The Role of Longleaf Groundcherry (*Physalis longifolia*) in Zebra Chip Epidemiology

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Authorization to Submit Thesis

This thesis of Cesar Alejandro Reyes Corral submitted for the degree of Master of Science with a Major in Plant Pathology and titled "The Role of Longleaf Groundcherry (*Physalis longifolia*) in Zebra Chip Epidemiology," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

The potato psyllid (*Bactericera cockerelli*) has been considered a major economic pest of potato (*Solanum tuberosum*) for over a decade because it is associated with the "psyllid yellows" crop condition and more importantly, it is a vector of the bacterial Zebra Chip pathogen, "*Candidatus* Liberibacter solanacearum". This psyllid develops on a wide range of host plants within the Solanaceae and Convolvulaceae and exists as distinct haplotypes that differ in host plant preference and use. In-field management of *B. cockerelli* using insecticides is not effective because *B. cockerelli* exists on a whole landscape level and recolonizes fields from non-crop hosts. Perennial weeds provide a 'bridge' between winter dormancy and the period when potato and annual weeds are available, which allow large populations of psyllids to accumulate and eventually move into potato fields during the growing season. Previous research on psyllid ecology focused primarily on the role of the perennial host plants, *Lycium barbarum* (matrimony vine) and *Solanum dulcamara* (bittersweet nightshade), as sources of psyllids colonizing potato. Both plant species are hosts for *B. cockerelli* but might not necessarily be the driving force of zebra chip epidemiology in Idaho; *L. barbarum* does not appear to be a host for "*Candidatus* Liberibacter solanacearum" while *S. dulcamara* is largely limited to riparian zones. *Physalis longifolia* (longleaf groundcherry) is a perennial non-crop solanaceous weedy host native to the East of the Rocky Mountains and found in all counties and states in the Pacific Northwest but has been largely overlooked by researchers as a potential host for *B. cockerelli* and Liberibacter. This thesis reports results from a series of field, laboratory and greenhouse experiments conducted during 2018 and 2019 to assess the host quality of *P. longifolia* to *B. cockerelli* and the zebra chip pathogen, *Candidatus* Liberibacter solanacearum. This thesis includes three independent chapters that are organized for submission to peer-reviewed journals.

Chapter 1 reports results of *B. cockerelli* preference and performance assays conducted in laboratory and greenhouse settings. These assays demonstrated that *P. longifolia* is a better host than potato for *B. cockerelli*. Psyllids produce more offspring on *P. longifolia* than on potato and prefer *P. longifolia* over potato when given a choice. Plant disease susceptibility and insect disease acquisition assays showed that *P. longifolia* is highly susceptible to Liberibacter. The rhizomes of infected *P. longifolia* plants successfully overwinter and produce infected plants before potato is available, potentially allowing psyllid colonization and acquisition of the pathogen before potato is available.

Chapter 2 reports results of a field survey conducted during 2018 and 2019 to confirm that *B. cockerelli* readily colonizes wild stands of *P. longifolia.* Over 200 psyllids were collected from wild *P. longifolia* stands in WA and ID, and many of these stands were adjacent to commercial potato fields. Molecular gut content analysis confirmed psyllid movement between *P. longifolia* and potato.

During 2019, I was approached by a cooperator from Saltillo, Mexico to examine whether *B. cockerelli* that were infesting a commercial field of *Physalis ixocarpa* (tomatillo) were infected with Liberibacter, and whether foliar symptoms exhibited by those plants were associated with the pathogen. Results confirmed presence of Liberibacter, and gut content analysis confirmed movement between non-crop solanaceous hosts and tomatillo in Saltillo, Mexico. Results of these surveys are reported in chapter 3.

Overall, the results presented provide evidence that *Physalis longifolia* is a metapopulation source for *B. cockerelli* and Liberibacter, which emphasizes the need to further evaluate more *Physalis* species and solanaceous weeds as hosts for *B. cockerelli*. Implications of these results could potentially help predict when and where infective psyllids will first colonize potato and develop landscape-level management tactics against *B. cockerelli* and Liberibacter.

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Dedication

To my friends, family and ferret who have stuck with me through thick and thin, without whom none of this would have been possible. Gracias totales.

Table of Contents

Authorization to Submit Thesis ii

Abstract iii

Acknowledgements v

Dedication vi

Table of Contents vii

List of Tables ix

List of Figures xi

Chapter 1: Susceptibility of *Physalis longifolia* Nutt. (Solanales: Solanaceae) to *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) and "*Candidatus* Liberibacter solanacearum" 1

- 1.1 Abstract 1
- 1.2 Introduction 1
- 1.3 Materials and Methods 4
  - 1.3.1 Sources of psyllids and plants 4
  - 1.3.2 *B. cockerelli* performance and preference 5
  - 1.3.3 Susceptibility to Liberibacter 7
  - 1.3.4 Diagnostic PCR and identification of *B. cockerelli* and "*Ca*. L. solanacearum" haplotypes 8
- 1.4 Results and Discussion 10
  - 1.4.1 *B. cockerelli* performance and preference 10
  - 1.4.2 Susceptibility to Liberibacter 11
- 1.5 Conclusions 13
- 1.6 Acknowledgements 13
- 1.7 References 14

Chapter 2: Using gut content analysis to confirm movement by *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) between potato and *Physalis longifolia* Nutt. (Solanales: Solanaceae) 31

- 2.1 Abstract 31
- 2.2 Introduction 31
- 2.3 Materials and Methods 33

2.3.1 Plant and Psyllid sources 33
2.3.2 Liberibacter detection and psyllid haplotyping 34
2.3.3 Molecular Gut Content Analysis 35
2.4 Results and Discussion 36
2.4.1 Field Surveys, Liberibacter Detection and Psyllid Haplotyping 36
2.4.2 Molecular Gut Content Analysis 37
2.6 Conclusions 38
2.7 Acknowledgements 38
2.8 References 39
Chapter 3: Detection of "*Candidatus* Liberibacter solanacearum" on *Physalis ixocarpa* Brot. (Solanales: Solanaceae) and Gut Content Analysis of *Bactericera cockerelli* Šulc (Hemiptera: Triozidae) in Saltillo, Mexico 52
3.1 Abstract 52
3.2 Introduction 53
3.3 Materials and Methods 54
3.3.1 Psyllid and Plant Sources 54
3.3.2 Liberibacter Detection and Haplotyping 54
3.3.3 Gut Content Analysis 55
3.4 Results and Discussion 56
3.4.1 Liberibacter Detection and Haplotyping 56
3.4.2 Gut Content Analysis 57
3.4.3 Conclusions 57
3.5 Acknowledgements 57
3.6 References 58

List of Tables

Table 1.1. Mean number (±S.E.) of *B. cockerelli* offspring present on plants 22 days after confining three females and one male on each plant. 23

Table 1.2. Number of *P. longifolia* plants displaying Liberibacter-associated foliar symptoms (severe yellowing), plants with Liberibacter-positive leaves, mean number (±S.E.) of fruit harvested, and seed weight per plant at the end of the 2018 growing season from both greenhouse and field assays. 24

Table 1.3. Number of re-sprouted *P. longifolia* plants, average Julian day for re-sprout, plants with Liberibacter-positive rhizomes, mean number (±S.E.) of fruit harvested, and seed weight per plant at the end of the 2019 growing season from both greenhouse (A) and field (B) assays. 25

Table 2.1. Location and site characteristics for wild *Physalis longifolia* stands monitored in Washington and Idaho. Washington site was monitored weekly and Idaho sites were monitored monthly. Asterisk denotes multiple stands on site. 47

Table 2.2. Number of *B. cockerelli* adults collected during the 2018 potato growing seasons. Asterisk denotes multiple stands on site. Only stands where psyllids were captured are included. 48

Table 2.3. Number of B. cockerelli adults collected during the 2019 potato growing seasons. Asterisk denotes multiple stands on site. 49

Table 2.5. Plant species, families and developmental hosts identified from *Bactericera cockerelli* collected during the 2019 growing season in Zillah, WA using a D-Vac. The proportion shows the number of samples with returned sequences using PCR primers ITS and trnF for each collection month. No returned sequences for the given PCR primer are denoted with (-). A threshold of 15 reads per plant species was implemented in order to ensure the validity of the results............................. 51

Table 2.6. Plant species, families and developmental hosts identified from *Bactericera cockerelli* collected during the 2018 growing season in Caldwell, ID using a D-Vac. The proportion shows the number of samples with returned sequences using PCR primers ITS and trnF for each collection month. No returned sequences for the given PCR primer are denoted with (-). A threshold of 15 reads per plant species was implemented in order to ensure the validity of the results............................. 52

Table 2.7. Plant species, families and developmental hosts identified from *Bactericera cockerelli* collected during the 2019 growing season in Caldwell, ID using a D-Vac. The proportion shows the number of samples with returned sequences using PCR primers ITS and trnF for each collection

month. No returned sequences for the given PCR primer are denoted with (-). A threshold of 15 reads per plant species was implemented in order to ensure the validity of the results. 53

Table 2.8. Number of different returned sequence combinations of solanaceous host plants and mean number of non-solanaceous transient plant families identified from *Bactericera cockerelli* samples collected in Zillah, WA during the 2018 and 2019 growing season. The numbers correspond to the returned sequences using PCR primer trnF. A threshold of 15 reads per plant species was implemented in order to ensure the validity of the results. 54

Table 2.9. Number of different returned sequence combinations of solanaceous host plants and mean number of non-solanaceous transient plant families identified from *Bactericera cockerelli* samples collected in Caldwell, ID during the 2018 and 2019 growing season. The numbers correspond to the returned sequences using PCR primer trnF. A threshold of 15 reads per plant species was implemented in order to ensure the validity of the results. 55

Table 3.1. Returned sequence combinations of solanaceous host plants and mean number of transient plant families identified via gut content analysis of *Bactericera cockerelli* samples collected in Saltillo, Mexico during November 2019. A threshold of 15 reads per plant species was implemented in order to ensure the validity of the results. 67

List of Figures

Fig. 1.1. Timeline of greenhouse and field Liberibacter susceptibility assays during the 2018 and 2019 growing seasons. 26

Fig. 1.2. (A) Mean number (±S.E.) of *B. cockerelli* offspring present on plants 22 days after confining three females and one male on each plant. (B) Weighted mean age index (±S.E.). Different letters denote significant differences among means. 27

Fig. 1.3. (A) Mean number (±S.E.) of ovarioles found in *B. cockerelli* F1 generation females after 21 days of development on *P. longifolia* and potato plants, (B) proportion of mature ovarioles found on female psyllids developed on potato and *Physalis longifolia,* and representative photos of ovarioles from a female that developed on potato (C) and *Physalis* (D). 28

Fig. 1.4. Proportion of total no. of *B. cockerelli* adults (A) and eggs (B) found on *P. longifolia* and potato plants 48 h after release in preference assays. 29

Fig. 1.5. Comparison between uninfected and Liberibacter-infected *P. longifolia* leaves developing foliar symptoms (severe yellowing) within two weeks after inoculation (A), re-sprouted *P. longifolia* plants in greenhouse Liberibacter susceptibility assays showing the growth difference between a control plant and haplotype A and B Liberibacter-inoculated plants (B), development of brown coloring stripes in *P. longifolia* rhizomes (C). 30

Fig. 2.1. Herbaria records (orange markers) showing the presence of *Physalis longifolia* in the Pacific Northwestern United States (http://www.pnwherbaria.org). The red shaded areas represent the primary potato growing regions in the Pacific Northwest. 56

Fig. 2.2. Orange marks denoting the location of collection sites in Zillah, WA (A) and Caldwell, ID (B) during the 2018 and 2019 potato growing season. All collection sites in Caldwell, ID are located within 1 km from potato fields 57

Fig. 2.3. *Physalis* stand in Zillah, WA during mid-July 2019 (A), *Physalis longifolia* growing next to commercially grown potato fields in Caldwell, ID (B), undisturbed “insect nurseries” (red circle) between potato circles hosting a wide variety of plant species including *P. longifolia* (C). 50

Fig. 3.1. *Physalis ixocarpa* plants in an experimental plot at the Universidad Autonoma Agraria Antonio Narro in Saltillo, Mexico during November 2019 (A). Close-up of *P. ixocarpa* exhibiting severe yellowing and curling symptoms (B). 68

Chapter 1: Susceptibility of *Physalis longifolia* Nutt. (Solanales: Solanaceae) to *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) and "*Candidatus* Liberibacter solanacearum"

1.1 Abstract

The potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) is a major pest of potato (*Solanum tuberosum* L.; Solanales: Solanaceae) as a vector of the pathogen that causes zebra chip disease, "*Candidatus* Liberibacter solanacearum". This psyllid occurs as several distinct haplotypes – Central, Northwestern, and Western – that differ in biological traits including host plant preference. The pathogen also occurs as several distinct haplotypes that differ in disease virulence. The objective of this study was to assess the suitability of longleaf groundcherry (*Physalis longifolia* Nutt.; Solanaceae) to the three haplotypes of *B. cockerelli* and the two haplotypes of "*Ca.* L. solanacearum" that occur in western North America. This perennial plant has largely been overlooked in prior research as a potential non-crop source of Liberibacter-infected *B. cockerelli* colonizing fields of potato. The results from preference, performance and fecundity assays of this study indicated that *B. cockerelli* of all three haplotypes produce more offspring on *P. longifolia* than on potato and prefer *P. longifolia* over potato when given the option. Laboratory studies also showed that *P. longifolia* is highly susceptible to Liberibacter and that infected rhizomes survive winter and produce infected plants in late spring that are then available for psyllid colonization and pathogen acquisition. Overall, results of this study suggest that *P. longifolia* is highly susceptible to both *B. cockerelli* and "*Ca.* L. solanacearum" and warrant further research to determine whether this plant is a source of infective *B. cockerelli* moving into potato fields.

Key Words: potato psyllid, *Bactericera cockerelli*, *Physalis longifolia*, Liberibacter

1.2 Introduction

The potato psyllid, *Bactericera cockerelli* (Šulc), (Hemiptera: Triozidae) is a major pest of solanaceous crops including potato, tomato, and pepper in Western North America and New Zealand. Large populations of nymphs cause a foliar disorder known as "psyllid yellows" that is thought to be caused by the phytotoxic effects of salivary proteins that are discharged into the plant phloem. The psyllid is also a vector of the bacterial plant pathogen, "*Candidatus* Liberibacter solanacearum"

(synonym *"Ca.* L. psyllaurous") (Hansen et al., 2008; L. W. Liefting et al., 2008). Liberibacter infection causes foliar dieback in plants known as "permanente" in all solanaceous crops examined and causes the production of discolored potato tubers known as zebra chip disease. There are no methods to directly control Liberibacter, so management of zebra chip disease currently relies upon prophylactic applications of insecticides to suppress populations of the insect vector.

A major challenge for the management of *B. cockerelli* and zebra chip disease is uncertainty of when or where Liberibacter-infected *B. cockerelli* will first colonize potato, and the inability to predict the risk of *B. cockerelli* infestation and Liberibacter infection during any given year. *Bactericera cockerelli* develops on a diversity of native and introduced plants within the Solanaceae and Convolvulaceae (Cooper et al., 2019; Horton et al., 2015; Kaur et al., 2018; Wallis, 1955). The diversity of available hosts varies over the large geographic distribution of the psyllid, with areas of high host plant diversity in Mexico and the southwestern United States and areas of lower host diversity in the Pacific Northwest. Many of these non-crop plants are also susceptible to "*Ca.* L. solanacearum" (Cooper et al., 2019). Identification of which non-crop host plants are most important for regional epidemiology of zebra chip disease will support the development of models to predict the risk for disease outbreaks and the development of areawide management of the psyllid vector.

