, Munyaneza 2012). Zebra chip has caused millions of dollars in losses to the potato industry in the United States, Mexico, Central America, and New Zealand (Munyaneza et al. 2007, Munyaneza 2012). Based on an analysis of the mitochondrial COI gene, four distinct genetic populations (or haplotypes) of the psyllid have been identified in North America (Swisher et al. 2012; 2013a, b, c; 2014a, b). The haplotypes are referred to as Central, Western, Northwestern, and Southwestern, depending on their perceived centers of geographic distribution. All four haplotypes have been reported on potatoes in the Pacific Northwest, but the Southwestern haplotype is less common in the region than the other
three haplotypes (Swisher et al. 2014b). Potato psyllid is also common on bittersweet nightshade 
(Solanum dulcamara), a perennial weed that was recently recognized to be an important 
overwintering source of this insect in the Pacific Northwest (Jensen et al. 2012, Murphy et al. 
2013). To date, only the Northwestern and Western haplotypes have been identified on 
bittersweet nightshade in the region (Swisher et al. 2013c).

Presence of different haplotypes of potato psyllid in the Pacific Northwest may 
complicate management of the psyllid if there are differences in biological traits among 
haplotypes. Variation among haplotypes in biological traits may determine the respective 
contributions of the different haplotypes to zebra chip epidemiology in commercial potato fields, 
complicating the development of effective management strategies for the disease and its insect 
vector. The objectives of this study were: (1) to compare bittersweet nightshade and potato as 
developmental hosts for the three psyllid haplotypes commonly found in Pacific Northwest 
potato crops, and (2) to assess effects of rearing host on body size of adults of all three psyllid 
haplotypes.

MATERIALS AND METHODS

Sources of Plants

Effects of host plant (potato and bittersweet nightshade) on developmental traits 
(preoviposition interval, egg incubation time, nymphal development time, and total [egg to adult 
] development time) of B. cockerelli were compared under laboratory conditions. Bittersweet 
nightshade was grown from insect-free plant cuttings collected locally near Wapato, WA. Potato 
(var. Russet Burbank) was grown from seed tubers (Skone and Connors Produce Inc., Warden,
WA). The plants were grown in 0.5-L pots (Kord Products, Toronto, Ontario, Canada) filled with a soil medium consisting of 86% sand, 13.4% peat moss, 0.5% Apex time release fertilizer (J. R. Simplot Co., Lathrop, CA), and 0.1% Micromax micronutrients (Scotts Co., Marysville, OH) in a greenhouse at USDA-ARS, Wapato, WA. Prior to conducting assays, the plants were tested for ‘Ca. L. solanacearum’ by PCR (Munyaneza et al. 2010) to ensure they were free of the bacterium.

**Sources of Insects**

Potato psyllid colonies were established at the USDA-ARS facility in Wapato, WA with insects collected from Weslaco, Texas (Central haplotype), southern California (Western haplotype), and Prosser, Washington (Northwestern haplotype). The colonies were maintained on either bittersweet nightshade or potato under laboratory conditions of 25±1 °C, 40±5% RH, with a photoperiod of 16:8 (L: D) h. Haplotype status of each of each colony was confirmed at intervals by examination of mitochondrial DNA (mtDNA) sequences, using high resolution melting analysis methods (Swisher et al. 2012, Swisher et al. 2014a). To confirm that the colonies were free of the Liberibacter pathogen, samples of psyllids from each colony were tested by conventional PCR (Munyaneza et al. 2010) prior to beginning an assay.

**Description of Insect Rearing Cages Used in Assays**

Developmental traits of psyllids from each of the three haplotypes were assessed by rearing individual insects in small transparent insect rearing cages as described by Mustafa et al. (2013). The cages were constructed using two 5-oz clear Polystyrene 40 Dram capped plastic vials (Thornton Plastics, Salt Lake City, UT), joined together by gluing the tops of the two lids to one another such that the two plastic vials were assembled top to top (Fig. 1). The top and two
sides of each upper vial were covered with mesh to allow ventilation (Fig. 1). A 10-mm diameter hole was drilled through the center of the two joined lids. Clippings from pathogen-free bittersweet nightshade or potato were added to each cage, with cut stem ends inserted through the small hole and into the water-filled reservoir (Fig. 1). Each clipping included two or three leaves. The clippings were held in place with a piece of cotton swab placed in the hole joining upper and lower vials.

**Pre-oviposition and Incubation Period and Nymphal Development**

The pre-oviposition period, incubation period, total nymphal developmental time, and total development time (egg incubation + nymphal development) were examined for psyllids of each haplotype on either bittersweet nightshade or potato. Psyllid developmental parameters were measured and monitored in the transparent insect rearing cages described above (Fig. 1) under conditions of 25±1 °C, 40±5% RH, and a photoperiod of 16:8 (L: D) h.

To obtain virgin adults for the assays, fifth instar nymphs of each haplotype were removed from the nightshade or potato-reared psyllid colonies. Nymphs were placed individually on clippings of the same host species from which they had been obtained, and held individually in the standard rearing cages (Fig. 1) until adult emergence. Newly-emerged adults were sexed, and male/female pairs of new adults were transferred to new cages containing a new clipping of the rearing host species until first appearance of eggs. The adult pairs were then allowed to lay eggs for an additional 24 h, after which the parental insects were collected and destroyed. The total time from the date of adult emergence to the date of the first egg laying was recorded to estimate the preoviposition period. The incubation period was determined as the time from the onset of egg laying to initial egg hatch. Newly hatched first instar nymphs were then carefully
transferred individually into rearing cages (Fig. 1) and monitored until eclosion. Total nymphal developmental time (hatch to adult eclosion) was recorded. A total of 40 individual nymphs, collected from 20 females, were monitored for each haplotype on each host plant.

**Body Size Measurements**

All newly-emerged psyllid adults were collected on the first day of their emergence, sexed, and preserved at -80°C, pending body measurements to estimate their size. A total of eight measurements were obtained from each individual (Fig. 2): distance between eyes (vertex), width of mesopraescutum and mesoscutum, wing length (2 measures), maximum width of wing, length of tibia of a hind leg, and length of antennae. To obtain these measurement’s, the adult psyllid was placed in a petri dish filled with alcohol and a layer of fine sand on the dish bottom to position the insect for measurement of the vertex, mesopraescutum, and mesoscutum. Once those three measurements had been made, the right antennae, right forewing, and right hind leg were carefully removed using Dumont # 5 11cm tweezer (World Precision Instruments, Sarasota, FL) and placed in 2-3 drops of alcohol on a microscope slide. The structures were oriented, covered with a cover slip to flatten them, and measured. All measurements were made with a Leica MZ6 dissecting scope (Leica Microsystems Inc., Buffalo Grove, IL) equipped with an ocular micrometer at 37.5x.

**Data Analysis**

Effects of haplotype and host species on developmental traits, pre-oviposition period, incubation period, total nymphal developmental time, and total life cycle, were analyzed using a $3 \times 2$ factorial analysis of variance (ANOVA). The analyses were done using the MIXED procedure in SAS (SAS Institute 2012). If the haplotype × host effect was significant, host
effects were examined separately for each haplotype using the SLICE command. If the haplotype effect in the ANOVA was significant, haplotype means were compared using Tukey’s tests. Principal components analysis (PCA) was used to examine whether the eight body measurements separated specimens by haplotype and rearing host. The analyses were done in PROC PRINCOMP (SAS Institute 2012). Statistical separation of haplotypes or host was done using a 3 × 2 (haplotype × host) factorial analysis of variance conducted on the scores from the first and second principal components. The analyses were done using PROC MIXED (SAS Institute 2012).

RESULTS

Development time

Summary statistics are presented as haplotype × host plant means as well as host and haplotype main effect means (Fig. 3-4). The host × haplotype interaction was significant for duration of the pre-oviposition period (Fig. 3A: $F_{2, 234} = 8.25, P= 0.0003$), suggesting that magnitude or direction of host effects depended upon haplotype. Only the Central haplotype showed significant host effects (Fig. 3A); the preoviposition period was significantly longer (by over 5 d) for psyllids reared on nightshade than potato. Averaged over host plant, the pre-oviposition period was affected by haplotype (Fig. 3C; $F_{2, 234} = 24.05, P < 0.0001$). The period was longest in the Central haplotype (Fig. 3C), due to the extended length of time for psyllids reared on nightshade (Fig. 3A). Duration of the egg incubation period was affected by host plant (Fig. 3E: $F_{1, 234} = 14.18, P= 0.0002$) and haplotype (Fig. 3F: $F_{2, 234} = 6.11, P= 0.0026$), but not by the host × haplotype interaction (Fig. 3D: $F_{2, 234} = 0.95, P = 0.39$). Incubation times were longest for the Western haplotype, whereas the Northwestern and Central haplotype showed similar
trend in incubation duration. Averaged over haplotype, incubation times were slightly longer for eggs reared on nightshade (5.9 d) than potato (5.6 d) (Fig. 3E).

Total nymphal development times were affected by host plant, with development times slightly slower on nightshade than potato (Fig. 4B; $F_{1, 234} = 4.47$, $P = 0.03$); a similar trend was noted for overall development times (Fig. 4E; $F_{1, 234} = 6.86$, $P = 0.009$). Haplotype effects were also significant for both traits (Fig. 4C and F; nymphal development times: $F_{2, 234} = 23.26$, $P < 0.0001$; total development times: $F_{2, 234} = 19.51$, $P < 0.0001$). The Northwestern haplotype required longer time to develop (averaged over host plant) than either the Western or Central haplotypes (Fig. 4C and F). The haplotype × host interaction was not significant for either measure ($P > 0.5$ for both traits).