The introduced perennial plants, matrimony vine (*Lycium barbarum* L.; Solanaceae), bittersweet nightshade (*Solanum dulcamara* L.; Solanaceae), and field bindweed (*Convolvulus arvensis* L.; Convolvulaceae), have been recently examined as potential early-season sources of psyllids colonizing potato in the growing regions of the Pacific Northwest (Washington, Oregon, and Idaho) (Cooper et al., 2020; Murphy et al., 2013; Thinakaran et al., 2017b; Wenninger et al., 2019a). Research in this region has focused particularly on these perennial plants because they provide *B. cockerelli* with an early and late season refugia when annual plants including potato are not available (Horton et al., 2016; 2015). Although previous reports provide evidence that these plants are likely sources of *B. cockerelli* colonizing potato, it is yet unclear whether these plants are sources of Liberibacter-infected psyllids. *Lycium barbarum* is common in the potato growing regions of Washington State and has phenotypic traits that force psyllids to disperse in mid-summer when potato is most susceptible to infestation (Cooper et al., 2020; Horton et al., 2016, 2015; Thinakaran et al., 2017b). However, there are conflicting reports in the literature about whether *L. barbarum* is susceptible to Liberibacter (Cooper et al., 2019; Galaviz et al., 2010; Henne et al., 2010; Wen et al., 2009), and this plant is not commonly found in the potato growing regions of Idaho where the incidence of zebra chip disease is

greater compared with the Columbia Basin of Washington (Dahan et al., 2019; Swisher et al., 2015; Wenninger et al., 2017, 2019b). *Solanum dulcamara* is susceptible to the pathogen (Cooper et al., 2019; Murphy et al., 2013; Swisher et al., 2013b), but psyllids occurring on *S. dulcamara* do not appear to readily disperse to potato (Fu et al., 2017; Swisher et al., 2013a, 2015). *Convolvulus arvensis* is a widely distributed host that is common in the potato growing regions of the Pacific Northwest, but is a poor host of the psyllid and does not appear to be susceptible to Liberibacter (Cooper et al., 2019; Sengoda et al., 2013; Torres et al., 2015; Wenninger et al., 2019b). This lack of clear evidence that these perennial host plants of *B. cockerelli* are sources of Liberibacter-infected psyllids colonizing potato suggests that a currently unrecognized plant may be serving this role.

Longleaf groundcherry (*Physalis longifolia* Nutt.; Solanaceae) is a perennial plant that has been largely overlooked as a potential non-crop source of potato-colonizing psyllids in the Pacific Northwest. *Physalis longifolia* propagates aggressively via an extensive rhizome system and is considered to be a threat to cultivated fields due to its high resistance to herbicides and adaptation abilities (Schaeffer et al., 1963; Yeoumans, 1962). According to herbaria records (*Consortium of Pacific Northwest Herbaria Specimen Database*, 2019) and personal observations made during field surveys conducted in 2018 and 2019 (Reyes Corral et. al., unpublished), *P. longifolia* occurs extensively throughout the Pacific Northwest, but is more abundant in the potato growing regions of Idaho than in the Columbia Basin of Washington and Oregon. Previous reports have indicated that *B. cockerelli* develops on *P. longifolia* and that these plants are susceptible to Liberibacter (Cooper et al., 2019; Wallis, 1955), but those reports relied upon mostly qualitative observations. Rigorous comparisons of host quality of *P. longifolia* and potato for the vector and pathogen are lacking. Additionally, *P. longifolia* has only been shown to be susceptible to Liberibacter, but symptoms associated with Liberibacter infection of *P. longifolia* have not yet been described.

Another challenge to the management of *B. cockerelli* and Liberibacter is the occurrence of different haplotypes of both the vector and pathogen (Horton et al., 2015). The psyllid occurs as biologically distinct haplotypes named for the regions within the United States from which they were first identified and where they predominately occur: Central, Western, and Northwestern (Swisher et al., 2012). The Central haplotype primarily occurs east of the Rocky Mountains from Mexico to south-central Canada and Western haplotype occurs west of the Rocky Mountains from the Baja peninsula to Washington State. Relative to the Central and Western haplotypes, the Northwestern haplotype appears to have a narrower geographic range which may be limited to the Pacific Northwest. All three

haplotypes occur in the potato growing regions of Washington, Oregon, and Idaho. These three haplotypes differ in certain biological traits including host plant preference and use (Cooper et al., 2019; Mustafa et al., 2015a,, 2015b, 2015c; Swisher et al., 2013b). For example, the Northwestern haplotype appears to prefer *S. dulcamara* over potato under field conditions and does not survive on hairy nightshade (*S. physalifolium*), whereas psyllids of the Western haplotype commonly occurs on potato and completes development on hairy nightshade (Cooper et al., 2019; Swisher et al., 2013a). *"Ca.* L. solanacearum" also occurs as distinct haplotypes, with two haplotypes – Liberibacter haplotypes A and B – of primary importance in the United States. Both Liberibacter haplotypes cause disease symptoms in potato and other crops, but Liberibacter haplotype B appears to be more virulent than haplotype A (Harrison et al., 2019; Mendoza-Herrera et al., 2018; Swisher et al., 2018). It is therefore important to consider haplotype variability when assessing the susceptibility of potential weedy hosts to *B. cockerelli* and Liberibacter.

This study had two primary objectives. First, *P. longifolia* was examined as a host for the three haplotypes of *B. cockerelli* that occur in the Pacific Northwest. For this objective, greenhouse assays were used to compare survival, development, and fecundity of *B. cockerelli* on *P. longifolia* and potato. Choice assays were used to examine whether *B. cockerelli* exhibits a preference for *P. longifolia* or potato. In addition, the susceptibility of *P. longifolia* to both Liberibacter haplotypes A and B was assessed and disease symptoms caused by Liberibacter on this plant were described.

1.3 Materials and Methods

1.3.1 Sources of psyllids and plants

Liberibacter-infected and non-infected *B. cockerelli* of the Central, Western and Northwestern haplotypes were obtained from colonies raised at the USDA-ARS research station in Wapato, WA. The colonies were maintained at 25°C with a 16:8 (L:D) h photoperiod on potato ('Russet Burbank'). Psyllid haplotypes and the presence or absence of Liberibacter within colonies was periodically tested as described below.

Potato 'Ranger Russet' plants were grown from seed tubers obtained from 3 Rivers Potato Services (Pasco, WA) during the 2018 and 2019 growing seasons. *Physalis longifolia* plants were originally grown from seed collected from wild stands located in Caldwell, ID. Voucher specimens were submitted to the Stillinger Herbarium (Accession # 181247, barcode ID186547) at the University of

Idaho, Moscow, ID and the Burke Museum of Natural History and Culture (voucher numbers pending) at the University of Washington, Seattle, WA. Digital records of the specimens can be found in the Consortium of Pacific Northwest Herbaria (http://www.pnwherbaria.org). Plants used in experiments were established by propagating 3.5 cm rhizome cuttings from established plants maintained in a greenhouse (Hitchcock & Clothier, 1898; K. Kindsher, personal communication, March 12, 2018; Yeoumans, 1962;). The rhizome cuttings were soaked in gibberellic acid (200 ppm in water, J.L. Hudson Seedsman, La Honda, CA) for 24 h prior to establishment in soil in order to ensure uniform re-sprout from each batch of rhizome cuttings. Plants were maintained in a greenhouse at 25°C with a 16:8 (L:D) h photoperiod. Potato and *P. longifolia* were used in experiments when the plants were between 15-20 cm tall. Plants were maintained in pots containing commercial potting soil (Miracle-Gro Moisture Control Potting Mix, Scotts Company, Marysville, OH).

1.3.2 *B. cockerelli* performance and preference

Performance assays were conducted in a greenhouse at 25°C with a 16:8 (L:D) h photoperiod to assess whether *P. longifolia* is a suitable host plant for *B. cockerelli*. Four psyllids (three ♀ and one ♂) of the Central, Western or Northwestern haplotypes were released onto each of 10 potato or 10 *P. longifolia* plants (60 plants total). Psyllids were confined to plants using mesh cages. The number of eggs, early instars (1st - 3rd), late instars (4th – 5th), newly emerged adults and sclerotized adults per plant were counted after 22 days. Psyllid development was estimated using the weighted mean age on each plant (Pfeiffer & Burts, 1983) with eggs assigned a value of 1, early instars assigned a value of 2, late instars assigned a value of 3, newly emerged adults a value of 4 and sclerotized adults assigned a value of 5. The experiment was conducted twice (trial). Data were analyzed using SAS 9.4 (PROC GLIMMIX) with treatment, psyllid haplotype, and their interaction as fixed effects and trial as a random variable. Count data were analyzed assuming a Poisson distribution (DIST=P of the MODEL statement) and the weighted mean age was analyzed assuming a Gaussian distribution (DIST=G). The validity of the models was assessed by examining the fit statistics (generalized chi square/df). When the overall treatment effects were significant, differences among means were determined using Tukey's test for multiplicity (ADJUST=Tukey option of the LSMEANS statement). The SLICEDIFF option of the LSMEANS statement was included to compare means within main effect factors (i.e. psyllid haplotype or plant host) when the interaction term did not indicate significance.

Lifetime fecundity of psyllids reared on *P. longifolia* and potato was estimated by counting the number of ovarioles present in newly emerged adults (Hopper et al., 2013; Uechi & Iwanami, 2012). This experiment was performed using only psyllids of the Central haplotype because Liberibacter-uninfected psyllids of the other two haplotypes were not available at the start of the experiment. Three psyllids (two ♀ and one ♂) were released onto each of 10 potato or 10 *P. longifolia* and the development of the resulting offspring was monitored daily for the presence of newly emerged adults. Three newly emerged females from each plant (60 females total) were dissected under magnification in phosphate-buffered saline (Cooper et al., 2014). Images of the ovarioles were captured using a scope-mounted digital camera (OLYMPUS DP47) connected to a computer imaging processing software (OLYMPUS cellSens Standard 2.1). The number of ovarioles present in each female was counted as a measure of lifetime fecundity (Hopper et al., 2013; Uechi & Iwanami, 2012). The presence of mature versus immature eggs was also recorded for each female. The average number of ovarioles per female collected from each plant was compared between psyllids reared on potato or *P. longifolia* using analysis of variance while the proportion of psyllids from each plant with mature eggs was assessed using a logistic regression. Both analyses were performed using PROC GLIMMIX of SAS 9.4.

Greenhouse assays were conducted to assess whether *B. cockerelli* shows preference when given a choice between potato and *P. longifolia*. A potato and *P. longifolia* plant were both placed into each of three 20 L plastic containers that were modified to allow ventilation and watering. Twenty mixed-gender psyllids of the Central, Western or Northwestern haplotype were released in the center of each plastic container. Preference by each haplotype was assessed in separate experiments. Buckets were maintained for 48 h in a greenhouse at 25°C with a 16:8 (L:D) h photoperiod. The number of psyllids present on each plant was counted inside a 4°C cold room in order to ensure psyllid latency for counting. The experiment was conducted twice (trial) and psyllid preference was analyzed using logistic regression (PROC GLIMMIX). In separate analyses, the proportion of adults or eggs on each plant to the total number of adults or eggs within each cage was included as dependent variables. In each analysis, treatment, psyllid haplotype, and their interaction were included as the fixed effects. The RANDOM statement designated cage as the experimental unit. When the overall statistic indicated significant differences among treatments, the SLICEDIFF option of the LSMEANS statement was used to determine treatment differences within each haplotype.

1.3.3 Susceptibility to Liberibacter

Susceptibility to infection by Liberibacter was compared between *Physalis* and potato plants in a non-heated detached greenhouse during the 2018 and 2019 growing seasons (Fig. 1.1). Plants grown in 19 L pots were infected with either Liberibacter haplotypes A or B by confining three Liberibacter-infected psyllids (two ♀ and one ♂) on leaves for one week. After the one-week inoculation access period, the cages were removed by excising the leaf covered with the sleeve cage. Leaf samples from each plant were collected every two weeks (10 weeks total) after inoculation to test for the presence of Liberibacter in leaf tissue. The ability for psyllids to acquire Liberibacter from inoculated plants was examined by confining five non-infected psyllids to each plant using sleeve cages one month after inoculation. Psyllids were removed from the plants two weeks later and each group was tested for the presence of Liberibacter.

Potato tubers were harvested at the end of 2018 and cut to observe the presence or absence of zebra chip symptoms. The data collected from potato plants was used to confirm that infective psyllids were used in the assays and that they successfully transmitted Liberibacter to plants. *P. longifolia* plants were overwintered in the non-heated greenhouse after the growing season of 2018. The plants were insulated with straw placed around the plant pots. The following spring, plants were monitored for re-sprout, and leaf samples of re-sprouted plants were collected weekly (8 weeks total) and tested for the presence of Liberibacter. The ability for psyllids to acquire Liberibacter from inoculated plants was retested in 2019 as described for 2018.

A field experiment was also conducted to examine the susceptibility of *P. longifolia* to Liberibacter haplotype B. Potato and *P. longifolia* plants were transplanted to field plots located at the USDA experimental farm in Moxee, WA. Plants were inoculated with Liberibacter haplotype B by confining three infected psyllids (two ♀ and one ♂) to each plant as described for the greenhouse assay. Leaf samples from each plant were collected every two weeks (10 weeks total) after inoculation to test for the presence of Liberibacter in leaf tissue. Plants were also monitored for the development of foliar symptoms associated with Liberibacter infection. Re-sprout of *Physalis* was monitored during the spring of 2019, and leaf tissue was collected every week (8 weeks total) from the new plants and tested for presence of Liberibacter using PCR (described below).

Fruit was collected and counted from plants using in both assays (greenhouse and field) in autumn of both 2018 and 2019 and seed mass was measured after drying the fruit. In autumn of 2019, rhizomes of *P. longifolia* plants used in both assays were removed from the soil and cut to observe the

presence or absence of zebra chip-like symptoms. Rhizomes were also tested for the presence or absence of Liberibacter using diagnostic PCR. The number of fruit and the seed weight was compared among treatments using SAS 9.4 with treatment as the fixed effect. Differences among means were assessed using the ADJUST=TUKEY option of the LSMEANS statement. Fruit count data were analyzed assuming a negative binomial distribution (DIST=NB option of the MODEL statement) and seed weight was analyzed assuming a gamma distribution (DIST=GAMMA).

1.3.4 Diagnostic PCR and identification of *B. cockerelli* and "*Ca*. L. solanacearum" haplotypes

DNA was extracted from both insect and plant samples by using a cetyltrimethylammonium bromide (CTAB) precipitation method (Zhang et al., 1998) with a few modifications. Each individual psyllid sample was placed in a 1.5 ml tube and ground using 600 µl of a CTAB and β-mercaptoethanol (ME) mix (2 µl of ME per ml of CTAB) and a micropestle. Samples were then incubated for 15 min at 65°C using a water bath. 600 µl of cold chloroform was then added to each sample and vortexed until an emulsion was formed. Samples were spun once again for 3 min at 20,000 x G and 500 µl of the upper aqueous phase (DNA layer) was transferred to a 1.5 ml tube containing 500 µl of isopropanol and 1 µl of GlycoBlue co-precipitant (15 mg/mL, Thermo Fisher Scientific, Waltham, MA). Following a 10 min centrifuge spin at 20,000 x G, the precipitated DNA pellets were washed using 500 µl of 70% EtOH and a 3 min spin at 20,000 x G. While keeping the pellets and decanting most of the EtOH used for the wash, the pellets were dried at for 45 min in a 37°C incubator. Leaf tissue DNA extraction was a similar process, where 5 *P. longifolia* leaves per sample were ground in universal extraction bags (Bioreba AG, Kanton Reinach, Switzerland), incubated for 30 min at 37°C in 300 µl of CTAB with 80 µl of lysozyme stock before incubating the samples again for 30 min at 65°C and adding 500 µl of CTAB + ME buffer. The following steps were carried out as mentioned previously for the psyllid samples. DNA extraction from rhizomes was achieved by freezing the samples in liquid nitrogen prior to grinding them using a mortar and pestle and following the leaf extraction protocol. Insect and plant samples were resuspended in 50 µl and 100 µl of nuclease-free water, respectively.

The presence or absence of Liberibacter in both insect and plant samples was assessed by diagnostic PCR (Crosslin et al., 2011) using primers OA2 (5′-GCG CTT ATT TTT AAT AGG AGC GGC A-3′) and OI2c (5′-GCC TCG CGA CTT CGC AAC CCA T-3′) to amplify a region of the 16S rRNA gene of Liberibacter. PCR conditions consisted of an initial denaturation step of 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s, followed by a final extension at 72°C for 5 min.

Each 20 µl reaction contained Amplitaq Gold 360 PCR Master Mix (Invitrogen, Carlsbad, CA), 5µM of each primer, and DNA template (10–20 ng/ml for insect samples and 50-150 ng/ml for plant samples). Liberibacter-positive samples from any of the insect and/or plant samples were classified as A or B based upon simple sequence repeat (SSR) markers (Lin et al., 2012b). PCR for SSR markers was performed in 25 µl reactions containing 0.5 µl of Advantage Taq polymerase (Takara Bio, Mountain View, CA), 2.5 µl of 10X Advantage buffer, 0.5 µl of 10 µM dNTPs, 0.5 µl of 100 nM of each SSR primer (Lso-SSR-1F/Lso-SSR-1R) and 5 µl of DNA template. PCR conditions for Liberibacter haplotyping consisted of an initial denaturation at 94°C for 5 min, followed by 40 cycles of 94°C for 10 s, 58°C for 10 s and 72°C for 15 s, with a 72°C final extension step for 5 min. The presence or size of amplicons was observed on a 1.5% agarose gel stained with ethidium bromide.

Psyllids were classified as Central, Western or Northwestern haplotype based upon sequences of cytochrome oxidase 1 gene (CO1) using a two-step process involving real-time quantitative PCR (qPCR) and High-Resolution Melting (HRM) analysis (Smith et al., 2010; Swisher et al., 2012). The analysis of the samples was conducted using a Lightcycler 480 (Roche Applied Science, Indianapolis, IN). Plates were loaded with 20 µl reactions consisting of 10 µl of Lightcycler 480 HRM Master Mix, 0.5 µl of each 20 µM primer, 5.8 µl of DNA-free water, 1.6 µl of 25 mM $MgCl_2$, and 1.6 µl of DNA template. Two different sets of primers were used in order to successfully classify the psyllid haplotypes (Chapman et al., 2012; Swisher et al., 2012). The first set of primers CO1 F3 (5'-TAC GCC ATA CTA GCA ATC GG-3') and CO1 meltR (5'-TGA AAT AGG AAT CAA-3') distinguish the Central haplotype from Northwestern, Western and Southwestern haplotypes. The second set of primers CO1 meltF (5'-GGA TTC ATT GTT TGA GCA CAT C-3') and CO1 meltR distinguish the Western and Southwestern haplotype from the Northwestern and Central haplotype. Cycling temperatures for both of the primer sets were: a preincubation step of 95°C for 10 min with a 4.4°C/s ramp rate following 35 amplification cycles consisting of 95°C for 10 s with 4.4°C/s ramp rate, 60°C with a second target of 53°C at a step size of 0.5°C with one cycle step delay for 15 s with 2.2°C/s ramp state and 72°C single acquisition for 25s with 4.4°C/s ramp rate. Melting conditions were set at 95°C for 1 s with a ramp rate of 4.4°C/s, 40°C for 1 s with a ramp rate of 2.2°C/s, 60°C for 20 s with a ramp rate of 1°C, 95°C for continuous acquisition at 25 acquisitions/°C with a cooling step of 40°Cfor 10 s with a ramp rate of 2.2°C/s. Results were analyzed using the LightCycler 480 Gene Scanning Software (Roche Applied Science) and manually classifying each sample based on melting curves from known haplotype standard samples.

1.4 Results and Discussion

1.4.1 *B. cockerelli* performance and preference

There was no significant haplotype by plant species interaction for analysis of the total number of offspring produced by *B. cockerelli* (*F*=1.3; d.f.=2, 100; *P*=0.279) indicating that the effects of host plant on the production of offspring was similar among the three psyllid haplotypes. Psyllids of the central haplotype produced fewer offspring (178.2 ± 32.40) than did psyllids of the northwestern haplotype (215.7 ± 39.21) and western haplotype (222.2 ± 40.41) regardless of plant host (*F*=1340.6; d.f.=1, 100; *P*=<0.001). There was also a significant difference in the total number of offspring produced by *B. cockerelli* on *P. longifolia* and potato (Fig. 1.2A; *F*=99.9; d.f.=2, 100; *P*=<0.001). Psyllids of all three haplotypes produced >30% more offspring on *P. longifolia* than on potato (Fig. 1.2A). This trend was observed for every life stage of the psyllid (eggs, early instars, late instars and adults) (Table 1.1).

There was no significant main effect interaction for analysis of the weighted mean age of psyllids (*F*=1.5; d.f.=2, 99; *P*=0.222). There were also no differences in age of psyllids developing on *P. longifolia* and potato (Fig. 1.2B; *F*=0.2; d.f.=1, 99; *P*=0.644) suggesting that the psyllids had similar development rates on these two host plants. Psyllids of the Central haplotype developed quicker than those of the Northwestern and Western haplotype regardless of plant species (Fig. 1.2B; *F*=24.6; d.f.=2, 99; *P*=<0.001), which is consistent with the report by Mustafa et al. (Tariq Mustafa, Horton, et al., 2015).

There was a significant difference in the number of ovarioles present in females that developed on potato versus *P. longifolia* (*F*=6.98; d.f.=1, 18; *P*=0.017). Psyllids that developed on *Physalis* produced more ovarioles than the females developed on potato (Fig. 1.3A). These results suggest that psyllids which develop on *P. longifolia* have greater lifetime fecundity compared with those which develop on potato. The proportion of females with mature ovarioles did not differ significantly between those reared on *P. longifolia* or potato (Fig. 1.3B; *F*=2.38; d.f.=1, 17; *P*=0.142), although nearly 20% more female psyllids that were reared on potato had mature ovarioles within 2 days of adult eclosion compared with those reared on *P. longifolia* (Fig. 1.3B, C, and D).

There were also significant differences in adult settling by three psyllid haplotypes of the central (Central: *F*=169.9; d.f.=1, 5; *P*=<0.001, Western: *F*=67.2; d.f.=1, 5; *P*=<0.001, Northwestern: *F*=28.9; d.f.=1, 5; *P*=0.003). Nearly 80% of all adults, regardless of haplotype, preferred to settle on *P. longifolia* rather than on potato in the choice assays (Fig. 1.4A). The adults also oviposited more eggs

on *P. longifolia* than on potato (Fig. 1.4B; Central: *F*=169.9; d.f.=1, 5; *P*=<0.001, Western: *F*=88.4; d.f.=1, 5; *P*=<0.001, Northwestern: *F*=119.8; d.f.=1, 5; *P*=<0.001).

Results of these laboratory assays indicate that *P. longifolia* is a highly suitable host for *B. cockerelli* when compared with potato. *Bactericera cockerelli* produced more offspring on *P. longifolia* than on potato, and females that developed on *P. longifolia* as nymphs had a larger quantity of ovarioles than those that developed on potato suggesting a greater lifetime fecundity. In addition, psyllids preferred to settle and oviposit on *P. longifolia* than on potato in choice assays. These results are consistent with the report by Cooper et al. (2019) which examined whether psyllids of the Northwestern and Western haplotypes could complete development on *P. longifolia*, and with the observation by Wallis (1955) that *B. cockerelli* adults and nymphs were present on wild stands of *Physalis* spp. This study is the first to examine the suitability of *P. longifolia* for all three psyllid haplotypes and provides the first statistical comparison of host quality between *P. longifolia* and potato.

1.4.2 Susceptibility to Liberibacter

Greenhouse experiments were conducted to examine the susceptibility of *P. longifolia* to Liberibacter haplotypes A and B. Potato plants inoculated with psyllids infected with Liberibacter haplotypes A and B produced visible foliar and tuber symptoms confirming that psyllids used for inoculation transmitted the pathogen to experimental plants. Disease symptoms were not observed on uninfected potato or *P. longifolia* plants. Foliar symptoms were observed on *P. longifolia* plants within two weeks after inoculation with either Liberibacter haplotype (Table 1.2A). Symptoms were observed as severe yellowing of leaves (Fig. 1.5A) and stunted plant growth (Fig. 1.5B). Liberibacter was detected by diagnostic PCR in plants inoculated with haplotype B eight weeks after inoculation, but the pathogen was not detected from *P. longifolia* plants inoculated with haplotype A in 2018 despite the development of symptoms in these plants (Table 2A). Liberibacter infection also affected the production of fruit and seeds. Although plants inoculated with Liberibacter haplotype A produced about equal number of fruit as uninfected plants, those inoculated with haplotype B produced more than twice as many fruit as uninfected plants or plants infected with haplotype A (Table 1.2A; *F*=5.63; d.f.=2, 12; *P*=0.019). Despite the increased number of fruit per plant, plants infected with Liberibacter haplotype B produced less than half the seed dry weight produced by uninfected plants or plants infected with haplotype A (Table 1.2A; *F*=3.89; d.f.=2, 12; *P*=0.050).

The plants were maintained in a non-heated greenhouse over the winter to examine whether rhizomes produced by plants infected with Liberibacter emerged the following spring and to determine whether Liberibacter overwinters within the rhizomes of *P. longifolia*. Nearly all plants emerged the following spring from overwintered rhizomes regardless of infection status (Table 1.3A). Plants produced by infected rhizomes showed foliar symptoms as early as two weeks after plant emergence (Fig. 1.5A). Plants were not caged during these assays. Although no psyllids were observed on plants after overwintering, two uninfected plants from 2018 showed foliar symptoms in 2019. PCR confirmed that these two plants, and all plants inoculated with Liberibacter in 2018 via infected psyllids were indeed infected with Liberibacter in spring of 2019. PCR detected the presence of Liberibacter as soon as one week after plant emergence, and one week before the development of visible symptoms. Uninfected *B. cockerelli* were confined to the leaves of each plant in mid-summer to confirm that the psyllids can acquire the pathogen. Psyllids acquired both Liberibacter haplotypes from infected plants, which is consistent with a previous report on Liberibacter haplotype B (Cooper et al., 2019). At harvest, plants infected with Liberibacter in 2018 produced fewer fruit than did plants inoculated with uninfected psyllids in 2018 (Table 1.3A).

At the end of the 2019 assays (Figure 1.1), rhizomes were removed from the soil and cut to observe the presence or absence of visible symptoms. Zebra chip-like symptoms were observed in rhizomes produced by *P. longifolia* plants infected with Liberibacter (Fig. 1.5C; Table 1.3A). These symptoms were generally more severe in plants infected with Liberibacter haplotype B than in those infected with haplotype A (Fig. 1.5C), which is consistent with a previous reports where a higher degree of severity of the Liberibacter haplotype B over haplotype A was demonstrated in potato and tomato plants (Harrison et al., 2019; Mendoza-Herrera et al., 2018; Swisher et al., 2018).

Field experiments using Liberibacter haplotype B were conducted to confirm results of the greenhouse assays. As observed in the greenhouse, plants infected with Liberibacter haplotype B developed foliar symptoms characterized by severe yellowing and stunted growth (Tables 2.2B and 2.3B). Diagnostic PCR confirmed the presence of the pathogen in inoculated plants both study years (Tables 2.2B and 2.3B). Half of the infected plants and all the control plants emerged in the spring of 2019. Plants produced by infected rhizomes developed disease symptoms in foliage and in the rhizomes as described for the greenhouse assays.

Results of these studies demonstrate that *P. longifolia* is susceptible to both Liberibacter haplotypes A and B and that uninfected psyllids can acquire the pathogen from infected plants.

Symptoms associated with Liberibacter infection of *P. longifolia* are similar to those associated with infection in potato: severe yellowing of leaves and striped patterns in the underground rhizomes. More importantly, overwintered rhizomes produced by infected plants readily produce infected plants the following spring. This contrasts to reports using symptomatic potato tubers, which have low rates of resprout the following spring, and do not produce plants that are infected with Liberibacter (Swisher et al. 2019; but see Pitman et al. 2011). The production of infected plants from overwintered rhizomes could be an early season source of pathogen acquisition by psyllids before they disperse into potato.

1.5 Conclusions

Although widely distributed throughout Washington, Oregon and Idaho, *Physalis longifolia* has not been thoroughly examined as a source of infective psyllids into potato fields. *P. longifolia* was only considered a minor concern until 1945, where interest in its control increased due to its ability to spread quickly and densely, compete with crops and its resistance to 2,4-D (Schaeffer et al., 1963; Yeoumans, 1962). There were no recorded associations between *Physalis* spp. and *B. cockerelli* until 1955, when adult and nymphs were collected from several *Physalis* species including *P. longifolia* (Wallis, 1955). However, only recently have researchers considered *Physalis* as a potential source of *B. cockerelli* and *"Ca.* L. solanacearum" (Cooper et al., 2019; Horton et al., 2015). Results of these experiments demonstrate that *P. longifolia* is likely a better host than potato for all three haplotypes of *B. cockerelli and* is also preferred by psyllids in choice assays. More importantly, *Physalis* is highly susceptible to both Liberibacter haplotypes A and B and that Liberibacter overwinters in *P. longifolia* rhizomes, which produce infected plants the following spring. These results suggest *Physalis* as Liberibacter acquisition source for *B. cockerelli* even before the emergence of potato. This information will help researchers and growers to better predict zebra chip outbreaks and will improve management decisions for *B. cockerelli* and zebra chip disease in the future. This research warrants more extensive field-studies on the role of *Physalis* as a source of infective psyllids colonizing potato fields, which is the subject of Chapter 3 of my Thesis.

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Table 1.1. Mean number (±S.E.) of *B. cockerelli* offspring present on plants 22 days after confining three females and one male on each plant.