**Body measurements**

Two principal components were extracted explaining 62% (axis 1) and 14% (axis 2) of the variation in male measurements, and 64% (axis 1) and 12% (axis 2) of the variation in female measurements (Fig. 5). For both sexes, all variables showed positive loadings along the first principal component (Table 1), suggesting that this component reflects variation in overall body size. The second component for both sexes appeared to reflect a contrast between breadth of the body (mesopraescutum, mesoscutum) and length of the antennae and tibia (Table 1). Large, positive values along axis 2 reflect scores of specimens having a relatively wide body but short antennae and tibia (Figure 5). The scatter plot of component scores showed that psyllids clustered by haplotype, albeit with overlap among clusters (Figure 5; host plants pooled for clarity). Mean haplotype × host plant scores are shown as circles (nightshade host) or inverted
triangles (potato host) horizontally along the top axis (PC1 means) or vertically along the right axis (PC2 means) of the scatter plots (Figure 5).

Analysis of variance showed that mean component scores for both male and female psyllids varied with haplotype along the first axis (means shown on horizontal axis at top of both graphs in Figure 5; males: \( F_{2,105} = 50.2, P < 0.0001 \); females: \( F_{2,121} = 58.5, P < 0.0001 \)). Overall body size (=PC1) was largest in psyllids of the Northwestern haplotype (red symbols) and smallest for psyllids of the Central haplotype (gray symbols). Mean scores also varied significantly with haplotype on the second axis for both sexes (means shown on the vertical axis at right of both graphs in Figure 5; males: \( F_{2,105} = 26.1, P < 0.0001 \); females: \( F_{2,121} = 32.7, P < 0.0001 \)). The mean scores suggest that psyllids of the Northwestern haplotype were narrower but had relatively longer antennae and tibiae than psyllids of the Western haplotype.

The effects of host plant on body size were less clear than haplotype effects. Host plant affected PC2 scores for both male and female psyllids (males: \( F_{2,105} = 8.0, P = 0.006 \); females: \( F_{2,121} = 7.8, P = 0.006 \)). For male psyllids, an examination of mean scores (along vertical axis) indicated that psyllids that were reared on nightshade (circles) had higher mean scores than psyllids that had been reared on potatoes (triangles), suggesting that nightshade had a positive effect on body width and a negative effect on tibial/antennal lengths. For female psyllids, the host effect actually depended upon haplotype (host x haplotype: \( F_{2,121} = 7.3, P = 0.001 \)). Extraction of simple effects contrasts indicated that host effects were significant for females of the Western and Northwestern haplotypes (\( P < 0.01 \) for both contrasts), but not for females of the Central haplotype (\( P = 0.14 \)). The patterns in mean PC2 scores for the Western and Northwestern females were similar to the host effects shown by male psyllids (i.e., nightshade was associated
with increases in body width and decreases in antennal/tibial lengths). The host x haplotype interaction was also significant for overall body size (PC1) of females ($F_{2,121} = 6.8, P = 0.002$). An examination of mean scores (horizontal axis in Figure 5) and extraction of simple effects contrasts indicated that nightshade had a negative effect on overall body size of Central females, a positive effect on body size of Western females, and no effect on Northwestern females.

**DISCUSSION**

More than 50% of U.S. potatoes are produced in the Pacific Northwest, where zebra chip was first observed in 2011 (Crosslin et al. 2012a,b). Currently, the only means to effectively manage zebra chip is by targeting the potato psyllid, its insect vector, primarily with insecticide applications (Munyaneza 2012). All four haplotypes of potato psyllid have been documented in the Pacific Northwest (Swisher et al. 2012; 2013a, b, c; 2014a,b) and it has been determined that the insect survives winter in the region on bittersweet nightshade (Jensen et al. 2012, Murphy et al. 2013). Little is known on the comparative biology of the different psyllid haplotypes, which could hamper development of effective management strategies for this insect pest in this important potato growing region of the U.S. For example, variation in developmental traits among haplotypes could affect how rapidly psyllid populations of a given haplotype develop in the field and contribute to spread of zebra chip.

Effects of host plant on developmental traits of psyllids from the three haplotypes most common in the potato growing region of the Pacific Northwest (Swisher et al. 2014b) were examined during this study. Results showed that host plant affected the preoviposition period, egg incubation times, and nymphal development times. For all traits, development was slower on
bittersweet nightshade than potato. The largest host effect was for the preoviposition period of the Central haplotype reared on nightshade (Fig. 3A). Central haplotype psyllids on nightshade had substantially lengthened preoviposition periods, in some pairs reaching over 30 d. These results may indicate that bittersweet nightshade is not as suitable for egg laying as potato for the Central haplotype. Egg incubation times and nymphal development times were slightly longer for psyllids reared on nightshade than potato, for all three haplotypes. The egg incubation times of 5.7-6.0 d (depending upon haplotype) fell within the range (3-15 d) shown in previous studies on a variety of solanaceous hosts and at several temperatures (Compere 1916, Knowlton and Janes 1931, Davis 1937, Abdullah 2008, Yang and Liu 2009, Yang et al 2010, Yang et al. 2013). Potato psyllid host species in the families Solanaceae and Convolvulaceae have also been shown to affect nymphal or total development times of this insect in other studies (Liu and Trumble 2007; Yang and Liu 2009; Puketapu and Roskruge 2011). The results of the present study contribute to previous reports on host plant effect on potato psyllid development.

Variation in body size measurements among haplotypes confirmed results of a similar analysis published elsewhere (Horton et al. 2014). That study explored the effects of photoperiod on body size of female potato psyllids of all three haplotypes, and found that psyllids of the Northwestern haplotype were larger in overall body size than psyllids of either the Central or Western haplotypes, but were narrower than psyllids of those two haplotypes (as found also in this study; Fig. 5). These results suggest that it might be possible to easily identify haplotypes from external traits, rather than having to rely on molecular methods that may be more costly, labor intensive, or not readily accessible. In the earlier study by Horton et al. (2014), psyllids were reared only on potato, and it was unclear whether haplotype differences in body size would
be maintained on other host plants. The present study indicated that rearing host affected body measurements of psyllids, but that the host effects were less evident and less consistent than the variation in body size associated with haplotypes. Vargas-Madríz et al. (2013) showed that morphometrics of both adult and nymphal potato psyllid were affected by cultivar of its tomato rearing host.

In sum, results of this study indicated that there were significant differences in developmental traits among the Central, Northwestern, and Western haplotypes of potato psyllid. The preoviposition period, egg incubation, nymphal and total developmental times of potato psyllid were affected by whether the insects were reared on potato or bittersweet nightshade. The psyllid required a slightly longer egg and nymphal developmental period on bittersweet nightshade compared to potato, and (for the Central haplotype) exhibited a substantially delayed onset of egg laying by newly eclosed pairs when reared on nightshade than on potato. Collectively, the results of this study indicated that there were significant differences in developmental traits among three psyllid haplotypes. These differences could lead to differences among haplotypes under field conditions in population development, possibly requiring haplotype-specific strategies to optimize management of potato psyllid and zebra chip in the potato growing regions of the Pacific Northwest.
REFERENCES


(http://zebrachipscri.tamu.edu/files/2013/04/2012-Proceedings.pdf)


Pletsch, D. J. 1947. The potato psyllid Paratrioza cockerelli (Šulc) its biology and control.


Table 1. Eigenvectors for the first two principal component axes.

<table>
<thead>
<tr>
<th>Body measure</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Axis 1</td>
<td>Axis 2</td>
</tr>
<tr>
<td>Vertex</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Mesopraescutum</td>
<td>0.27</td>
<td>0.46</td>
</tr>
<tr>
<td>Mesoscutum</td>
<td>0.31</td>
<td>0.51</td>
</tr>
<tr>
<td>Wing measure A</td>
<td>0.42</td>
<td>-0.15</td>
</tr>
<tr>
<td>Wing measure B</td>
<td>0.41</td>
<td>-0.16</td>
</tr>
<tr>
<td>Wing measure C</td>
<td>0.38</td>
<td>0.11</td>
</tr>
<tr>
<td>Tibia length</td>
<td>0.38</td>
<td>-0.30</td>
</tr>
<tr>
<td>Antennal length</td>
<td>0.32</td>
<td>-0.53</td>
</tr>
</tbody>
</table>
**Figure 1.** Example of small rearing cage used in all assays. Each cage consists of two 5-oz clear Polystyrene 40 Dram capped plastic vials glued together top to top and containing potato or bittersweet nightshade clipping onto which fifth instar nymphs or psyllid adult pairs were placed.
Figure 2. Measurements used for examining the effects of bittersweet nightshade and potato on size of male and female psyllids. Measurements include, distance between eyes (vertex), width of mesopraescutum, mesoscutum, 3 wing measures (2 lengths, 1 width), length of tibia of hind leg, antennal length.
Figure 3. Mean (± SEM) preoviposition (A-C) and egg incubation times (D-F) for three haplotypes of potato psyllid reared on two host plant species. Panels show the host x haplotype means (A, D) and the main effect means for host species (B, E) and haplotype (C, F). Asterisks depict significant host plant effects. Capital letters above bars (C, F) show haplotypic differences in mean fecundity (by Tukey’s test).
Figure 4. Mean (± SEM) nymphal development (A-C) and total development times (D-F) for three haplotypes of potato psyllid reared on two host plant species; total development time is the sum of the egg incubation period (Fig. 3 D-F) and nymphal development times. Panels show the host x haplotype means (A, D) and the main effect means for host species (B, E) and haplotype (C, F). Asterisks depict significant host plant effects. Capital letters above bars (C, F) show haplotypic differences in mean fecundity (by Tukey’s test).
Figure 5. Scatter plots showing clustering of haplotypes along first and second principal component axes for male (upper panel) and female (lower panel) potato psyllids. Symbols along top and right axes of the plots are haplotype x host plant means: triangles indicate potato rearing host; circles indicate nightshade rearing host.
CHAPTER THREE