		Central	**Northwestern**	**Western**	**Combined Haplotype**
A) Eggs					
	Potato	28.5 ± 10.09	61.4 ± 21.85	63.4 ± 23.07	**48.07 ± 15.92 b**
	Physalis	62.3 ± 22	124.7 ± 43.9	125.1 ± 46.2	**99.1 ± 32.82 a**
	Combined host plant	**42.2 ± 14.15 b**	**87.5 ± 29.39 a**	**89.1 ± 30.56 a**	
	Haplotype: *F*= 14.2; d.f.=2, 97; *P*=<0.001				
	Plant Species: *F*= 28.7; d.f.=1, 97; *P* =<0.001				
	Interaction: *F*= 0.05; d.f.=2, 97; *P*=0.951				
B) Early Instars					
	Potato	27.5 ± 8.74	56.2 ± 18.27	62.4 ± 21.37	**45.8 ± 12.27 b**
	Physalis	39.8 ± 12.62	102.2 ± 32.31	82.5 ± 29.27	**69.5 ± 18.65 a**
	Combined host plant	**33.0 ± 9.19 b**	**75.8 ± 21.20 a**	**71.8 ± 21.30 a**	
	Haplotype: *F*=8.6; d.f.=2, 99; *P* =0.001				
	Plant Species: *F*=4.8; d.f.=1, 99; *P*=0.031				
	Interaction: *F*=0.3; d.f.=2,99; *P*=0.782				
C) Late Instars					
	Potato	54.6 ± 12.45	45.9 ± 10.80	37.9 ± 9.48	**45.7 ± 8.6 b**
	Physalis	93.0 ± 21.13	50.0 ± 11.41	76.6 ± 19.76	**70.9 ± 13.4 a**
	Combined host plant	71.3 ± 13.96 a	47.9 ± 9.50	53.9 ± 11.44	
	Haplotype: *F*=3.0; d.f.=2, 99; *P* =0.055				
	Plant Species: *F*=9.2; d.f.=1, 99; *P* =0.003				
	Interaction: *F*=1.6; d.f.=2, 99; *P* =0.205				
D) Adults					
	Potato	**24.3 ± 2.23 a**	**6.0 ± 0.75 b**	**7.0 ± 0.89 a**	10.0 ± 0.94
	Physalis	**27.4 ± 2.48 a**	**9.7 ± 1.04 a**	**8.8 ± 1.09 a**	13.3 ± 1.20
	Combined host plant	25.8 ± 2.21	7.6 ± 0.76	7.8 ± 0.82	
	Haplotype: *F*=248.4; d.f=2,100; *P* =<0.001				
	Plant Species: *F*=19.3; d.f.=1, 100; *P* =<0.001				
	Interaction: *F*=3.78; d.f.=2, 100; *P*=0.026				

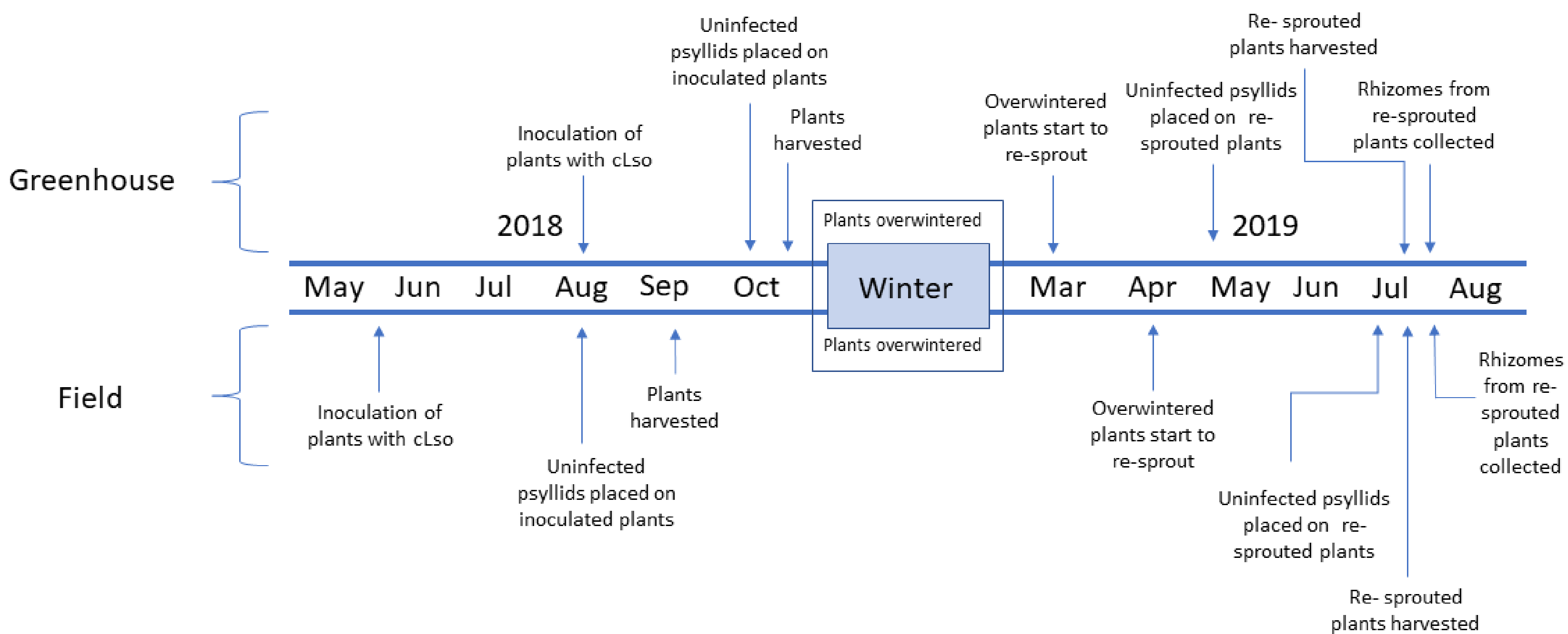
Table 1.2. Number of *P. longifolia* plants displaying Liberibacter-associated foliar symptoms (severe yellowing), plants with Liberibacter-positive leaves, mean number (±S.E.) of fruit harvested, and seed weight per plant at the end of the 2018 growing season from both greenhouse and field assays.

Treatment	Foliar symptoms	Liberibacter positive leaves	Fruit count	Seed weight (g)/plant
A) Greenhouse				
control	0/5	0/5	99.8 ± 41.24 b	15.1 ± 3.81 ab
cLso A	5/5	0/5	84.8 ± 17.19 b	21.6 ± 5.44 a
cLso B	5/5	3/5	207.0 ± 41.24 a	8.1 ± 2.04 b
B) Field				
Control	0/9	0/9	85.6 ± 33.39 a	57.7 ± 6.49 a
cLso B	2/9	2/9	85.3 ± 31.11 a	34.3 ± 3.60 b

Table 1.3. Number of re-sprouted *P. longifolia* plants, average Julian day for re-sprout, plants with Liberibacter-positive rhizomes, mean number (±S.E.) of fruit harvested, and seed weight per plant at the end of the 2019 growing season from both greenhouse (A) and field (B) assays.

2019						
Treatment	**Re-sprouted plants**	**Average Julian day**	**Lso + rhizomes**	**Rhizome symptoms**	**Fruit count**	**Seed weight (g)/plant**
A) GH						
control	5/5	83	1/5	2/5	53.0 ± 27.75 a	0.02 ± 0.001 a
cLso A	5/5	86	3/5	5/5	23.0 ± 21.17 b	0.02 ± 0.002 a
cLso B	4/5	91	3/4	5/5	0	0
B) Field						
Control	9/9	119	5/9	9/9	0	0
cLso B	5/9	113	5/9	9/9	0	0

Fig. 1.1. Timeline of greenhouse and field Liberibacter susceptibility assays during the 2018 and 2019 growing seasons.



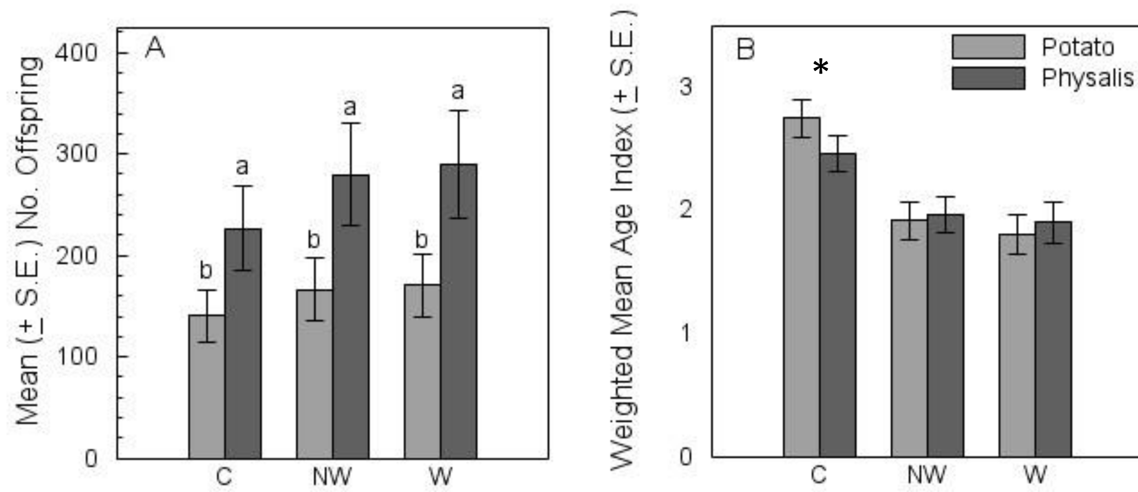


Fig. 1.2. (A) Mean number (±S.E.) of *B. cockerelli* offspring present on plants 22 days after confining three females and one male on each plant. (B) Weighted mean age index (±S.E.). Different letters denote significant differences among means.

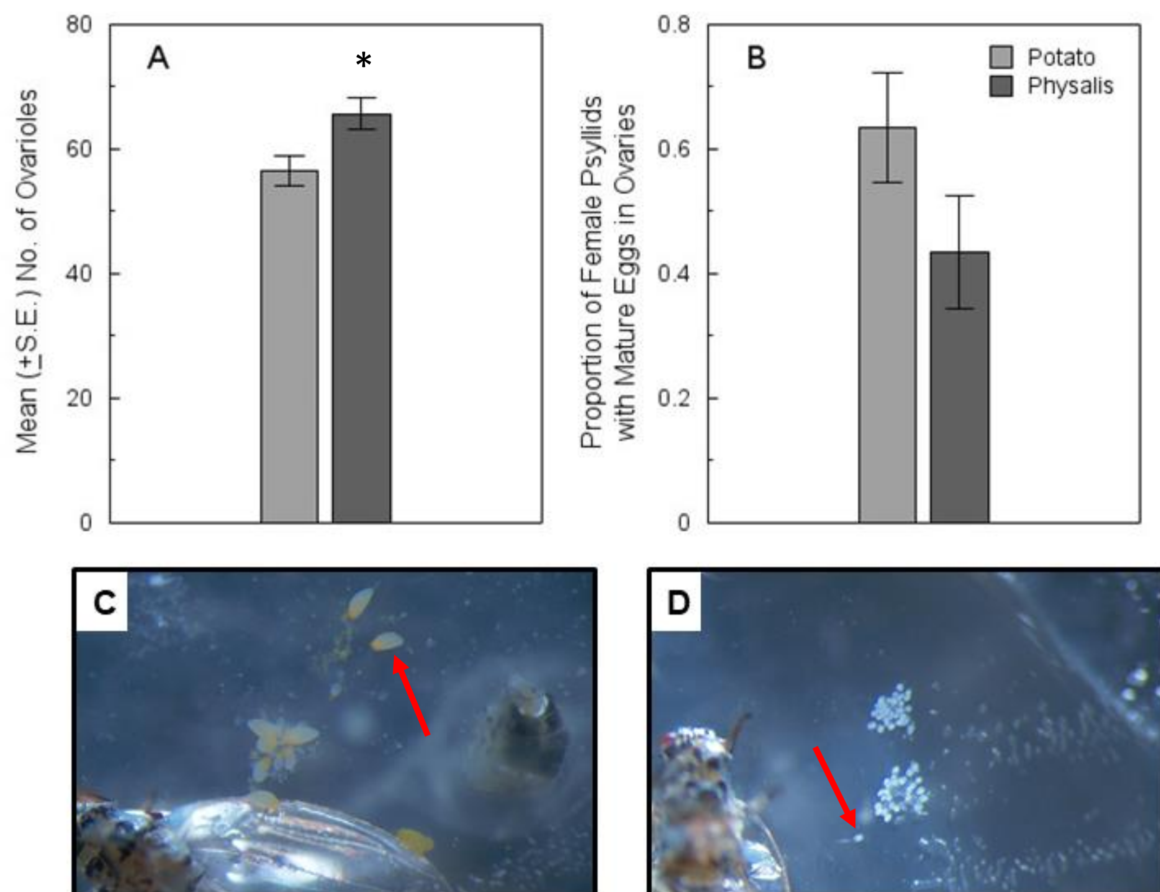


Fig. 1.3. (A) Mean number (±S.E.) of ovarioles found in *B. cockerelli* F1 generation females after 21 days of development on *P. longifolia* and potato plants, (B) proportion of mature ovarioles found on female psyllids developed on potato and *Physalis longifolia,* and representative photos of ovarioles from a female that developed on potato (C) and *Physalis* (D).

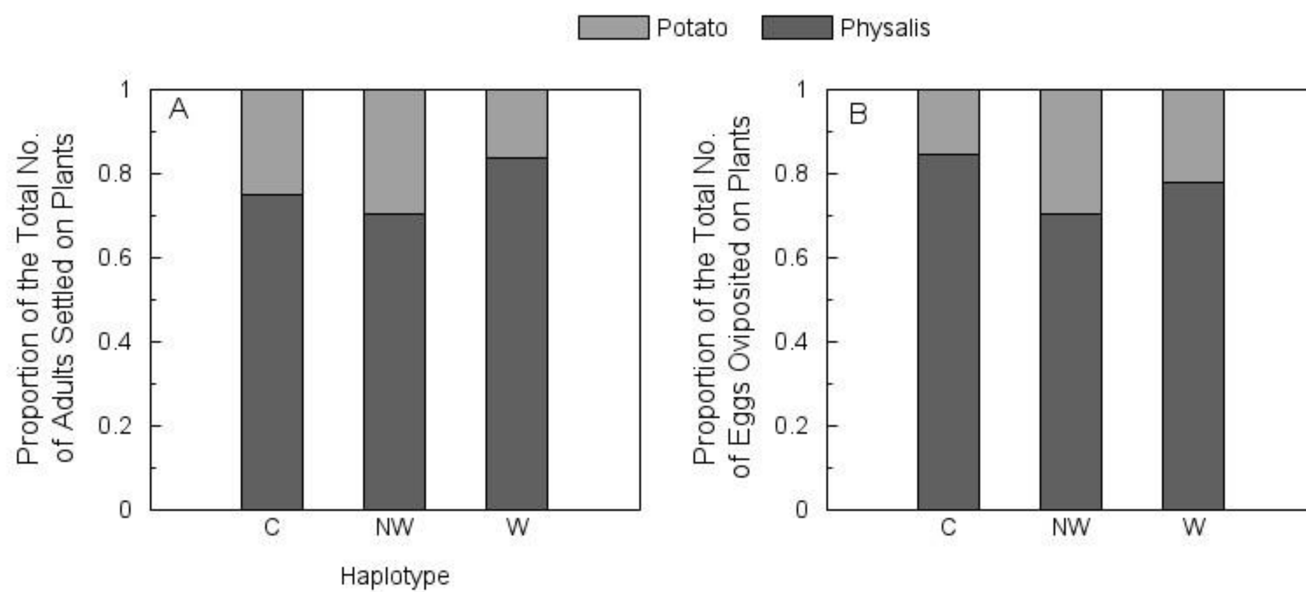


Fig. 1.4. Proportion of total no. of *B. cockerelli* adults (A) and eggs (B) found on *P. longifolia* and potato plants 48 h after release in preference assays.