INTERHAPLOTYPE FERTILITY AND EFFECTS OF HOST PLANT ON REPRODUCTIVE TRAITS OF THREE HAPLOTYPES OF BACTERICERA COCKERELLI (HEMIPTERA: TRIOZIDAE)

(JOURNAL: ENVIRONMENTAL ENTOMOLOGY)

ABSTRACT

Potato psyllid, Bactericera cockerelli (Šulc) (Hemiptera: Triozidae), is a serious pest of solanaceous crops in North and Central America and New Zealand. This insect vectors the bacterium that causes zebra chip disease of potato (Solanum tuberosum L.). Four distinct genetic populations, or haplotypes, of B. cockerelli have been identified. Three of the haplotypes may co-occur in potato fields in the Pacific Northwest of U.S. Solanaceous weeds, including the perennial Solanum dulcamara (bittersweet nightshade), may provide refuge for psyllid populations which then migrate to potato crops. This study tested whether fecundity, fertility (% egg hatch), and adult longevity of potato psyllid were affected by host plant (S. dulcamara or potato) and whether these reproductive traits were similar among the three haplotypes that are most common in the Pacific Northwest: Northwestern, Central, and Western. We hypothesized that the locally resident haplotype (Northwestern), which is known to overwinter extensively on S. dulcamara, would show relatively higher fitness on nightshade than the other two haplotypes. Fecundity differed significantly among haplotypes, with an average lifetime fecundity of 1050, 877, and 629 eggs for Northwestern, Western, and Central females, respectively. Egg hatch was
significantly reduced in psyllids reared on bittersweet nightshade (61.9%) versus potato (81.3%). Adult psyllids lived longer on nightshade than on potato, averaging 113.9 and 108.4 d on nightshade and 79.0 and 85.5 d on potato for males and females, respectively. However, the longer life span of psyllids on nightshade than potato failed to lead to higher fecundity, because females on nightshade often ended egg laying well before death, unlike those on potato. There was no evidence for any of the fitness traits to suggest that the locally resident haplotype (Northwestern) performed relatively better on nightshade than the other two haplotypes. Lastly, we examined whether mating between psyllids of different haplotypes affected sperm transfer and egg hatch rates. Females of the Northwestern haplotype failed to produce viable eggs when mated by males of either the Western or Central haplotypes.

INTRODUCTION

Potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a phloem feeding insect with a wide host range (Knowlton and Thomas 1934, Pletsch 1947, Wallis 1955). The psyllid is economically detrimental to potato (*Solanum tuberosum* L.) and other crops in the Solanaceae. Damage is caused directly by its feeding activities, and by its vectoring of the bacterium “*Candidatus Liberibacter solanacearum*”, the putative causal agent of zebra chip disease of potato (Munyaneza et al. 2007, Munyaneza 2012). Potato psyllid is distributed in the United States, Mexico, and Central America (Pletsch 1947, Wallis 1955, Munyaneza 2012). This insect pest is also widespread in New Zealand, where it was recently introduced (Gill 2006, Teulon et al. 2009, Thomas et al. 2011). In recent years, potato psyllid outbreaks in southern California, Baja California in Mexico, the Pacific Northwest of the United States, and Central
America led to the discovery of genetically distinct populations (or haplotypes) of the psyllid (Liu et al. 2006, Liu and Trumble 2007, Jackson et al 2009; Swisher et al. 2012, 2013b, 2014a). Four haplotypes of the psyllid have been described in the Americas (Swisher et al. 2012, 2013a,b,c; 2014a,b). Depending on the perceived centers of their geographic distributions, these haplotypes are referred to as “Central” (Texas and central U.S., eastern Mexico, and Central America), “Western” (southern California, Baja California, Mexico), “Northwestern” (Washington, Idaho, Oregon) and “Southwestern” (New Mexico, southern Colorado, north eastern Colorado). All four haplotypes have been reported in the Pacific Northwest (Swisher et al. 2012, 2013a,c; 2014b), although only the Northwestern haplotype actually appears to be resident in the region (Swisher et al. 2013c, 2014b). Three of the haplotypes (Central, Western, and Northwestern) may regularly occur as mixtures in potato fields within certain growing regions of the Pacific Northwest (Swisher et al. 2014b).

Currently, the only means to effectively manage zebra chip is by targeting potato psyllid for control (Munyaneza 2012). Potato psyllid management in Pacific Northwest potato crops is made more difficult due to the presence of these different psyllid haplotypes, which may differ in biological traits that determine their respective contributions to zebra chip epidemiology. These traits could include haplotype differences in vectoring efficiencies, interhaplotype fertility, fitness traits, host plant use, and overwintering capabilities. The perennial bittersweet nightshade (Solanum dulcamara L.) has now been recognized to be a critical resource for potato psyllid overwintering in the Pacific Northwest (Jensen et al. 2012, Murphy et al. 2013), particularly for the Northwestern haplotype (Swisher et al. 2013c). Bittersweet nightshade is also a host of “Ca. L. solanacearum” (Murphy et al. 2014, Munyaneza, unpublished). Advances in understanding
potato psyllid haplotype biology and in defining suitability of bittersweet nightshade for potato psyllids of each haplotype will help the potato industry in the Pacific Northwest make informed decisions about the need for psyllid controls as components of their pest management programs. The objectives of the current study were to: (1) evaluate host effects of bittersweet nightshade and potato on reproductive traits of three potato psyllid haplotypes commonly found in Pacific Northwest potato crops, and examine whether there is evidence for local adaptation to this host plant by the locally resident haplotype (Northwestern); and, (2) assess interhaplotype fertility among haplotypes.

MATERIALS AND METHODS

Sources of Plants

Potato and bittersweet nightshade were compared for effects on fitness traits of B. cockerelli. Potato (var. Russet Burbank) was grown from seed tubers (Skone and Connors Produce Inc., Warden, WA), and bittersweet nightshade was grown from cuttings collected locally (Wapato, WA). Plants were grown in 0.5-L pots (Kord Products, Toronto, Ontario, Canada) filled with a soil medium consisting of 86% sand, 13.4% peat moss, 0.5% Apex time release fertilizer (J. R. Simplot Co., Lathrop, CA), and 0.1% Micromax micronutrients (Scotts Co., Marysville, OH) in a greenhouse at USDA-ARS, Wapato, WA. Prior to conducting experiments, the plants were tested for “Ca. L. solanacearum” by PCR (Munyaneza et al. 2010) to ensure they were free of the bacterium.

Sources of Insects

Colonies of three potato psyllid haplotypes were established at the USDA-ARS facility in Wapato, WA from insects collected in Texas (Central haplotype), southern California (Western
haplotype), and Prosser, WA (Northwestern haplotype). The insects were maintained on bittersweet nightshade or potato in separate colonies. All of the colonies were maintained in the laboratory at 25 °C ± 1, 40±5% RH, with a photoperiod of 16:8 (L: D) h. Haplotype status of each of the psyllid colonies was confirmed at desired intervals by examination of mitrocondrial DNA (mtDNA) sequences, using high resolution melting analysis methods (Swisher et al. 2012, 2013a, 2014a). In addition, psyllid adults used in the experiments were collected at the end of each trial and tested to confirm the haplotype status.

**Construction of Cages Used in Assays**

Reproductive traits of psyllids from each haplotype were assessed by rearing individual insects in small transparent cages constructed of two 5-oz clear Polystyrene 40 Dram capped plastic vials (Thornton Plastics, Salt Lake City, UT), joined together by gluing the tops of the two lids to one another such that the two plastic vials were assembled top to top (Fig. 1). The upper vial included mesh-covered openings on two sides and on the top for ventilation (Fig. 1). A 10-mm diameter hole was drilled through the center of the two joined lids. Clippings from pathogen-free potato or bittersweet nightshade were added to each cage, with cut stem ends inserted through the small hole and into the water-filled reservoir (Fig. 1). Each clipping included two or three leaves. The clippings were held in place with a piece of cotton swab placed in the hole joining upper and lower vials.

**Fecundity, Egg Fertility, and Adult Longevity**

Fecundity, egg fertility, and adult longevity were examined for psyllids of each haplotype on either bittersweet nightshade or potato. Psyllid fitness measures were monitored in the transparent insect cages (Fig. 1) under conditions of 25 °C ± 1, 40±5% RH, and a photoperiod of
16:8 (L: D) h. To confirm absence of the Liberibacter pathogen in psyllids, samples of potato psyllids from each colony were tested by conventional PCR (Munyaneza et al. 2010) prior to beginning an assay.