Fig. 1.5. Comparison between uninfected and Liberibacter-infected *P. longifolia* leaves developing foliar symptoms (severe yellowing) within two weeks after inoculation (A), re-sprouted *P. longifolia* plants in greenhouse Liberibacter susceptibility assays showing the growth difference between a control plant and haplotype A and B Liberibacter-inoculated plants (B), development of brown coloring stripes in *P. longifolia* rhizomes (C).

Chapter 2: Using gut content analysis to confirm movement of *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) between potato and *Physalis longifolia* Nutt. (Solanales: Solanaceae)

2.1 Abstract

The potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a major pest of potato (*Solanum tuberosum* L.; Solanaceae) primarily because it is a vector to the bacterium associated with zebra chip disease, "*Candidatus* Liberibacter solanacearum". *Bactericera cockerelli* occurs not only on cultivated solanaceous crops, but on numerous uncultivated perennial species within the Solanaceae and Convolvulaceae. Understanding where infective psyllids come from has become an intricate task, as many psyllid pests colonize fields from non-crop hosts and transitory shelter plants. Recent controlled experiments demonstrated that longleaf groundcherry (*Physalis longifolia* Nutt.; Solanaceae) is a highly suitable host for *B. cockerelli* and the zebra chip pathogen, but evidence that wild psyllid populations occur on *P. longifolia* in the potato growing regions of Idaho is lacking. Over 200 psyllids were collected from *P. longifolia* plants located in WA and ID during the 2018 and 2019 potato growing seasons, confirming that *B. cockerelli* readily colonizes this non-crop host. High-throughput gut content analysis of plant-derived internal transcribed spacer (ITS) and the chloroplast *trnF* gene was used to identify the previous dietary history of psyllids and to infer their landscape movements prior to capture. Results of gut content analysis confirmed movement by *B. cockerelli* between *P. longifolia* and potato. This information could improve the ability to predict which fields are at risk of being colonized by psyllids.

Key words: potato psyllid, *Bactericera cockerelli*, *Physalis longifolia,* Liberibacter

2.2 Introduction

Potato (*Solanum tuberosum* L.; Solanaceae) is an economically important crop in the Pacific Northwest (PNW) of the United States. However, this crop is constantly challenged by pests and diseases, which result in significant losses for growers. One of the most important pests associated with potato is the potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), which is a vector to the bacterium that is commonly associated with zebra chip disease, "*Candidatus* Liberibacter solanacearum" (syn "*Candidatus* Liberibacter psyllaurous") (Hansen et al., 2008; Liefting, et al., 2009a,

2009b). Zebra chip disease is characterized by the development of brown necrotic lesions in raw tubers which become more noticeable when fried and render the tubers unmarketable (Crosslin et al., 2012; Munyaneza, 2012, 2015). High densities of *B. cockerelli* are also associated with "psyllid yellows", a crop disorder that affects plants in the Solanaceae (Edmunson et al., 1951; Richards & Blood, 1933; Wallis, 1955). Currently, there are no methods to directly control Liberibacter, so growers rely upon applications of insecticides to suppress vector populations within crop fields.

B. cockerelli and Liberibacter occur as biologically distinct haplotypes. Psyllids are classified as Central, Western and Northwestern (Swisher et al., 2012) haplotypes, which are named after the regions in the United States which they were first identified. Each psyllid haplotype differs in plant host preference, use, and biological development (Cooper, et al., 2019; Mustafa et al., 2015a, 2015b, 2015c; Swisher et al., 2013b). Liberibacter haplotypes (A-F, U), also differ in host plant biology and virulence. For example, Liberibacter haplotypes A and B cause disease symptoms in potato, but haplotype B has been shown to be more virulent than haplotype A (Harrison et al., 2019; Mendoza-Herrera et al., 2018; Swisher et al., 2018).

Previous research has shown that *B. cockerelli* completes development on a diversity of plants within the Solanaceae and Convolvulaceae (Cooper, et al., 2019; Kaur et al., 2018; Wallis, 1955). Because *B. cockerelli* moves from non-crop habitats, identifying potential non-crop solanaceous hosts for *B. cockerelli* is vital for a successful landscape-level management strategy for psyllid control. Previous research has demonstrated the importance of perennial host plants including *Lycium barbarum* (matrimony vine) and *Solanum dulcamara* (bittersweet nightshade) as sources of psyllids colonizing potato (Horton et al., 2016, 2015; Swisher et al., 2013b; Thinakaran et al., 2017a). These perennial weeds act as 'bridge hosts' between winter dormancy and emergence of annual hosts including potato, thus providing a refugia for *B. cockerelli* populations to increase before the growing season and become readily available to move into potato. Longleaf groundcherry (*Physalis longifolia* Nutt.; Solanaceae) is another perennial solanaceous non-crop host commonly found throughout the potato growing regions of Idaho, Oregon and Washington (*Consortium of Pacific Northwest Herbaria Specimen Database*, 2019; Kindscher et al., 2012) (Fig. 2.1). Previous studies have recorded psyllid development on *P. longifolia* (Cooper et al., 2019; Wallis, 1955) and have also shown its resistance to 2,4-D herbicidal control (Schaeffer et al., 1963; Yeoumans, 1962). The role of *Physalis longifolia* as a source of infective psyllids has not thoroughly been studied. Research presented in chapter 1 demonstrated that *P. longifolia* as a highly suitable host for *B. cockerelli* and the zebra chip pathogen

(Reyes Corral et al., unpublished). However, that research relied upon controlled greenhouse and field plot trials, so evidence that *B. cockerelli* occurs on wild stands of *P. longifolia* in the potato growing regions of Idaho is still lacking.

Recently, molecular gut content analysis using high-throughput sequencing of plant barcoding genes has been developed to identify the dietary history of *B. cockerelli* and to infer the landscape-level movements of the insects prior to capture (Cooper et al., 2016, 2019). This novel tool could be used to determine whether *B. cockerelli* readily migrates between potato and wild stands of *P. longifolia* or whether the psyllid primarily stays in *P. longifolia.* A better understanding of the landscape-level movements of *B. cockerelli* during and after the growing season could lead to more dedicated and effective cultural practices. The goal of this study had three main objectives. Field surveys were conducted to assess the presence and abundance of *Physalis longifolia* and *B. cockerelli* in the growing regions of Idaho and Washington. The second objective was to assess the presence of Liberibacter from insect and leaf samples collected off *Physalis longifolia* during the field surveys. Lastly, the third objective was to infer psyllid movement and previous dietary history using high-throughput gut content analysis.

2.3 Materials and Methods

2.3.1 Plant and Psyllid sources

Wild *P. longifolia* stands were identified in Zillah, WA and Caldwell, ID during 2018 and 2019 (Table 2.1). Upon identification of the stands, weekly and monthly field surveys were conducted throughout the growing seasons (Table 2.1). Field surveys consisted of a visual examination of ten *P. longifolia* terminals and the collection of five leaves per stand. Leaf samples were placed in universal extraction bags (Bioreba AG, Kanton Reinach, Switzerland) and stored in a cooler for transportation. Following the visual inspection, the stand was sampled for three minutes using a D-vac collection apparatus made from a leaf blower and its vacuum attachment (Homelite 150 MPH 400 26cc Gas Handheld Blower Vacuum, model UT26HBV, Homelite Corporation, Charlotte, NC). The intake tube of the vacuum device was fitted with an organdy bag held in place with rubber tubing to capture insects. D-vac samples were transferred to 4.6 L plastic re-sealable bags which were immediately placed in a cooler chilled with ice packs for transportation.

The majority of the plant material from the collection bags was removed by using a sieve with approximately 3 mm mesh size. All *B. cockerelli* were transferred to individual 1.5 ml centrifuge tubes and stored at -30°C. The insect bycatch of each collection bag was also stored at -30°C upon sorting and is available for future examination of natural enemies occurring within stands of *P. longifolia*.

2.3.2 Liberibacter detection and psyllid haplotyping

Prior to DNA extraction, *B. cockerelli* specimens were surface sterilized in 70% EtOH for 2 s, deionized (DI) water for 2 s, 1% bleach for 1 min, and finally rinsed in DI water for 2 s twice. DNA was extracted from *B. cockerelli* using a DNA extraction kit (DNeasy Blood & Tissue Kit, QUIAGEN, Germany) with some modifications to the manufacturer's protocol. Psyllid samples were ground in 1.2 ml tubes with 180 µl of ATL Buffer and incubated at 56°C overnight after adding 20 µl of Proteinase K. After incubation, 200 µl of AI Buffer were added to each sample and vortexed before and after adding 200 µl of 96% EtOH following a quick spin down. Samples were transferred into a DNeasy Mini Spin Column and centrifuged for 1 min at 6,000 x G. The flow-through was discarded and the column was placed in a new 2 ml collection tube. Five hundred microliters of AW1 Buffer were added to each sample before a 1 min spin at 6,000 x G. The column was then transferred to a new 2 ml collection tube and each sample was added 500 µl of AW2 Buffer before a 3 min spin at 20,000 x G. Columns for each sample were then transferred to a new 1.5 ml tube, added 50 µl of AE Buffer and spun for 1 min at 6,000 x G. Finally, 50 µl of AE Buffer were added to each column and spun for 1 min at 6,000 x G for a second time. The column was discarded and the flow-through was stored at -20°C.

DNA from *P. longifolia* samples was extracted using a cetyltrimethylammonium bromide (CTAB) precipitation DNA extraction method (Zhang et al., 1998). Five leaves per sample were ground in universal extraction bags (Bioreba AG, Kanton Reinach, Switzerland), incubated for 30 min at 37°C in 300 µl of CTAB with 80 µl of lysozyme stock before incubating the samples again for 30 min at 65°C and adding 500 µl of CTAB + β-mercaptoethanol (ME) buffer. Six hundred microliters of cold chloroform were then added to each sample and vortexed until an emulsion was formed. Samples were spun once again for 3 min at 20,000 x G and 500 µl of the upper aqueous phase (DNA layer) was transferred to a 1.5 ml tube containing 500 µl of isopropanol and 1 µl of GlycoBlue co-precipitant (15 mg/mL, Thermo Fisher Scientific, Waltham, MA). Following a 10 min centrifuge spin at 20,000 x G, the precipitated DNA pellets were washed using 500 µl of 70% EtOH and a 3 min spin at 20,000 x G. While

keeping the pellets and decanting most of the EtOH used for the wash, the pellets were dried at for 45 min in a 37°C incubator. Pellets were resuspended in 100 µl of nuclease-free water.

The presence or absence of Liberibacter for both insect and plant samples was confirmed by conventional PCR using primers OA2 (5'-GCG CTT ATT TTT AAT AGG AGC GGC A-3') and OI2c (5'-GCC TCG CGA CTT CGC AAC CCA T-3') to amplify a region of the 16S rRNA gene of Liberibacter (Crosslin et al., 2011). PCR conditions for Liberibacter detection for both insect and leaf samples consisted of an initial denaturation step of 94˚C for 2 min followed by 35 cycles of 94˚C for 30 s, 65˚C for 30 s, and 72˚C for 60 s, followed by a final extension at 72˚C for 5 min. Each 20 µl reaction contained Amplitaq Gold 360 PCR Master Mix (Invitrogen, Carlsbad, CA), 5µM of each primer, and DNA template (10–20 ng/ml for insect samples and 50-150 ng/ml for plant samples).

Psyllid haplotypes were determined by sequencing the cytochrome c oxidase 1 gene (CO1) amplicons produced by conventional PCR using the CO1 F3 (5'-TAC GCC ATA CTA GCA ATC GG-3') and CO1 R3 (5'-GAG TAA CGT CGT GGT ATT CC-3') primer set (Crosslin et al., 2011). Visible PCR products were purified using Exosap-IT PCR product cleanup (78200. 200.UL, Thermo Fisher Scientific, Waltham, MA). Samples were then submitted to MC Laboratories (San Francisco, CA) for direct DNA sequencing. Sequence analyses were conducted using Geneious R10 (Biomatters, Ltd., Auckland, New Zealand). The sequences were aligned and compared to sequences of known psyllid haplotypes of the Northwestern, Western and Central haplotypes and were analyzed using the BLAST alignment tool of the NCBI database (Kearse et al., 2012).

2.3.3 Molecular Gut Content Analysis

Psyllid dietary history and landscape movement prior to capture was inferred using high-throughput gut content analysis of plant-derived internal transcribed spacer (ITS) and the chloroplast *trnF* gene. ITS primers were ITS2F (5'-ATG CGA TAC TTG GTG TGA AT-3') and ITS3R (5'-GAC GCT TCT CCA GAC TAC AAT-3') (Chen et al., 2010; Cooper et al., 2019). Primers for *trnF* were B49873-e (5'-GGT TCA AGT CCC TCT ATC CC-3') and A50272-F (5'-ATT TGA ACT GGT GAC ACG AG-3') (Cooper et al., 2019; Taberlet et al., 1991). Individual barcoded primers (Pacific Biosciences, 2014) were used for each sample. PCR conditions for the ITS primer set started with a denaturation step of 94°C for 5 min and followed by 34 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 45 s. The final extension was set to 72°C for 10 min. PCR conditions for the *trnF* primer were similar to ITS. Initial denaturation was set to 94°C for 10 min, followed by 39 cycles of 94°C for 30 s, 52°C (52-58°C depending on barcoded primer used)

and 72°C for 45 s and a final extension of 72°C for 7 min. Each 50 µl reaction contained Invitrogen Amplitaq Gold 360 PCR Master Mix (Invitrogen, Carlsbad, CA), 250 nM of each primer, and 5 µl of DNA template. Amplicons (400-600bp) were observed on 1% agarose TBE (Tris-borate-EDTA) gels stained with ethidium bromide (EtBr).

PCR products were combined in a single vial and then sent to the Washington State University Laboratory for Biotechnology and Bioanalysis (WSU LBB) for direct sequencing using the Pacific Biosciences (PacBio) sequencing platform. The volume of PCR product to be pooled together per sample was determined based on the brightness of the amplicons. Ten microliters of PCR product were transferred if the amplicon was clearly visible on the gel, 20 µl if there was a faint band, and 30 µl if there were no visible amplicons. Amplicons were ligated to the hairpin SMRT bell adapters using PacBio Template kit v 1.0 and purified the library using AMPureXP beads (Beckman-Coulter). Libraries were quantified and bound to the P6 polymerase, loaded into a single SMRT cell, and observed for 6 h using C4 chemistry on a PacBioRSII. Raw movies were processed into reads and subsequently into high quality reads of interest. Barcodes were separated using SMRT Portal version 2.3. Average read-length was 24.6Kb, which produced an average single molecule coverage of 33x. This resulted in nearly all the data being very high quality, with Phred scores between 35 and 45 (99.95 and 99.995 accuracy). Operational taxonomic units (OTUs) were grouped using the *de novo* assembly alignment tool from Geneious R10 and the custom sensitivity setting with a minimum overlap identity of 95%, maximum gaps per read set to 1%, and 2% maximum mismatches per read. OTUs were analyzed using the Basic Local Alignment Search Tool (BLAST) function of the National Center for Biotechnology Information (NCBI) to accurately identify plant taxa (Altschul et al., 1990) from each sample.

2.4 Results and Discussion

2.4.1 Field Surveys, Liberibacter Detection and Psyllid Haplotyping

B. cockerelli adults were captured from *P. longifolia* during both 2018 and 2019 growing seasons in Washington and Idaho. In 2018, 53% of the psyllid specimens were captured in ID, and 47% in WA. The following year, 83% of the psyllid samples were captured in ID and 17% in WA. The number of psyllids collected per site was low in 2018 (Table 2.2) relative to 2019 (Table 2.3). CO1 sequences from psyllid specimens collected in 2018 identified two psyllid haplotypes: 80% (12) of the samples were classified as Northwestern, 13% (2) as Western, and 7% (1) as Unknown. The Western psyllid haplotype was collected only in Washington from September samples. All psyllid samples collected in

2019 in ID and WA were classified as Northwestern haplotype based upon returned sequences and compared to known psyllid haplotype sequences.

Only one psyllid sample was collected from a site adjacent to a commercial potato field in 2018. However, in 2019, 71% of the psyllids captured were collected from wild *P. longifolia* stands that were directly adjacent to commercially grown potato fields (Fig. 2.2A and 2.2B). Not only were these stands adjacent to potato fields but were also located in unmanaged areas of dense native or naturalized plants that may have included other solanaceous host plants of the psyllid (Fig. 2.3C). These unmanaged non-crop areas were often located in corners of crops circles where vehicle access and/or herbicidal control is limited. Regardless of the location and sampling year, the highest number of psyllids captured during field surveys were observed during the first two weeks of September (Fig. 2.4). The number of captured psyllids coincided with and could be attributed to potato harvest in adjacent crop fields. In Idaho, potato harvest in the Pacific Northwest begins in July for early varieties, but most harvest activities occur in early September and continues through most of October, depending on the cultivar. The peaks for the number of adult psyllids found for both collection years correspond to peak harvest in the potato growing regions of Idaho. The presence of '*Candidatus* Liberibacter solanacearum' was assessed in a total of 185 *B. cockerelli* samples (Table 2.2 and 2.3) and 307 *P. longifolia* leaf samples during the 2018 and 2019 growing seasons in Zillah, WA and Caldwell, ID. Liberibacter was not detected in any of the insect or leaf samples collected during field surveys.

2.4.2 Molecular Gut Content Analysis

Ninety-three psyllids captured in field surveys (15 from 2018 and 78 from 2019) were submitted for gut content analysis. Analyses of sequences identified 11 plant families from psyllids collected in 2018 in WA (Table 2.4) and 14 plant families from psyllids collected in 2019 (Table 2.5). Twelve plant families were identified from psyllids collected in ID in 2018 (Table 2.6) and 15 from psyllids collected in 2019 (Table 2.7). *Physalis* spp. was detected in 100% of the psyllids collected in WA and 85% of psyllids collected in Idaho using the conservative cutoff of 15 reads for inclusion of plant taxa but was detected in a greater percentage of psyllids if this cutoff is not used.

Results of gut content analysis were used to infer psyllid feeding behavior/movement prior to capture, and to infer the plant on which each psyllid likely completed development. The mean number of non-solanaceous plant families (transient feeding plants) identified per sample in the analysis was

also recorded. We can use the data to infer whether the psyllids developed on *Physalis* or arrived there from a different host. Psyllids from which *Physalis* spp. was the only known host plant identified were assumed to have developed on *Physalis* whereas psyllids from which *Physalis* plus another Solanaceae or Convolvulaceae host plant was identified were assumed to have developed on a different host plant before migrating to *Physalis.* Results demonstrate that some psyllids had arrived in the *Physalis* from potato, providing evidence for movement between *Physalis* and cultivated potato, particularly in August and September (Table 2.9B) when potato was harvested. While this result was expected in Idaho where potato fields are abundant, the detection of potato and tomato from psyllids collected near Zillah, WA was not expected. Although Zillah, WA is not considered a major potato growing region of Washington, a follow up survey revealed the presence of a mixed-crop farm located nearby where both potato and tomato were grown (Fig. 2.2A). Additional stands of *P. longifolia* were also discovered within the boundaries of this farm. Results of gut content analysis also provide evidence for movement of psyllids between *Physalis* and other solanaceous weeds including *Lycium barbarum* (matrimony vine) and *Solanum dulcamara* (bittersweet nightshade).

2.6 Conclusions

Results of this study confirm that *B. cockerelli* readily colonizes wild stands of *P. longifolia* in Washington and Idaho from both cultivated and uncultivated habitats. Importantly, many wild stands of *P. longifolia* surveyed in Idaho were adjacent to commercial potato fields. The presence of these stands may serve as a continuous unmanaged sources of *B. cockerelli* colonizing potato fields. Overall, results of my study indicate that *P. longifolia* should be considered a high risk weed as a source of *B. cockerelli* and potentially the zebra chip pathogen during outbreak years.

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Table 2.1. Location and site characteristics for wild *Physalis longifolia* stands monitored in Washington and Idaho. Washington site was monitored weekly and Idaho sites were monitored monthly. Asterisk denotes multiple stands on site.

Site	City, State (County)	Stand area (m^2)	Neighboring habitat	Months sampled (No. of dates)
A) 2018				
Canal Rd	Zillah, WA (Yakima)	80	Riparian/Agricultural (irrigated)	Jul - Oct (10)
10th Ave*	Caldwell, Idaho (Canyon)	100	Residential/Agricultural (irrigated)	May - Sep (4)
Sand Hollow Rd	Caldwell, Idaho (Canyon)	20	Roadside/Agricultural (irrigated)	May - Sep (4)
Highway 30	Caldwell, Idaho (Canyon)	10	Roadside/Bureau of Land Management	Jun - Sep (3)
Fruitland	Fruitland, Idaho (Payette)	180	Commercially grown orchard (irrigated)	Jun (1)
Potato Fields*	Caldwell, Idaho (Canyon)	60	Commercially grown fields/Agricultural (irrigated)	Jul – Sep (2)
B) 2019				
Canal Rd	Zillah, WA (Yakima)	80	Riparian/Agricultural (irrigated)	May - Sept (17)
10th Ave*	Caldwell, Idaho (Canyon)	100	Residential/Agricultural (irrigated)	Jun - Sep (4)
Sand Hollow Rd	Caldwell, Idaho (Canyon)	20	Roadside/Agricultural (irrigated)	Jun - Jul (2)
Farmway Rd	Caldwell, Idaho (Canyon)	10	Commercially grown fields (irrigated)	Jun - Sep (4)
Potato Fields*	Caldwell, Idaho (Canyon)	60	Commercially grown fields (irrigated)	Jun - Sep (4)
SE 9th Ave*	Caldwell, Idaho (Canyon)	1000	Commercially grown fields (irrigated)	Jun - Sep (3)

Table 2.2. Number of *B. cockerelli* adults collected during the 2018 potato growing seasons. Asterisk denotes multiple stands on site. Only stands where psyllids were captured are included.

2018		Psyllids collected							
Site	**City, State (County)**	**30 Jul**	**31 Jul**	**27 Aug**	**3 Sep**	**7 Sep**	**17 Sep**	**24 Sep**	**Total**
Canal Rd	Zillah, WA (Yakima)	0	0	1	2	0	3	1	7
10th Ave*	Caldwell, Idaho (Canyon)	1	0	0	0	6	0	0	7
Sand Hollow Rd	Caldwell, Idaho (Canyon)	0	1	0	0	0	0	0	1
	Total	1	1	1	2	6	3	1	15

Table 2.3. Number of B. cockerelli adults collected during the 2019 potato growing seasons. Asterisk denotes multiple stands on site.

2019		**Psyllids collected**															
Site	**City, State (County)**	**3 Jun**	**12 Jun**	**13 Jun**	**17 Jul**	**18 Jul**	**22 Jul**	**29 Jul**	**14 Aug**	**15 Aug**	**27 Aug**	**06 Sep**	**09 Sep**	**18 Sep**	**19 Sep**	**24 Sep**	**Total**
Canal Rd	Zillah, WA (Yakima)	1	0	0	0	0	1	2	0	0	3	10	2	0	0	3	22
10th Ave*	Caldwell, Idaho (Canyon)	0	1	0	5	0	0	0	0	2	0	0	0	20	0	0	28
Sand Hollow Rd	Caldwell, Idaho (Canyon)	0	0	0	21	0	0	0	0	0	0	0	0	0	0	0	21
SE 9th Ave*	Caldwell, Idaho (Canyon)	0	0	0	0	2	0	0	0	26	0	0	0	0	43	0	71
Farmway Rd	Caldwell, Idaho (Canyon)	0	0	1	0	6	0	0	7	0	0	0	0	0	0	0	14
Potato Fields*	Caldwell, Idaho (Canyon)	0	0	0	0	4	0	0	3	0	0	0	0	0	7	0	14
	Total	1	1	1	26	12	1	2	10	28	3	10	2	20	50	3	170

Table 2.4. Plant species, families and developmental hosts identified from *Bactericera cockerelli* collected during the 2018 growing season in Zillah, WA using a D-Vac. The proportion shows the number of samples with returned sequences using PCR primers ITS and trnF for each collection month. No returned sequences for the given PCR primer are denoted with (-). A threshold of 15 reads per plant species was implemented in order to ensure the validity of the results.

Plant species	August		September	
Solanaceae	ITS	trnF	ITS	trnF
Nicotiana spp.	-	1/1	-	-
Physalis spp.	1/1	1/1	2/6	6/6
Solanum lycopersicum	1/1	-	1/6	-
Solanum tuberosum	1/1	1/1	1/6	2/6
Convolvulaceae	-	-	2/6	3/6
Non-host plant families				
Amaranthaceae	1/1	1/1	1/6	2/6
Apiaceae	-	-	-	1/6
Asteraceae	-	1/1	-	3/6
Brassicaceae	1/1	-	-	-
Caryophyllaceae	-	-	-	1/6
Oleaceae	1/1	1/1	1/6	1/6
Poaceae	-	1/1	-	3/6
Rosaceae	-	-	-	2/6
Salicaceae	-	-	1/6	2/6

Table 2.5. Plant species, families and developmental hosts identified from *Bactericera cockerelli* collected during the 2019 growing season in Zillah, WA using a D-Vac. The proportion shows the number of samples with returned sequences using PCR primers ITS and trnF for each collection month. No returned sequences for the given PCR primer are denoted with (-). A threshold of 15 reads per plant species was implemented in order to ensure the validity of the results.

Plant species	July		August		September	
Solanaceae	ITS	trnF	ITS	trnF	ITS	trnF
Lycium spp.	-	1/2	-	-	-	-
Nicandra physalodes	-	1/2	-	2/3	-	1/3
Physalis spp.	1/2	2/2	3/3	3/3	2/3	3/3
Solanum dulcamara	-	2/2	3/3	2/3	-	2/3
Solanum lycopersicum	-	-	1/3	-	-	-
Solanum tuberosum	-	1/2	2/3	3/3	1/3	1/3
Non-host plant families						
Amaranthaceae	-	1/2	2/3	2/3	-	-
Asteraceae	-	2/2	3/3	3/3	1/3	2/3
Betulaceae	-	-	1/3	-	0/3	-
Brassicaceae	-	2/2	1/3	-	-	-
Cucurbitaceae	-	-	-	-	1/3	-
Fabaceae	1/2	-	1/3	-	-	-
Lamiaceae	-	1/2	-	-	-	-
Poaceae	1/2	2/2	3/3	2/3	-	1/3
Polygonaceae	-	2/2	-	-	-	-
Potamogetonaceae	-	1/2	-	-	-	-
Rosaceae	1/2	1/2	-	-	-	-
Theaceae	-	1/2	-	-	1/3	2/3
Urticaceae	-	1/2	1/3	1/3	-	-

Table 2.6. Plant species, families and developmental hosts identified from *Bactericera cockerelli* collected during the 2018 growing season in Caldwell, ID using a D-Vac. The proportion shows the number of samples with returned sequences using PCR primers ITS and trnF for each collection month. No returned sequences for the given PCR primer are denoted with (-). A threshold of 15 reads per plant species was implemented in order to ensure the validity of the results.

Plant species	July		September	
Solanaceae	ITS	trnF	ITS	trnF
Lycium spp.	-	-	-	1/6
Physalis spp.	-	2/2	-	4/6
Solanum dulcamara	-	-	-	2/6
Solanum tuberosum	-	1/2	-	2/6
Convolvulaceae	-	1/2	-	2/6
Non-host plant families				
Amaranthaceae	-	-	-	1/6
Anacardiaceae	-	-	-	1/6
Apiaceae	-	-	-	1/6
Asteraceae	-	-	-	3/6
Brassicaceae	-	-	-	1/6
Malvaceae	-		-	1/6
Pinaceae	-	1/2	-	1/6
Poaceae	-	-	-	2/6
Salicaceae	-	1/2	-	1/6
Scrophulariaceae	-	-	-	1/6

Table 2.7. Plant species, families and developmental hosts identified from *Bactericera cockerelli* collected during the 2019 growing season in Caldwell, ID using a D-Vac. The proportion shows the number of samples with returned sequences using PCR primers ITS and trnF for each collection month. No returned sequences for the given PCR primer are denoted with (-). A threshold of 15 reads per plant species was implemented in order to ensure the validity of the results.

Plant species	July		August			September
Solanaceae	ITS	trnF	ITS	trnF	ITS	trnF
Hyoscyamus niger	-	-	-	-	14/32	-
Lycium spp.	-	-	-	2/20	1/32	2/32
Nicandra physalodes	-	4/18	-	2/20	-	1/32
Physalis spp.	-	14/18	2/20	17/2	7/32	14/32
Solanum dulcamara	-	-	-	-	-	4/32
Solanum lycopersicum	-	-	-	-	3/32	-
Solanum nigrum	-	-	-	-	1/32	-
Solanum tuberosum	-	7/18	-	3/20	7/32	4/32
Convolvulaceae	-	-	-	-	-	1/32
Non-host plant families						
Amaranthaceae	-	1/18	1/20	2/20	7/32	11/32
Amaryllidaceae	-	-	1/20	-	-	-
Asteraceae	-	-	-	2/20	14/32	10/32
Brassicaceae	-	-	-	-	1/32	-
Ephedraceae	-	-	-		1/32	-
Malvaceae	-	-	-	-	1/32	1/32
Moraceae	-	-	-	1/20	-	1/32
Pinaceae	-	-	-	-	-	1/32
Poaceae	-	-	-	-	-	8/32
Rosaceae	-	-	1/20	-	-	-
Rutaceae	1/18	-	-	-	-	-
Sapindaceae	-	-	-	-	-	1/32
Ulmaceae	-	-	-	-	-	1/32

Table 2.8. Number of different returned sequence combinations of solanaceous host plants and mean number of non-solanaceous transient plant families identified from *Bactericera cockerelli* samples collected in Zillah, WA during the 2018 and 2019 growing season. The numbers correspond to the returned sequences using PCR primer trnF. A threshold of 15 reads per plant species was implemented in order to ensure the validity of the results.

Plant association	**July**	**August**	**September**
A) 2018			
Total no. psyllids analyzed	0	1	6
Solanaceae			
Physalis only	0	0	4
Physalis + potato	0	0	2
Three or more Solanaceae	0	1	0
Transient plant families			
Mean (min – max) no. transient host plants visited	0	5.0	4.2 (3-6)
B) 2019	**July**	**August**	**September**
Total no. psyllids analyzed	2	3	3
Solanaceae			
Physalis only	1	2	1
Physalis + potato	0	0	1
Physalis + *S. dulcamara*	0	0	1
Three or more Solanaceae	1	1	0
Transient plant families			
Mean (min – max) no. transient host plants visited	7.0 (6-8)	2.7 (1-3)	1.7 (0-3)

Table 2.9. Number of different returned sequence combinations of solanaceous host plants and mean number of non-solanaceous transient plant families identified from *Bactericera cockerelli* samples collected in Caldwell, ID during the 2018 and 2019 growing season. The numbers correspond to the returned sequences using PCR primer trnF. A threshold of 15 reads per plant species was implemented in order to ensure the validity of the results.