To obtain virgin adults for the fitness assays, fifth instar nymphs of each haplotype were gently removed from the nightshade-reared and potato-reared colonies of psyllids with a soft painting brush. The nymphs were placed individually on clippings of the same host species from which they had been reared, and held individually in the standard rearing cages (Fig. 1) until adult emergence. Newly-emerged adults were sexed, and male/female pairs of new adults were transferred to new cages containing a new clipping of the rearing host species. Extra males of the same age not used in the initial pairings were kept as backup for replacing any males that died before the female of the pair died (see below). Lifetime fecundity and egg fertility were monitored for a total of 12 pairs of psyllids per haplotype and host plant species (producing a total of 3 haplotypes x 2 host species x 12 pairs = 72 pairs of psyllids). Numbers of eggs deposited per female were recorded under a binocular stereomicroscope (EMZ 13; Meija Techno America, San Jose, CA) every four days, starting with the first oviposition, so the count is done before the eggs have had a chance to hatch. Following each egg count, the psyllid pair was transferred to a new cage with new clippings of the appropriate host species. The process was repeated for each pair until the female had died. The total number of eggs per female over her lifetime was calculated by summing the individual 4-day counts. If the male psyllid in a given cage died before the female, the male was replaced with a new insect of the same age and haplotype, reared from the same host species. Eggs were monitored for hatch using a binocular stereomicroscope (EMZ 13; Meija Techno America, San Jose, CA) and nymphal emergence was
recorded. Lifetime percent egg fertility was determined for each pair as: egg fertility = (total number of eggs that hatched/ total number of eggs) x 100.

Adult longevity was estimated for each pair by determining the total life span duration (time from adult emergence to time of death) of both the male and female in each pair. Longevity of males was recorded only for the initial male in those pairs in which male death preceded female death (i.e., longevity was not recorded for replacement males).

**Interhaplotype Fertility**

Crosses were conducted between all possible pairings of haplotypes; producing 9 types of pairings (the total includes the 3 intrahaplotype crosses). The assays were limited to psyllids reared on potato. Newly-emerged male and female psyllids of each haplotype were obtained using the rearing methods described above, and paired on potato clippings in the small rearing cages. Fifteen and five pairs of psyllids per inter- and intrahaplotype crosses, respectively, were monitored (a total of 105 pairs). Each pair was examined daily for the onset of egg laying. Once oviposition had begun, egg laying was allowed to continue for five days. Each pair was then transferred to a new cage and potato clipping, and was allowed for another four days for oviposition. Adults were removed at the end of this second 4-day period, and the females were set aside for dissection (see below). The eggs were monitored for nine days and then the total number of nymphs and shriveled (non-hatching) eggs were determined on each clipping. Fertility was determined from the total numbers of nymphs and unhatched eggs for each cross. Female psyllids were dissected under a microscope and checked for mating status (presence or absence of spermatophores; Guédot et al. 2013). Psyllid haplotype was verified following the
dissection for both male and female psyllids in each pair by examination of mtDNA (Swisher et al. 2012, 2013a, 2014a).

**Data Analysis**

All statistical analyses were done using SAS (SAS Institute 2012). Effects of haplotype and host species on adult longevities were analyzed using a 3 x 2 factorial analysis of variance (ANOVA). The analyses were done using the GLM procedure. Analysis of variance could not be used to examine egg hatch or lifetime fecundity due to significant departures of the data from assumptions of normality and constant variance. The count data (lifetime fecundity) were instead modeled assuming a negative binomial distribution. The analysis was done using the GLIMMIX procedure. Model fit was evaluated by use of the chi-square/df statistic provided by GLIMMIX (Littell et al. 2006). Probability of egg hatch was modeled as a binomial response (total eggs hatching/total eggs monitored) by use of PROC GENMOD. Model fit was poor due to over dispersion (shown by Pearson chi-square statistic), thus the model was refitted with a scaling factor using the SCALE = PEARSON option (Stokes et al. 2005). Means were back-transformed into proportions using the ILINK option. In all analyses, if the haplotype effect in the ANOVA was significant, haplotype means were compared using Tukey tests. The proportion of hatched eggs to the total number of eggs was compared among haplotype crosses using the LOGISTIC procedure of SAS (SAS Institute 2012). The FIRTH option and LINK=LOGIT function were included in the MODEL statement. Differences in hatch rates among the nine crosses were compared using 95% confidence intervals.
RESULTS

Fecundity, Egg Fertility, and Adult Longevity. Numbers of eggs deposited per female per 4-d mating interval and female survival over those intervals are summarized for all three haplotypes and both host species (Fig. 2). Egg laying rates for all haplotypes rapidly climbed over the first three sampling intervals and remained high on both host species through days 40-50, and then declined sharply thereafter (Fig. 2). Peak egg laying rates ranged approximately between 75 and 100 eggs per 4-day interval for the Northwestern haplotype, 50 to 100 eggs per interval for the Western haplotype, and 40 to 60 eggs per interval for females of the Central haplotype females (Fig. 2). No obvious host effects were noted except in the second half of the monitoring period for the Northwestern haplotype, where egg laying rates were somewhat higher for females on the potato host (Fig. 2). Percentage survival of females (Fig. 2: solid and dashed lines) was high well into 60-80 d in age, but then began a fairly sharp decline following that interval. Females appeared to survive longer on nightshade than on potato, particularly females of the Western and Central haplotypes (see also the adult longevity results, below).

Summary statistics for egg hatch percentages, lifetime fecundity, and male and female life spans are presented as haplotype x host plant means (to search for evidence of local adaptation to nightshade by the Northwestern haplotype) as well as host and haplotype main effect means (Fig. 3-4). Mean lifetime fecundity was not affected by host species (Fig. 3B: $F_{1,66} = 0.02, P = 0.66$); mean egg production on either plant species was approximately 800 eggs per female when results are averaged over haplotype. Fecundity was affected by haplotype (Fig. 3C; $F_{2,66} = 15.85, P < 0.0001$). Egg production was highest in the Northwestern and Western
haplotypes. The host x haplotype interaction was non-significant (Fig. 3A; $F_{2,66} = 1.52, P=0.23$).

The host x haplotype interaction was non-significant for the egg hatch data (Fig. 3D; $F_{2,66} = 2.00, P=0.14$). Averaged over haplotype, percentage egg hatch was higher (by almost 20 percentage points) for eggs deposited on potato than eggs deposited on nightshade (Fig. 3E; $F_{1,66} = 83.3, P < 0.0001$). The three haplotypes exhibited overall similar rates of egg hatch (Fig. 3F; $F_{2,66} = 0.11, P=0.89$).

Host- and haplotype-associated patterns in adult longevity were similar between female and male psyllids (Fig. 4). For both sexes, the haplotype x host interaction term in the ANOVAs were not significant (Figs. 4A, D: females, $F_{2,66} = 1.71; P=0.19$; males, $F_{2,66} = 2.1, P=0.13$). Psyllids of both sexes showed a significantly longer life span on nightshade than potato (Figs. 4B, E: females, $F_{1,66} = 10.35; P=0.002$; males, $F_{1,66} = 23.7, P < 0.0001$). The increase in longevity on nightshade was about 23 and 35 d for female and male psyllids, respectively, averaged over haplotype (Figs. 4B, E). Haplotype effects were significant for male psyllids (Fig. 4F: $F_{2,66} = 6.5, P=0.003$), with males of the Northwestern haplotype having a significantly longer life span than males of the other two haplotypes (by 23-30 d).

The absence of host-associated differences in total fecundity (Fig. 3B) despite the longer lifespan of females on nightshade (Fig. 4B) appears to have been caused by an ending of egg laying well before death by females on nightshade, in contrast to what was generally observed for female psyllids on potato. This pattern is shown by the drop of the egg laying curve to zero for females on the nightshade host (Fig. 2: solid circles), often well before death of the females (Fig. 2: solid lines). This effect was particularly noticeable for the Western and Central
haplotypes. To examine this pattern more closely, we calculated the number of consecutive egg-free intervals immediately preceding death for each female, and visually compared this statistic for nightshade- vs potato-reared females (Fig. 5). Females on nightshade ended egg laying well-before death (reaching 25 monitoring intervals [or 100 days] preceding death for one Northwestern female; Fig. 5). Psyllids on potatoes exhibited a smaller egg-free interval preceding death, and often deposited eggs almost until time of female death. In sum, despite the longer life-span of females on nightshade, the absence of egg laying by nightshade-reared females during the several weeks preceding female death led to estimates of mean lifetime fecundity that were of similar magnitude between the potato and nightshade hosts (Fig. 3B).

**Interhaplotype Fertility.** Dissection showed that all females from every cross contained spermatophores, indicating that females in all crosses had mated. Rates of egg hatch clustered into two discrete categories (Table 1). Eggs deposited by Northwestern females that had been mated by Central or Western males all failed to hatch. The second group, comprising the remaining seven types of crosses, exhibited high rates of egg fertility (79-93% hatch); these high rates included eggs deposited by females that had been mated by males from a different haplotype (Table 1).

**DISCUSSION**

Potato psyllid has been identified as the vector of the bacterium “Ca. L. solanacearum”, putative causal agent of zebra chip disease of potato (Munyaneza et al. 2007, Munyaneza 2012). Expansion of zebra chip into the Pacific Northwest growing regions of the United States led to the discovery that the psyllid vector survives winter in the region on bittersweet nightshade (Jensen et al. 2012, Murphy et al. 2013). It is now known that the potato psyllid species is
composed of at least four unique genetic types or haplotypes (Swisher et al. 2012, 2014a). It is unclear whether growers in the Pacific Northwest or elsewhere should be concerned about all haplotypes of the psyllid or with a subset of haplotypes, due to a shortage of studies that compare biology of the different haplotypes. For example, it is not known whether all haplotypes are equally efficient at acquiring and transmitting the zebra chip pathogen, or whether life history traits such as development and reproduction are similar among the different haplotypes. Variation in reproductive traits among haplotypes could affect how rapidly psyllid populations of a given haplotype increase in the field and contribute to spread of the pathogen causing zebra chip disease.

This study examined the effects of host plant on reproduction and adult longevity of psyllids from the three haplotypes which can be found co-occurring during summer in potato fields of the Pacific Northwest (Swisher et al. 2014b). Host plant did not affect fecundity of psyllids (Fig. 3A). There were haplotype differences in egg oviposition. Females of the Northwestern and Western haplotypes had higher lifetime fecundity than females of the Central haplotype (Fig. 3C). The absence of host effects has been found in other studies, most notably the study by Yang and Liu (2009), who showed that fecundity was similar for psyllids on eggplant (*Solanum melongena*) and bell pepper (*Capsicum annuum*). However, Liu and Trumble (2007) found that fecundity was higher for Central haplotype psyllids than those of the Western haplotype when the insects were reared on tomato (*Solanum lycopersicum*), but not on pepper. Several previous studies showed that average fecundity of potato psyllid varied between 311 to 720 eggs per female on potato, tomato, chili pepper, eggplant and bell pepper (Compere 1916, Lehman 1930, Knowlton and Janes 1931, Knowlton 1933, Davis 1937, Abdullah 2008, Yang...
and Liu 2009, Yang et al. 2010, Prager et al. 2014). Results of the present study showed that average fecundity of potato psyllid was 1050, 877, and 629 eggs per female for the Northwestern, Western, and Central haplotypes, respectively. Interestingly, fecundity estimates for Central psyllids in the current study are similar to estimates reported in several of these other studies (Lehman 1930, Knowlton and Janes 1931, Knowlton 1933, Abdullah 2008, Yang and Liu 2009, Yang et al. 2010), which we believe (based upon geography) are likely to have used psyllids of the Central haplotype in determining fecundity. In sum, Northwestern and Western psyllids were more fecund than Central psyllids (Figs. 3A and C), and this trait could have an impact on population growth rates under field conditions depending upon the relative abundance of the three haplotypes in any particular field.

Previous studies showed that longevity of female potato psyllid ranged from 14.6 to 189 d on potato, and male longevity was reported averaging from 22 d on tomato to 64 d on potato (Knowlton and Janes 1931, Abdullah 2008, Yang and Liu 2009, Yang et al 2010). During the present study, both male and female psyllids lived longer on bittersweet nightshade than potato, with an average life span of 108 and 85 d for females and 114 and 79 for males on nightshade and potato, respectively (Fig. 4). In addition, overall life span was higher for Northwestern psyllids than Western and Central psyllids (Fig. 4F). Female life-span in all three haplotypes tended to exceed duration of the egg laying period, for unknown reasons. This pattern was especially noticeable on nightshade (Fig. 5). Extreme instances of this trait were shown by a few females on nightshade that permanently ended egg laying 80-100 d before death (Fig. 5).

Egg fertility was affected by host plant for each of the three psyllid haplotypes, with significantly higher percent egg hatch for psyllids reared on potato than those reared on
bittersweet nightshade, irrespective of haplotype (Fig. 3D-F). The cause of the reduced egg hatch on nightshade is not yet known, but conceivably could include effects through either the male or female parent. Diet has been shown to affect male quality in other insect species, leading to effects on size of testes and numbers of sperm produced or transferred to females (Guerra and Bhuiya 1977, Ward and Simmons 1991, Delisle and Hardy 1997, Droney 1998), with possible effects on egg fertilization. Suboptimal diets for female Lepidoptera have been shown to be associated with the production of eggs having reduced hatch rates (Geister et al. 2008, Wang et al. 2013). Indeed, reduced hatch of deposited eggs has been shown to accompany rearing of females on diets having high concentrations of certain plant allelochemicals, including allelochemicals known to occur in the Solanaceae (Büyükgüzel et al. 2013). Whether any of these explanations account for the reduced hatch of eggs produced by nightshade-reared psyllids has yet to be determined.

An unexpected finding of this study was that egg fertility was also affected by haplotyptic match of the parental psyllids. Northwestern females did not produce viable eggs when mated with males of Western or Central psyllids, but exhibited an average fertility rate of 85% when paired with Northwestern males (Table 1). All of these Northwestern females had indeed been mated, as confirmed by dissecting the insects and checking for presence of spermatophores (Guédot et al. 2013). The fertility effects were asymmetric, in that crosses between Northwestern males and Central or Western females produced viable eggs at a level similar to what was produced by intrahaplotype pairings (Table 1). This is the first report of partial interhaplotype reproductive incompatibility in potato psyllid. The cause of this asymmetric incompatibility is
not yet known, but studies are underway to examine factors that could be controlling the asymmetry.

In summary, results of this study showed that there were significant differences in reproductive biology among the three haplotypes of potato psyllid found to co-occur in the Pacific Northwest of the United States. However, the absence of significant host x haplotype effects in all analyses indicates that there was no evidence for host-dependent differences among the haplotypes. Thus, we have no evidence that the locally resident haplotype (Northwestern) was also locally adapted to the nightshade host. Fecundity was higher in Northwestern and Western psyllids, regardless of whether the insects were reared on potato or bittersweet nightshade. Fertility was higher for psyllids of all haplotypes when reared on potato than if reared on bittersweet nightshade, whereas adult longevity showed the opposite host trend. Finally, an asymmetric reproductive incompatibility between Northwestern psyllids and those of Central and Western haplotypes was discovered. Either of these two crosses led to a complete absence of egg hatch, despite spermatophores transfer. Because populations of potato psyllid in several potato growing regions of North America have been shown to comprise a mixture of haplotypes (Swisher et al. 2012, 2013a; 2014a,b), mating between non-like haplotypes under field conditions is conceivably possible, with potential effects on subsequent egg viability. Collectively, our results indicate that a full understanding of psyllid population biology in any growing region will require knowledge of haplotype composition within the region, as well as diversity of the host plant complex in the growing region.
REFERENCES


doi:10.1371/journal.pone.0094047


Table 1. Proportion of eggs that hatched (± 95% confidence intervals) in each of nine possible intra- and interhaplotype crosses.

<table>
<thead>
<tr>
<th></th>
<th>Western male</th>
<th>Central male</th>
<th>Northwestern male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western female</td>
<td>0.90 (0.872 - 0.926) a</td>
<td>0.83 (0.806 – 0.848) c</td>
<td>0.81 (0.786 – 0.829) c</td>
</tr>
<tr>
<td></td>
<td>(874)*</td>
<td>(2243)</td>
<td>(2442)</td>
</tr>
<tr>
<td>Central female</td>
<td>0.86 (0.844 – 0.882) b</td>
<td>0.85 (0.813 – 0.878) bc</td>
<td>0.86 (0.839 – 0.878) b</td>
</tr>
<tr>
<td></td>
<td>(2694)</td>
<td>(875)</td>
<td>(2441)</td>
</tr>
<tr>
<td>Northwestern female</td>
<td>0 d</td>
<td>0 d</td>
<td>0.85 (0.812 – 0.873) bc</td>
</tr>
<tr>
<td></td>
<td>(2710)</td>
<td>(2656)</td>
<td>(990)</td>
</tr>
</tbody>
</table>

Fifteen and five pairs were established for each of the inter- and intrahaplotype crosses, respectively. Numbers with different letters are significantly different (α=0.05), with Wald 95% confidence intervals.

* Total number of eggs per cross
Figure 1. Example of small rearing cage used in all assays. Each cage consists of two 5-oz clear Polystyrene 40 Dram capped plastic vials glued together top to top and containing potato or bittersweet nightshade clipping onto which fifth instar nymphs or psyllid adult pairs were placed.
Figure 2. Filled and open circles show mean (± SEM) number of eggs deposited per interval and surviving female for three haplotypes of potato psyllid reared on two host plant species, from the onset of egg laying until female death. Solid lines and dashed lines show percentage (of the original 12 females per haplotype and host plant) alive at each 4-d interval.
Figure 3. Mean (± SEM) lifetime fecundity (A-C) and percentage egg hatch (D-F) for three haplotypes of potato psyllid reared on two host plant species. Panels show the host x haplotype means (A, D) and the main effect means for host species (B, E) and haplotype (C, F). Asterisks depict significant host plant effects. Capital letters above bars (C) show haplotypic differences in mean fecundity (by Tukey’s test).
Figure 4. Mean (± SEM) longevity of adult female (A-C) and adult male (D-F) potato psyllids for three haplotypes of psyllid reared on two host plant species. Panels show the host x haplotype means (A, D) and the main effect means for host species (B, E) and haplotype (C, F). Asterisks depict significant host plant effects. Capital letters above bars (F) show haplotypic differences in male longevity (by Tukey’s test).
Figure 5. Box plot showing numbers of consecutive egg-free intervals (each of 4 d in length) preceding female death. Box boundaries depict 25th and 75th percentiles, horizontal line within box shows median, error bars depict 90th and 10th percentiles, and circles show outlying points.
ABSTRACT

The potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a vector of the phloem-limited bacterium ‘*Candidatus Liberibacter solanacearum*’ (Lso), the putative causal agent of zebra chip disease of potato. Little is known on the mechanisms by which the potato psyllid transmits Lso to potato. This lack of information is compounded by the recent discovery of at least four haplotypes of potato psyllid that often co-occur on potato crops in the Pacific Northwest. It is not known whether these haplotypes differ in their Lso transmission efficiency, which would necessitate haplotype-specific management approaches. The present study used the electrical penetration graph (EPG) technology to assess stylet probing behaviors and Lso transmission efficiency of three psyllid potato psyllid haplotypes (Central, Western, and Northwestern) that are commonly found in Pacific Northwest potato crops. Results do not provide evidence that feeding behaviors and Lso transmission rates differ among *B. cockerelli* haplotypes. EPG waveforms produced by the psyllids of all the three haplotypes were similar in appearance, frequency, and duration, suggesting that they probe and feed in a similar manner.
Results also showed that Lso transmission rates among the psyllids of the three haplotypes were not statistically different, suggesting that Lso transmission efficiency was similar among the haplotypes. Transmission of Lso occurred with as little as 10 min inoculation access period, and increased with increasing inoculation access period, regardless of psyllid haplotype. Average Lso transmission rate was < 10% with <2 h inoculation access period, but increased to ~40% and 60% after 3 h and 24 h inoculation access periods, respectively. Information from this research increases our understanding Lso transmission to potato by potato psyllid and could help in developing effective management strategies for zebra chip disease.