Plant association	July	August	September
A) 2018			
Total no. psyllids analyzed	2	0	6
Solanaceae			
Physalis only	1	0	0
Physalis + potato	1	0	0
Physalis + *S. dulcamara*	0	0	2
Physalis + *Lycium*	0	0	1
Three or more Solanaceae	0	0	0
Transient plant families			
Mean (min – max) no. transient host plants visited	4.0 (4-4)	0	4.2 (2-5)
B) 2019	**July**	**August**	**September**
Total no. psyllids analyzed	17	19	30
Solanaceae			
Physalis only	6	10	7
Physalis + potato	6	3	4
Physalis + *Lycium*	0	2	1
Physalis + *S. dulcamara*	0	0	3
Physalis + unidentified Solanaceae	4	2	1
Three or more Solanaceae	1	0	1
Transient plant families			
Mean (min – max) no. transient host plants visited	0.4 (0-3)	0.4 (0-2)	1.6 (0-6)

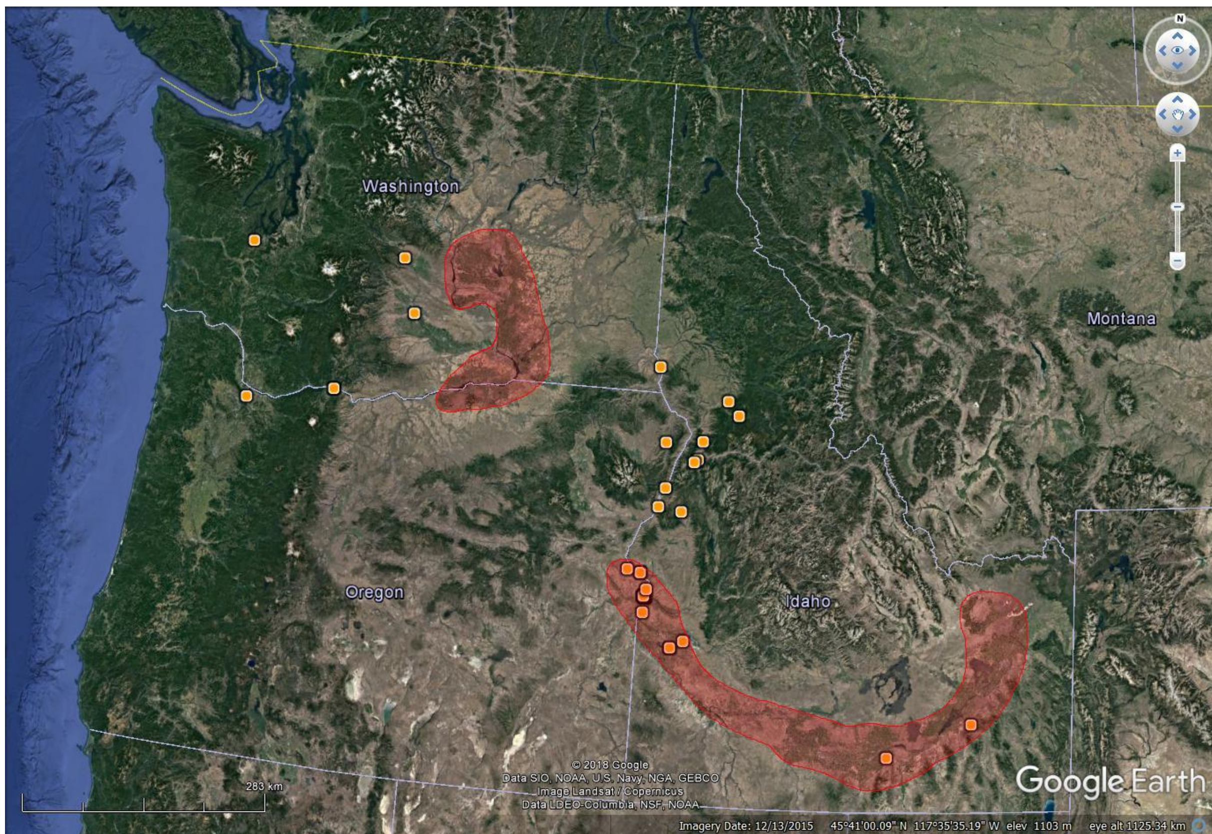

Fig. 2.1. Herbaria records (orange markers) showing the presence of *Physalis longifolia* in the Pacific Northwestern United States (http://www.pnwherbaria.org). The red shaded areas represent the primary potato growing regions in the Pacific Northwest.

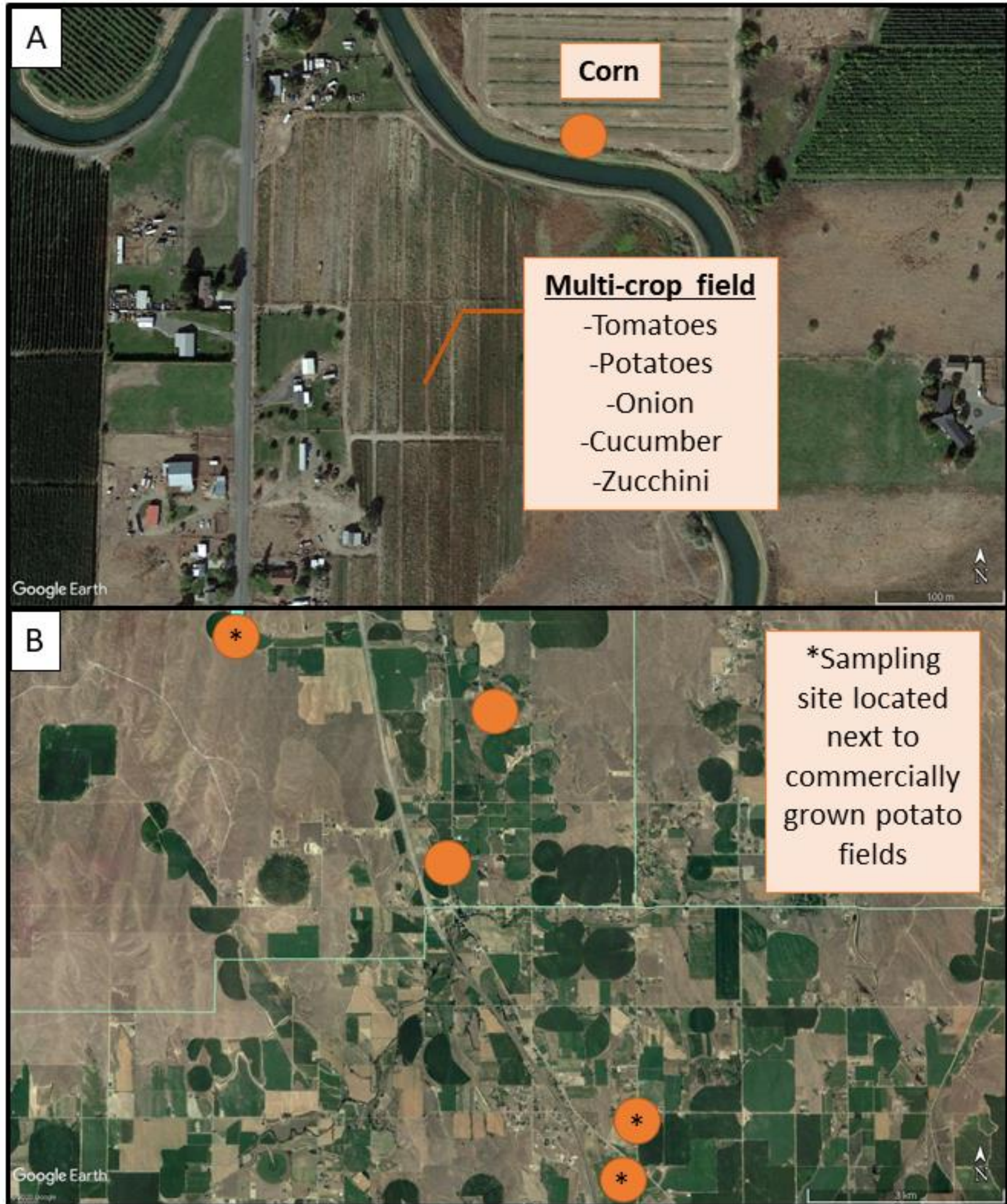

Fig. 2.2. Orange marks denoting the location of collection sites in Zillah, WA (A) and Caldwell, ID (B) during the 2018 and 2019 potato growing season. All collection sites in Caldwell, ID are located within 1 km from potato fields.

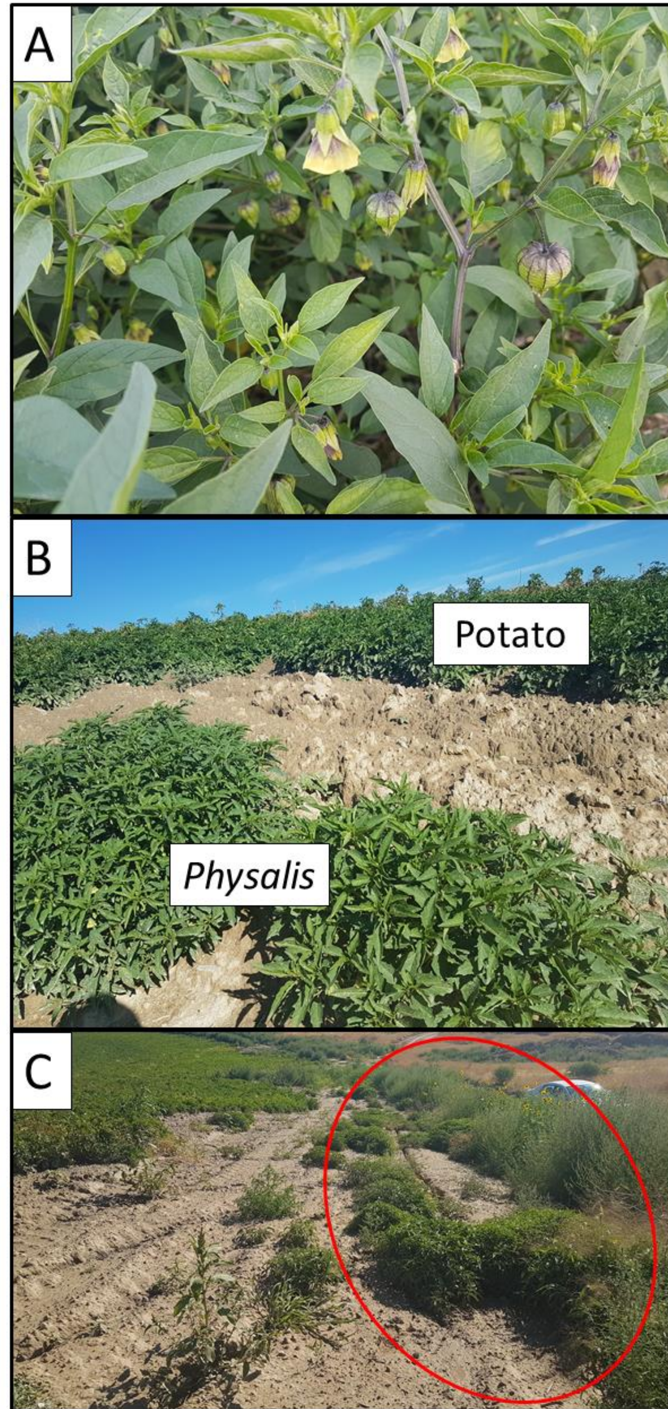

Fig. 2.3. *Physalis* stand in Zillah, WA during mid-July 2019 (A), *Physalis longifolia* growing next to commercially grown potato fields in Caldwell, ID (B), undisturbed "insect nurseries" (red circle) between potato circles hosting a wide variety of plant species including *P. longifolia* (C).

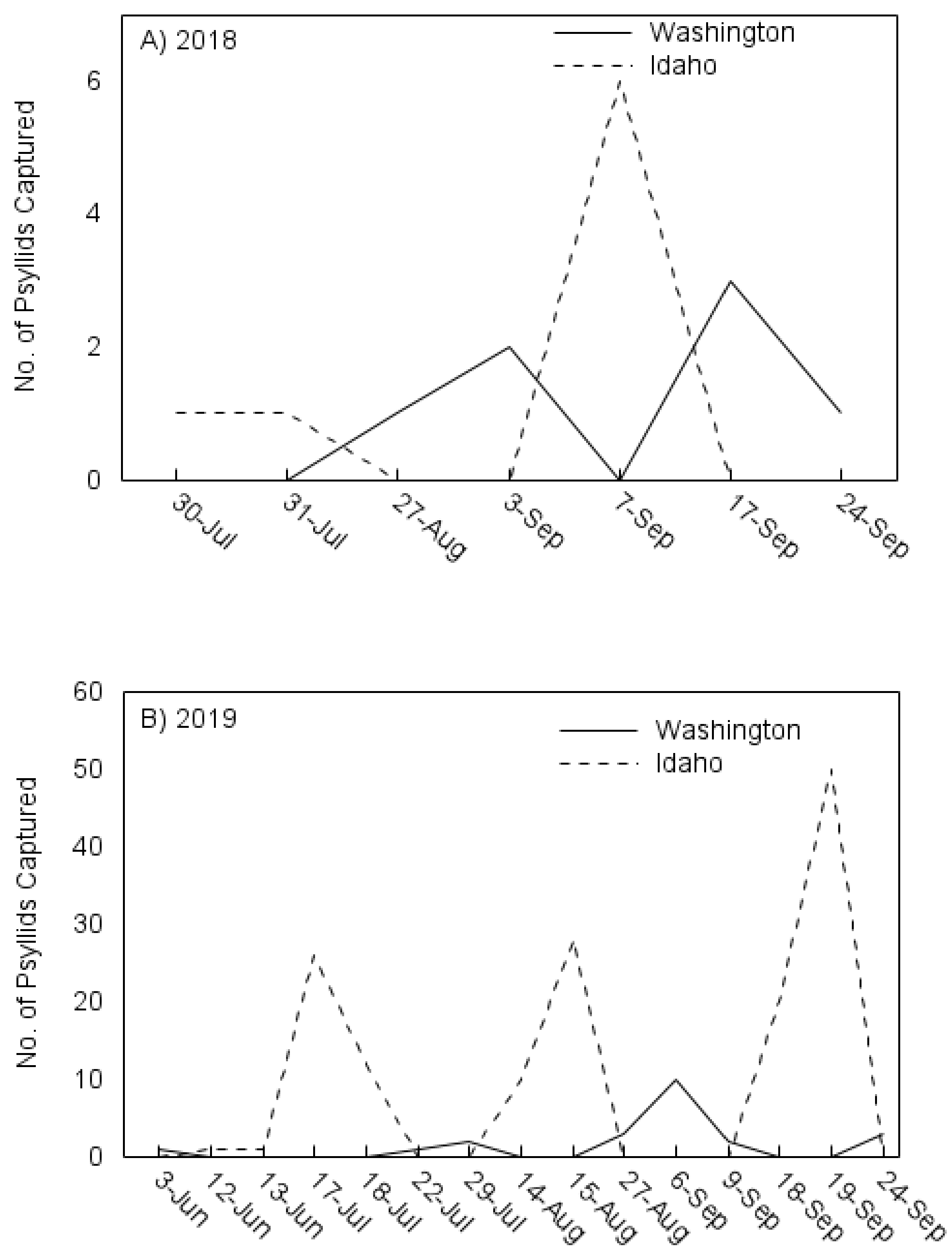

Fig. 2.4. Seasonality of psyllids collected in Zillah, WA and Caldwell, ID during the 2018 (A) and 2019 (B) potato growing seasons.