INTRODUCTION

Zebra chip disease of potato is caused by the phloem-limited bacterium ‘Candidatus Liberibacter solanacearum’ (Lso), vectored by the potato psyllid, Bactericera cockerelli (Šulc) (Hemiptera: Triozidae) (Munyaneza 2012). Zebra chip has caused millions of dollars in losses to the potato industry in the United States, Mexico, Central America, and New Zealand (Munyaneza 2012). While zebra chip was first reported in Mexico in 1994 and 2000 in Texas (Munyaneza 2012), the disease did not reach Pacific Northwest, the major potato growing region of the United States, until 2011 (Crosslin et al. 2012a,b). Zebra chip symptoms were not linked to potato psyllid and Lso until very recently (Munyaneza et al. 2007, Munyaneza 2012), hence little is known about psyllid-Lso interactions.

Little is known about the mechanisms by which the potato psyllid transmits Lso to potato. This lack of information is compounded by the recent discovery of at least four genetic
variants, or haplotypes, of potato psyllid that often co-occur in potato fields in the Pacific Northwest (Washington, Oregon, and Idaho). If Lso transmission efficiency differs between these psyllid haplotypes, then different psyllid management approaches may be required.

An innovative technology known as the Electrical Penetration Graph (EPG) (McLean & Kinsey, 1964; Backus & Bennett, 2009) is a very useful tool to assess stylet probing behavior of hemipterans and other piercing-sucking insects, including psyllids. This EPG technology can help observe and quantify the feeding of these insects whose mouth parts are normally not visible while probing into plant tissues. EPG technique electrically records the changes in resistance (in form of consistent and repeated patterns referred to as “waveforms”) that relate to the different stylet penetration activities of sucking insects, providing information on stylet tip position in specific plant tissues and time spent at each location (Tjallingii, 1985).

Pearson et al. (2014) recently identified and characterized EPG waveforms representing stylet penetration behaviors of the potato psyllid feeding on potato. Six waveform families and four types were identified: family A, initial penetration and saliva secretion; family B, penetration of epidermal cells; family C, secretion of most of the salivary sheath and stylet pathway in mesophyll/parenchyma, with two types, C1 and C2; family D, initial contact with phloem cells; family E, activities in phloem cells, with two types, E1, putative phloem salivation, and E2, phloem sap ingestion; and family G, xylem ingestion. Pearson et al. (2014) then suggested that active phloem sap ingestion during E2 plays a critical role in acquisition of Lso bacterial cells, whereas E1 plays a role in inoculation of the bacterium into phloem sieve elements, because it represents salivation into phloem sieve elements. These conclusions on Lso
inoculation and acquisition into and from potato plants by potato psyllid are shared by Sandanayaka et al. (2014).

EPG has been used to assess pathogen transmission by insect vectors (Powell, 1991; Fereres & Collar, 2001; Symmes et al., 2008; Sandanayaka et al., 2013, 2014; Pearson et al. 2014), by correlating stylet penetration activities to acquisition and inoculation of pathogens (Martin et al., 1997; Tjallingii & Prado, 2001; Stafford et al., 2009; Moreno et al., 2012). The current study used EPG technology to compare the stylet probing behavior and Lso transmission efficiency among three psyllid haplotypes (Central, Western, and Northwestern) that are commonly found in potato crops in the Pacific Northwest. In addition, the minimum time required for successful inoculation of Lso into potato plants by psyllids of the three haplotypes was assessed.

MATERIALS AND METHODS

Sources of Insects. Lso-free and Lso-infected potato psyllid colonies of three haplotypes (Central, Western, and Northwestern haplotypes) were established at the USDA-ARS facility in Wapato, WA; with insects collected from Weslaco, Texas (Central haplotype), southern California (Western haplotype), and Prosser, Washington (Northwestern haplotype). The colonies were started with isofemale lines and were maintained on Atlantic potatoes under laboratory conditions of 25±1 °C, 40±5% RH, with a photoperiod of 16:8 (L: D) h. Prior to conducting assays, the haplotype status of each psyllid colony was confirmed by examination of mitrocondrial DNA (mtDNA) sequences, using high resolution melting analysis as described by
Swisher et al. (2012). The insects were also screened for Lso by conventional PCR as described by Munyaneza et al. (2010) to confirm infection.

**Sources of Plants.** Certified disease-free potatoes (var. Atlantic) grown from seed tubers obtained from CSS Farms Inc. (Colorado City, CO) were used for this study. The Atlantic variety was selected due to its high susceptibility to zebra chip disease (Munyaneza et al. 2007a, b; 2008). The tubers were planted in 0.5-L pots (Kord Products, Toronto, Ontario, Canada) filled with a soil medium consisting of 86% sand, 13.4% peat moss, 0.5% Apex time release fertilizer (J. R. Simplot Co., Lathrop, CA), and 0.1% Micromax micronutrients (Scotts Co., Marysville, OH) in a greenhouse at USDA-ARS, Wapato, WA. Prior to conducting assays, the plants were tested for ‘Ca. L. solanacearum’ by PCR (Munyaneza et al. 2010) to ensure they were free of the bacterium.

**Assessing stylet probing behaviors.** Stylet probing behaviors of the three psyllid haplotypes were assessed using the electrical penetration graph (EPG) technology as described by Pearson et al. (2014). Twelve randomly selected Lso-free potato psyllid adults were used for each of the three haplotypes, for a total of 36 insects. Briefly, individual psyllids were first collected and starved for 2-3 h at 4 °C. Following starvation, the psyllids were held on ice pending the wiring process. An electrode consisting of a 3-cm long copper wire that was soldered to the head of 3 mm diameter brass nail was used to wire the insects. One end of the electrode made of a 3cm long, 25.4 µm diameter gold wire (sold as a 0.0010 in.; Sigmund Cohn., Mt. Vernon, NY, USA) was attached to the copper wire of the electrode. The starved psyllids were then secured by gently gripping their fore- and hind wings with a pair of soft grip forceps (Bio Quip, Rancho Dominguez, CA) and attaching their pronotum to the other end of the
electrode using a hand-mixed silver glue adhesive, under a Leica MZ6 stereo microscope (Buffalo Grove, Illinois, USA). The silver glue adhesive mixture consisted of 1:1:1 (v: v: w) of water-based white household glue, water and silver flake (Inframat Advanced Materials LLC, Manchester, CT, USA). The wired insects were left at room temperature for a 30-min recovery period, after which the electrode was inserted into the EPG amplifier and the psyllids were placed directly on the abaxial surface of a leaf of an potato ‘Atlantic’ potted plant and the probing behaviors recorded for 24 h. The EPG recordings were conducted in an experimental room under controlled conditions of 25±1 °C, 40±5% RH, with a photoperiod of 16:8 (L: D) h. The EPG waveforms resulting from the psyllid probing were acquired using a four-channel AC-DC EPG monitor (Backus and Bennett 2009; EPG Equipment Co., Otterville, MO). The EPG data output was digitized using a WinDaq DI-720 analog-to-digital (A-D) board and recorded with WinDaq Pro+ acquisition software (DATAQ Instruments, Akron, OH) at a sample rate of 100 Hz, input impedances (Ri) of 10^9 Ohms (Ω), and DC substrate voltage. The total number of waveform events and time duration spent in intracellular style penetration/pathway phase (C); xylem-ingesting (G); initial contact with phloem tissue (D), salivation into phloem sieve elements (E1), and phloem sap ingestion (E2) were recorded as described by Pearson et al. (2014). Furthermore, time to first probe and time to first E1 and E2 from 1st probe were determined.

**Assessing Lso transmission efficiency.** Lso transmission efficiency for each of the three psyllid haplotypes was assessed by using EPG. Potato psyllid adults of each of the three haplotypes were randomly collected from Lso-infected laboratory colonies and starved for 6 h at 4 °C. The psyllids were then tethered to the gold wire with a silver glue adhesive and placed on
the abaxial surface of Atlantic potato leaves for EPG recordings as described above. The psyllids were given inoculation access periods of 1, 2, 3, 4, 5, 6, 12, and 24 h. EPG recordings were terminated at the end of each inoculation access period, after which the psyllids were collected and tested for Lso by PCR to confirm infection. A total of 20 psyllids were used for each haplotype and for each inoculation access period. After the EPG recordings, the psyllid-exposed plants were transplanted into small field cages outdoors (Buchman et al. 2011) and monitored 4 to 6 weeks for zebra chip disease symptom development (Sengoda et al. 2014). In addition, the plants were tested for Lso by PCR at the end of the experiment to confirm infection. Lso transmission rate was determined for each of the psyllid haplotypes and inoculation access period by dividing the number of Lso-infected plants by the number of Lso-positive psyllids.

**Assessing minimum Lso inoculation time.** To determine the minimum time required for the successful Lso inoculation, EPG recording data was used to determine Lso-infected psyllids with 3 h-IAP or less and that had a single phloem salivation (E1) and whose exposed-potato plants developed zebra chip symptoms and/or tested positive for the bacterium.

**Data Analysis.** The effects of haplotypes and probing/feeding behaviors were examined by multivariate analysis of variance (MANOVA) using PROC GLM (SAS version 9.3). The dependent variables in the multivariate analysis were 1) intracellular style penetration/pathway phase (C), 2) initial contact with phloem tissue (D), 3) salivation into phloem sieve elements (E1), 4) phloem sap ingestion (E2), and 5) ingestion of xylem sap (G). The fixed effects were potato psyllid haplotypes. The Roy’s Greatest Root Statistic was used to test the overall significance of the multivariate model (Scheiner 2001). Data were examined for heterogeneity of
variance and non-normality of errors by inspecting residual and normal quantile-quantile plots respectively. Based on plots, data was log transformed prior to analysis. Time to first probe and time to first E1 and E2 from first probe was analyzed using the GLIMMIX (Restricted Maximum Likelihood) procedure of SAS 9.3 (SAS Institute 2012). Based on the plots, the data were adjusted using the DIST= LOGN option of the model statement prior to analysis. The Lso transmission rate was compared among the three haplotypes using the GLIMMIX procedure of SAS 9.3 (SAS Institute 2012).

RESULTS

Probing behaviors. The waveforms representing an intracellular style penetration/pathway phase (C), initial contact with phloem tissue (D), salivation into phloem sieve elements (E1), phloem sap ingestion (E2), and ingestion of xylem sap (G) variants (Gv1 and Gv2) produced by each of three psyllid haplotypes were visually compared to those described by Pearson et al. (2014). The waveforms were found to be similar in appearance with those developed by Pearson et al. (2014) (Figs. 1-3), suggesting that the psyllids of the three haplotypes exhibit similar feeding behaviors.

Average numbers of stylet penetration (C) events in 24 h were 24.7, 20, and 31 for Central, Northwestern, and Western haplotypes, respectively, whereas total duration of this probing event averaged 9.2, 6.5, and 7.9 h for Central, Northwestern, Western haplotypes, respectively (Fig. 4). During the xylem ingestion (G) probing event, Central, Northwestern, and Western haplotypes made 3, 2, and 1.9 attempts to feed on xylem contents, respectively, with a total average duration of 3.05, 1.0, and 1.5 h for Central Northwestern, and Western psyllids,
respectively (Fig. 4). The number of probing events during the initial contact with the phloem cells (D) averaged 7, 4.8, and 5 for Central, Northwestern, and Western haplotype psyllids, respectively, with an average time duration of 3.4, 3.9, and 8.2 min for Northwestern, Western, and Central haplotypes, respectively (Fig. 4). Central, Northwestern, and Western haplotype psyllids made an average of 10.9, 8.8, and 9.9 attempts to salivate in the phloem (E1), with the total phloem salivation probing duration averaging 3.7, 3.5, and 2.9 h for Central, Northwestern, and Western haplotypes, respectively (Fig. 4). On the other hand, Central, Northwestern, and Western haplotype made 5.5, 5.9, and 6.5 attempts to ingest phloem (E2), with an average total duration of 3.8, 4.3, and 6.4 h, respectively (Fig. 4). It took an average of 10.5, 5.3, and 7.9 min for Central, Northwestern, and Western haplotype psyllids, respectively, to make the first probe at the beginning of the EPG recordings process (Fig. 5). Then from the first probe, it took about 2.2, 4.0, and 4.1 h for Central, Northwestern, and Western psyllids, respectively, to first salivate in the phloem (E1) (Fig. 5).

Multivariate analysis of patterns in feeding behaviors (C, D, E1, E2, and G) during the 24 h EPG recording period did not reveal significant differences among the three psyllid haplotypes (number of events, $F_{5, 30}=1.80, P=0.14$; event durations, $F_{5, 30}=2.31, P=0.06$) (Fig. 4A and 4B). In addition, average time to first probe (C) from the start of the EPG recording was similar for the three psyllid haplotypes ($F_{2, 33}=2.14, P=0.13$). Furthermore, there was no significant difference in average time to first salivation into phloem sieve elements (E1) ($F_{2, 32}=0.97, P=0.39$) or phloem sap ingestion (E2) ($F_{2, 31}=0.03, P=0.97$) between the three psyllid haplotypes (Fig. 5).
**Lso transmission efficiency.** Statistical analysis of the data of Lso transmission rate among the psyllids of the three haplotypes (Fig. 6) showed that overall transmission of Lso to potato plants was not affected by the haplotype \((F_{2, 464} = 0.90, \ p = 0.41)\). However, the statistical analysis showed that Lso transmission rate increased with the inoculation access time period \((F_{7, 464} = 6.17, \ p = < 0.001)\). There was no haplotype by inoculation access period interaction indicating that the effects of inoculation access period on Lso transmission was similar among the three haplotypes \((F_{14, 464} = 0.26, \ p = 0.99)\).

**Minimum Lso inoculation time.** Using EPG recording data from selected Lso-infected psyllids that had access to potato plants for 3 h or less, exhibited a single event of phloem salivation \((E1)\), and whose exposed potato plants developed zebra chip symptoms and/or tested positive for the bacterium, the actual time required for successful inoculation of Lso by a single psyllid to potato plants was estimated to be less than 10 min (Fig. 7).

**DISCUSSION**

Lso that causes zebra chip in potatoes is vectored by potato psyllid. However, little is known on mechanisms by which this insect transmits the bacterium to potato and other host plants. This lack of information on Lso transmission biology by its insect vectors is compounded by the discovery of at least four haplotypes of potato psyllid that often co-occur in potato crops, particularly in the Pacific Northwest. It is not known whether psyllids of the different haplotypes exhibit different feeding/stylet probing behaviors, which may lead to differences in transmission efficiency of Lso by the psyllid haplotypes and may require different management strategies for this insect pest. During the present study, EPG technology was used to elucidate probing
behaviors and Lso transmission efficiency of three psyllid potato psyllid haplotypes (Central, Western, and Northwestern) that are commonly found in Pacific Northwest potato fields. The EPG waveform recordings on potato were compared with those originally described by Pearson et al. (2014).

Results showed that the shapes and forms of the EPG waveforms (C, D, E1, E2, and G) produced by the psyllids of all the three haplotypes were similar in appearance to the potato psyllid characteristic waveforms described by Pearson et al. 2014 (Figs. 1, 2, and 3), who used potato psyllids originating from Texas and presumed to be of the Central haplotype (Swisher et al. 2012, 2013). The waveforms were also similar to those reported by Butler et al. (2012) in California and Sandanayaka et al. (2014) in New Zealand, who presumably used potato psyllids of the Western haplotype (Thomas et al. 2011; Swisher et al. 2012, 2013). This study is the first to directly compare EPG quantitative data among different potato psyllid haplotypes. Statistical analysis of the EPG data collected during this study indicated that there were no significant differences in the number and duration of the different probing behavior events among the three psyllid haplotypes examined. The overall similarities in EPG waveforms, and number and duration of the different stylet probing events among the three psyllid haplotypes suggest that these insects feed on potato in a similar manner. Sandanayaka et al. (2014) also showed that there was no difference in feeding behavior between Lso-free and Lso-infected potato psyllids; however, these researchers only used psyllids presumably of Western haplotype.

The present study also examined Lso transmission efficiency among the three psyllid haplotypes by recording probing behaviors of the insects during selected inoculation access periods and then monitoring the inoculated plants for zebra chip symptoms and confirming Lso
infection by PCR testing. Particularly, attention was paid on the time the psyllids spent probing in the phloem tissue. The phloem salivation (E1) and ingestion (E2) phases (Fig. 3) are considered as the probing events during which inoculation and acquisition of pathogens, including Lso, take place (Pearson et al. 2014, Sandanayaka et al. 2014). Lso is presumably inoculated into healthy plants during the phloem salivation phase, whereas the bacterium is acquired from infected plants during the phloem ingestion.

Results showed that Lso transmission rates among the psyllids of the three psyllid haplotypes were not statistically different, suggesting that Lso transmission efficiency was similar among psyllids of the three haplotypes, regardless of inoculation access period (Fig. 6). However, Lso transmission rate for each of the psyllid haplotypes significantly increased with the inoculation access period (Fig. 6). Average Lso transmission rate remained low (< 10%) during the first two hours but significantly jumped with 3 h inoculation access period (up to 40%) and was highest when the insects were allowed access to the plants for 24 h (up to 60%) (Fig. 6). An earlier report by Buchman et al. (2011) indicated that single potato psyllids (of Central haplotype) were effective in inducing zebra chip, with an average of Lso transmission rate of 47%, when given an inoculation access period as short as 6 h. This report is consistent with the results of the present study, during which Lso transmission rate of 43% was observed for psyllids of Central haplotype, when allowed access to the plants for 6 h. Interestingly and in contrast to the report by Buchman et al. (2011), results of the present study showed that Lso transmission rate of psyllids with an even lower inoculation access period of 3 h was as high as one of the psyllids given access to the plants for 6 h, especially with the insects from Central and Northwestern haplotypes, which had an average Lso transmission rate of 35 and 41%,
respectively. These results provide further evidence that potato psyllid could be even harder to manage because of its rapid Lso transmission to potato plants, which may require an inoculation access period as short as 3 h to effectively inoculate the plants. This 3h IAP can easily be explained by the fact that it took about 2-4 h following the first stylet probe for potato psyllids to reach and salivate into the phloem sieve elements, as demonstrated by the EPG recording data (Fig. 5). These observations support results of a recent report from New Zealand by Sandanayaka et al. (2014), which indicated that the minimum plant access period required for Lso transmission by a single potato psyllid (presumably of Western haplotype) was about 2 h. During our study, even insects that were allowed to have access to plants for just 1 h induced zebra chip, but Lso transmission rate was significantly lower than those psyllids with a 3 h or higher inoculation access period (Fig. 6).

Moreover, a closer analysis using EPG recording data from selected Lso-infected psyllids that had access to potato plants for 3 h or less and were successful at inoculation the potato plants with Lso during a single event of phloem salivation (E1) showed that the actual inoculation time required for successful infection of potato plants with the bacterium ranged from 3.6 to 9.3 min (Fig. 7). While, Lso acquisition was not assessed during the present study, Sandanayaka et al. (2014) recently indicated that the minimum Lso acquisition threshold by potato psyllid was about 36 min.

Results of the present study and those by Buchman et al. (2011) and Sandanayaka et al. (2014) clearly underscore the high risk of Lso infection by potato psyllid and substantial challenges to controlling this insect vector to manage zebra chip disease. The studies demonstrate that psyllids of the three haplotypes examined are very effective in transmitting Lso
to potato plants and that it takes about 1-3 h inoculation access and less than 10 min for the insects to successfully inoculate Lso into potato plants and cause zebra chip. Both short potato psyllid inoculation access period and minimum Lso inoculation time have serious implications in managing potato psyllid because a single Lso infective psyllid feeding on potato for a couple of hours could lead to substantial spread of zebra chip disease within potato field in potato growing areas. Furthermore, insecticide applications may not kill potato psyllids fast enough before they can transmit Lso to potato plants.

While zebra chip was first reported in Mexico in 1994 and 2000 in Texas (Munyaneza 2012), the disease did not reach the Pacific Northwest of U.S. until 2011 (Crosslin et al. 2012a,b). Psyllids of Central, Western, and Northwestern were present in this important potato growing region when the zebra chip outbreak occurred in the Pacific Northwest but no Lso was detected in the Northwestern haplotype psyllids. Northwestern psyllids are considered as residents of the Pacific Northwest and this haplotype predominates in the Columbia Basin of Washington and Oregon (Swisher et al. 2014). This observation led to the conclusion that the 2011 zebra chip outbreak in the Pacific Northwest was due to potato psyllids other than those of the Northwestern haplotype (Swisher et al. 2013, 2014). To date, Lso infection has been rare in Northwestern psyllids and little zebra chip has been observed in the Columbia Basin after the 2011 potato growing season, prompting the question to whether these psyllids are efficient vectors of Lso. Results of the present study clearly indicate that Northwestern haplotype potato psyllids are equally efficient in transmitting Lso to potato as Central and Western psyllids. The results also suggest that the recent low incidence of zebra chip observed in the Columbia Basin is probably due to predominantly occurring Lso-free Northwestern haplotype psyllid populations.
However, potato growers in the regions should be cautious as the situation could change, especially if Lso ends up spreading throughout these psyllid populations through horizontal and vertical transmission. This could lead to zebra chip spreading further in the region and potentially causing serious damages to potato crops.

In summary, results of the present study showed that potato psyllids of Central, Western, and Northwestern exhibit similar stylet probing behaviors and are equally efficient in transmitting Lso to potato, which put these insects at the same risk level of spreading zebra chip disease in potato crops. Results of this research also showed that it takes about 2-3 h for psyllids of the three haplotypes to reach the phloem tissue of potato following the first stylet probe and less than 10 min to successfully inoculate Lso into potato plants. This rapid transmission of Lso to potato by psyllids pose serious challenges to managing potato psyllids to prevent zebra chip damage to potato crops, as insecticide applications targeted against the psyllids may not kill them fast enough to prevent Lso transmission. Therefore, it is recommended that other more effective psyllid management strategies for potato psyllid be developed, including use of antifeedent products and host plant resistance.
REFERENCES


Figure 1. EPG waveforms produced by psyllids of the three potato psyllid (*Bactericera cockerelli*) haplotypes during the Intracellular Stylet Penetration/Pathway Phase (C) and Initial contact with Phloem Tissue (D). The waveforms are compared to characteristic EPG waveforms identified and described by Pearson et al. (2014).
Figure 2. EPG waveforms produced by psyllids of the three potato psyllid (Bactericera cockerelli) haplotypes during the ingestion of xylem sap (G) variants (Gv1 and Gv2). The waveforms are compared to characteristic EPG waveforms identified and described by Pearson et al. (2014).
Figure 3. EPG waveforms produced by psyllids of the three potato psyllid (*Bactericera cockerelli*) haplotypes during the salivation into phloem sieve elements (E1), and ingestion of phloem sap (E2). The waveforms are compared to characteristic EPG waveforms identified and described by Pearson et al. (2014).
Figure 4. Average number of different stylet probing behavior events and average time spent in each probing behavior event in 24 h by individual psyllids of each of the three potato psyllid (Bactericera cockerelli) haplotypes.
Figure 5. Average time it took individual potato psyllids (Bactericera cockerelli) of each of the three haplotypes to make the first Probe (C), first Phloem Salivation (E1), and first Phloem Ingestion (E2).
Figure 6. Average ‘Candidatus Liberibacter solanacrum’ (Lso) transmission rate among potato psyllids (*Bactericera cockerelli*) of each of three psyllid haplotypes (Central, Western, and Northwestern). The psyllids were given inoculation access period of 1, 2, 3, 4, 5, 6, 12, and 24 h.
Table 1. Minimum inoculation time required for successful inoculation of *Candidatus Liberibacter solanacearum* (Lso) by a single potato psyllid (*Bactericera cockerelli*), estimated using two selected Lso-infected psyllids for each of the three haplotypes that had access to potato plants for 3 h or less, exhibited a single event of phloem salivation (E1), whose exposed potato plants developed zebra chip symptoms and/or tested positive for the bacterium, and had the lowest inoculation time.
CHAPTER FIVE

GENERAL CONCLUSIONS

Potatoes grown in the United States, Mexico, Central America, and New Zealand are threatened by a newly discovered bacterial disease known as zebra chip. The disease causal agent, *Candidatus Liberibacter solanacearum* (Lso) is vectored by potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae). The potato psyllid is an economic pest of solanaceous crops in North and Central America, and (as an introduction) in New Zealand. The psyllid feeds and completes its life cycle on over 40 host species including cultivated and wild solanaceous host plants. Recent discovery of four genetic variants (haplotypes) of potato psyllid, on the basis of their mitochondrial gene (CO1 gene), may complicate management of the insect, if differences in biological traits, including development, reproduction, and Lso transmission efficiency among the psyllid haplotypes. In addition, bittersweet nightshade is now recognized as a critical resource for potato psyllid overwintering in the Pacific Northwest.

Currently, the only means to effectively manage zebra chip is by targeting potato psyllid for control. Potato psyllid management in Pacific Northwest may be made more difficult due to the presence of different psyllid haplotypes. Advances in understanding potato psyllid haplotype biology and in defining suitability of bittersweet nightshade for potato psyllids of each haplotype will help the potato industry in the Pacific Northwest make informed decisions concerning the need for psyllid controls as components of their pest management programs. Our research evaluated the host effects of bittersweet nightshade and potato on developmental and
reproductive traits of the three potato psyllid haplotypes (Central, Western, and Northwestern) commonly found in Pacific Northwest potato crops. In addition, feeding and Lso transmission biology of psyllids of the three haplotypes was assessed, using the EPG technology.

The results show that the development times were longer for psyllids reared on nightshade than on potato. The duration of the pre-oviposition period, egg incubation requirements, nymphal development time, and total developmental time was higher on bittersweet nightshade compared to potato. The largest host effects were found for the Central haplotype, which exhibited a substantially extended preoviposition period by over 5 d on bittersweet nightshade compared to potato. The reproductive traits such as Fecundity differed significantly among haplotypes, with an average lifetime fecundity of 1050, 877, and 629 eggs respectively for Northwestern, Western, and Central females, respectively. Egg hatch was significantly reduced in psyllids reared on bittersweet nightshade (61.9%) versus potato (81.3%). Adult psyllids lived longer on nightshade than on potato, averaging 113.9 and 108.4 d on nightshade and 79.0 and 85.5 d on potato for males and females, respectively. However, the longer life span of psyllids on nightshade than potato failed to lead to higher fecundity, because females on nightshade often ended egg laying well before death, unlike those on potato. There was no evidence for any of the fitness traits to suggest that the locally resident haplotype (Northwestern) performed relatively better on nightshade than the other two haplotypes. Lastly, we examined whether mating between psyllids of different haplotypes affected sperm transfer and egg hatch rates. Females of the Northwestern haplotype failed to produce viable eggs when mated by males of either the Western or Central haplotypes.
Moreover, the results of stylet probing behaviors and Lso transmission efficiency of three potato psyllid haplotypes showed that the EPG waveforms produced by the psyllids of all the three haplotypes were similar in appearance, frequency, and duration, suggesting that they probe and feed in a same manner. Also, Lso transmission rate among the psyllids of the three psyllid haplotypes was similar. However, Lso transmission rate for each of the psyllid haplotypes significantly increased with inoculation access period. Average Lso transmission rate remained low (< 10%) during the first two hours but significantly jumped with 3 h inoculation access period (up to 40%) and was highest when the insects were allowed access to the plants for 24 h (up to 60%). Furthermore, it was determined that the actual inoculation time required for successful infection of potato plants with Lso was less than 10 min.

In conclusion, the results of current research will help increase understanding of potato psyllid haplotypes biology as it relates to zebra chip epidemiology and spread, leading to development of effective management strategies for this important potato disease.