Chapter 3: Detection of "*Candidatus* Liberibacter solanacearum" on *Physalis ixocarpa* Brot. (Solanales: Solanaceae) and Gut Content Analysis of *Bactericera cockerelli* Šulc (Hemiptera: Triozidae) in Saltillo, Mexico

3.1 Abstract

The potato psyllid, *Bactericera cockerelli* (Šulc), (Hemiptera: Triozidae) is a pest of potato (*Solanum tuberosum* L.; Solanaceae) and tomato (*Solanum lycopersicum* L.; Solanaceae) largely because it is a vector of "*Candidatus* Liberibacter solanacearum", the bacterium that is associated with "tomato permanent yellowing disease" ("enfermedad permanente del tomate") in solanaceous crops and with zebra chip disease of potato. In addition, feeding by large populations of nymphs causes foliar disorders known as "psyllid yellows". *Bactericera cockerelli* occurs as three or more major haplotypes (Central, Northwestern, and Western) which differ in plant host preference and biological development differences. Liberibacter is also classified into haplotypes (A-F, U), where haplotypes A and B infect *S. tuberosum* and *S. lycopersicum*. During the 2019 growing season, a tomatillo (*Physalis ixocarpa* Brot.; Solanaceae) experimental plot in Saltillo, Mexico exhibited severe yellowing symptoms similar to psyllid yellows or permanent yellowing disease. *Bactericera cockerelli* and tomatillo leaf samples were collected from affected fields and tested for Liberibacter using diagnostic polymerase chain reaction (PCR). High-through put gut content analysis of plant-derived internal transcribed spacer (ITS) and the chloroplast *trnF* gene was used to identify the previous dietary history of psyllids and to infer their landscape movements prior to capture. Liberibacter was detected in 71% of both insect and plant tissue samples. Liberibacter haplotype classification of insect and leaf samples was based upon simple sequence repeat (SSR) markers using conventional PCR. Liberibacter was classified as haplotype B in 78% and 80% of the insect and leaf samples, respectively. Gut content analysis confirmed movement between weedy hosts and commercial tomatillo. Results indicate that *Physalis ixocarpa* is susceptible to the zebra chip pathogen and a potential host of *B. cockerelli*.

Key words: potato psyllid, *Bactericera cockerelli*, *Physalis ixocarpa,* Liberibacter

3.2 Introduction

Tomatillo (*Physalis ixocarpa* Brot.; Solanaceae, synonym *Physalis philadelphica* Lam. var. *immaculata* Waterf.) or Mexican groundcherry is an economically important solanaceous crop in Mexico. About 771,000 metric tons of tomatillo are produced in Mexico annually, primarily in Sinaloa, Zacatecas, and Jalisco, and a large portion of harvested tomatillo are exported (Vargas-Ponce et al., 2015). Tomatillo production is challenged by a complex of insect pests and plant pathogens, including the potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), the vector of "*Candidatus* Liberibacter solanacearum" (syn. "*Ca.* L. psyllarous") (Hansen et al., 2008; Liefting et al., 2009a, 2009b). Liberibacter is associated with the development of foliar disease symptoms known as "tomato permanent/permanent yellowing disease" ("enfermedad permanente del tomate") in nearly all solanaceous crops, and with symptoms within potato tubers known as zebra chip. Although tomatillo is a documented host of *B. cockerelli* and is susceptible to infection by Liberibacter, the disease symptoms associated with Liberibacter infection have not been documented.

A major challenge to the management of zebra chip disease is the inability to predict when and where Liberibacter-infected psyllids will colonize crops. This challenge is due to uncertainty about what non-crop plants are sources of infected psyllids dispersing into crop fields. *Bactericera cockerelli* completes development on a diversity of plants within the Solanaceae and Convolvulaceae including many weeds that require management by growers (Cooper et al., 2019; Horton et al., 2015a, 2015b; Kaur et al., 2018; Thinakaran et al., 2017b; Wallis, 1955). Identification of which weedy hosts are primary sources of *B. cockerelli* colonizing potato fields could help growers predict the severity and timing of regional outbreaks of permanent yellowing disease and zebra chip, or to control weeds that are deemed to be a high risk for infective psyllids.

Another challenge to the management of *B. cockerelli* and Liberibacter is the occurrence of several distinct haplotypes of both the vector and pathogen. *Bactericera cockerelli* occurs as at least four haplotypes, but only three – named the central, western, and northwestern haplotypes – appear to be of primary concern in western North America. These psyllid haplotypes have distinct biological traits including differences in fecundity, size, presence of bacterial endosymbionts, and host plant preference or use (Cooper, et al., 2015; Mustafa et al., 2015a, 2015b, 2015c; Swisher et al., 2013a). "*Ca.* L. solanacearum" also occurs as different haplotypes with three haplotypes – Liberibacter haplotypes A, B, and F – infecting Solanaceae in North America. Little is known about haplotype F, but published reports indicate that haplotype B causes more severe disease symptoms in potato and

tomato than does haplotype A (Harrison et al., 2019; Mendoza-Herrera et al., 2018; Swisher & Garczynski, 2019; Swisher et al., 2018).

During November 2019, a tomatillo experimental plot in Saltillo, Coahuila, Mexico exhibited severe yellowing symptoms that could be associated with psyllid yellows and zebra chip disease (Edmunson et al., 1951; Munyaneza, 2012, 2015; Richards & Blood, 1933). Further inspections of the plot revealed high populations of *Bactericera cockerelli* on the tomatillo plants in the experimental plot. This study had three principal objectives. The first objective was to confirm the suspected presence or absence of '*Ca*. Liberibacter solanacearum' in both insect and plant samples collected from this experimental plot. The second objective was to identify *B. cockerelli* and Liberibacter haplotypes collected off *Physalis ixocarpa*. Finally, the third objective was to infer in *B. cockerelli* landscape movement and previous dietary history prior to capture by using high-throughput gut content analysis. I also provide descriptions of foliar symptoms associated with this *B. cockerelli* and Liberibacter outbreak.

3.3 Materials and Methods

3.3.1 Psyllid and Plant Sources

B. cockerelli and *P. ixocarpa* samples were collected from an experimental plot located at the Universidad Autonoma Agraria Antonio Narro, Saltillo, Mexico during November 2019. The experimental tomatillo plot was first established on 2 July 2019. On 5 October 2019, apparent symptoms of Liberibacter infection, analogous to those observed on potato and tomato, were observed and recorded (Fig. 3.1). Plants were sampled for *B. cockerelli* by gently shaking the plants 5-7 times over a 43 x 28 cm white paper sheet and collecting the psyllids into 96% ethanol using a fine brush. Partially expanded and fully expanded leaves were also collected, cut longitudinally in three sections, and placed in 96% ethanol. The samples were then shipped to the USDA laboratory in Wapato, WA for molecular diagnosis of *"Ca.* L. solanacearum" and for gut content analysis of psyllids.

3.3.2 Liberibacter Detection and Haplotyping

Individual psyllid and leaf samples were tested for the presence of Liberibacter by extracting DNA using a cetyltrimethylammonium bromide (CTAB) precipitation method (Zhang et al., 1998). Insect and leaf samples were resuspended in 50 µl and 100 µl of nuclease-free water, respectively. Conventional PCR using primers for the 16S rRNA gene of Liberibacter (OA2/OI2c) was used to confirm

the presence or absence of Liberibacter (Crosslin et al., 2011). PCR conditions for Liberibacter detection for both insect and leaf samples consisted of an initial denaturation step of 94˚C for 2 min followed by 35 cycles of 94˚C for 30 s, 65˚C for 30 s, and 72˚C for 60 s, followed by a final extension at 72˚C for 5 min. Each 20 µl reaction contained Amplitaq Gold 360 PCR Master Mix (Invitrogen, Carlsbad, CA), 5µM of each primer, and DNA template (10–20 ng/ml for insect samples and 50-150 ng/ml for plant samples). Liberibacter haplotypes for both insect and leaf samples were classified as A or B based upon simple sequence repeat (SSR) markers (Lin et al., 2012a). PCR for SSR markers was performed in 25 µl containing 0.5 µl of Advantage Taq polymerase (Takara Bio, Mountain View, CA), 2.5 µl of 10X Advantage buffer, 0.5 µl of 10 mM dNTPs, 0.5 µl of 100 nM of each SSR primer (Lso-SSR-1F/Lso-SSR-1R) and 5 µl of DNA template. Temperature conditions for Liberibacter haplotyping consisted of an initial denaturation at 94°C for 5 min, followed by 40 cycles of 94°C for 10 s, 58°C for 10 s and 72°C for 15 s, with a 72°C final extension step for 5 min. Samples were electrophoresed using a 1.5% agarose gel and 5 µl of amplification product to confirm the successful SSR products. High resolution melting analysis of the mitochondrial Cytochrome C Oxidase subunit 1-like gene was conducted to classify psyllid haplotypes as Central, Western or Northwestern (Swisher et al., 2012).

3.3.3 Gut Content Analysis

High-throughput gut content analysis of plant-derived internal transcribed spacer (ITS) and the chloroplast *trnF* gene was used to identify the previous dietary history of psyllids and to infer their landscape movements prior to capture. The primers for trnF were B49873-e (5'-GGT TCA AGT CCC TCT ATC CC-3') and A50272-F (5'-ATT TGA ACT GGT GAC ACG AG-3') (Taberlet et al., 1991) and the primers for ITS were ITS2F (5'-ATG CGA TAC TTG GTG TGA AT-3') and ITS3R (5'-GAC GCT TCT CCA GAC TAC AAT-3') (Chen et al., 2010). PCR conditions for reactions using all three primer pairs included an initial denaturation step of 94°C for 10 min followed by 39 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 45 s, followed by a final extension at 72°C for 10 min. Each 50 µl reaction contained Invitrogen Amplitaq Gold 360 PCR Master Mix (Invitrogen, Carlsbad, CA), 250 nM of each primer, and 5 µl of DNA template. Separate asymmetric-barcoded forward and reverse primers (Pacific Biosciences, 2014) were used for each collection. The presence of 400-600 bp amplicons were observed on 1% agarose TBE (Tris-borate-EDTA) gels stained with ethidium bromide.

PCR products were pooled into a single sample and shipped to the Washington State University Laboratory for Biotechnology and Bioanalysis and sequenced directly using the Pacific Biosciences

(PacBio) sequencing platform. Pooled barcoded amplicons were ligated to the hairpin SMRT bell adapters using PacBio Template kit v 1.0, and library purified using AMPureXP beads (Beckman-Coulter). Libraries were quantified and bound to the P6 polymerase, magbead loaded into a single SMRT cell, and observed for 6 hours using C4 chemistry on a PacBioRSII. Raw movies were processed into reads, reads processed into high quality reads of interest and barcodes separated using SMRT Portal version 2.3. Average read-length was 24.6Kb, which produced an average single molecule coverage of 33x. This resulted in nearly all the data being very high quality, with Phred scores between 35 and 45 (99.95 and 99.995 accuracy). Sequences were grouped into operational taxonomic units (OTUs) using the *de novo* assembly alignment tool of Geneious R10 using the custom sensitivity setting with a minimum overlap identity of 98% and 98% maximum mismatches per read. Each OTU was then analyzed using the BLAST function of National Center for Biotechnology Information (NCBI) database to putatively identify taxa to plant family or genus (Altschul et al., 1990). Single unique reads were not assembled into OTUs and were therefore discarded.

3.4 Results and Discussion

3.4.1 Liberibacter Detection and Haplotyping

The presence of "*Candidatus* Liberibacter solanacearum" was assessed in 21 psyllids and 7 tomatillo leaves. Liberibacter was detected in 71% of the psyllids collected from symptomatic *Physalis ixocarpa* plants and 78% of the positive insect samples were classified as Liberibacter haplotype B. *Physalis* leaves tested positive for Liberibacter in 71% of the samples as well, where 80% of the Liberibacter-positive samples were classified as Liberibacter haplotype B. Symptoms observed on infected plants included severe yellowing of leaves and plant dieback, which is consistent with symptoms observed in other solanaceous plants including *P. longifolia* (Pitman et al., 2011; Swisher et al., 2019; section 1.4.2). Analysis of the melting behavior of the 500 bp CO1 amplicon using the LightCycler Gene Scanning Software (Roche Applied Science) identified two different *B. cockerelli* haplotypes in the samples. 95% of the psyllid samples collected were classified as Central haplotype, with only one sample classified as Western haplotype. The presence of the central haplotype in this region of Mexico is consistent with previous reports (Jackson et al., 2009; Liu et al., 2006; Swisher et al., 2012).

3.4.2 Gut Content Analysis

Molecular gut content analysis confirmed movement of *B. cockerelli* among weedy solanaceous species and *Physalis* (Table 3.1). However, it was not possible to confidently identify most of the solanaceous weeds to species likely because the species are not currently represented in the NCBI database warranting the development of a custom sequence library from leaf samples collected from this region. *Physalis* was detected from 100% (21/21) of the psyllids confirming that the psyllids had fed upon *P. ixocarpa* before capture. At least one host plant representing a large number of sequences identified from psyllids appeared to belong to the Dulcamaroid clade of *Solanum. Solanum triquetrum* of the Dulcamaroid clade may occur in this region (Knapp, 2013), but field surveys are required to confirm its presence. *Datura* plants infested with *B. cockerelli* were observed adjacent to the *S. ixocarpa* field (Sanchez personal observation), but the plant was not identified from gut content sequences.

3.4.3 Conclusions

Results of study confirm that commercial tomatillo, *P. ixocarpa,* is a suitable host for *B. cockerelli* and is susceptible to infection by *"Ca.* L. solanacearum". Disease symptoms caused by Liberibacter of *P. ixocarpa* are similar to those observed in other solanaceous crops and weeds. Results indicate that the Central haplotype of *B. cockerelli* is prevalent in this region, and that the dominate Liberibacter haplotype is haplotype B, which is causes more severe disease symptoms than other known haplotypes. Finally, results of gut content analysis provide evidence that psyllids colonized commercial *P. ixocarpa* fields from wild hosts within the Solanaceae and Convolvulaceae, which warrants more extensive field surveys and the development of a custom plant sequence library to identify the which weed species are important for Liberibacter epidemiology in this region.

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Table 3.1. Returned sequence combinations of solanaceous host plants and mean number of transient plant families identified via gut content analysis of *Bactericera cockerelli* samples collected in Saltillo, Mexico during November 2019. A threshold of 15 reads per plant species was implemented in order to ensure the validity of the results.

Plant Association	**P1**		**P2**	
PCR Primer set	**trnF**	**ITS**	**trnF**	**ITS**
Total no. psyllids analyzed	10	5	11	8
Solanaceae				
Physalis only	0	0	0	0
Physalis + potato	0	0	0	0
Physalis + unknown Solanaceae	8	1	3	1
Three or more Solanaceae	2	1	8	2
Transient plant families				
Mean (min – max) no. transient host plants visited	0.6 (0-1)	0.7 (0-3)	0.7 (0-2)	1.2 (0-3)

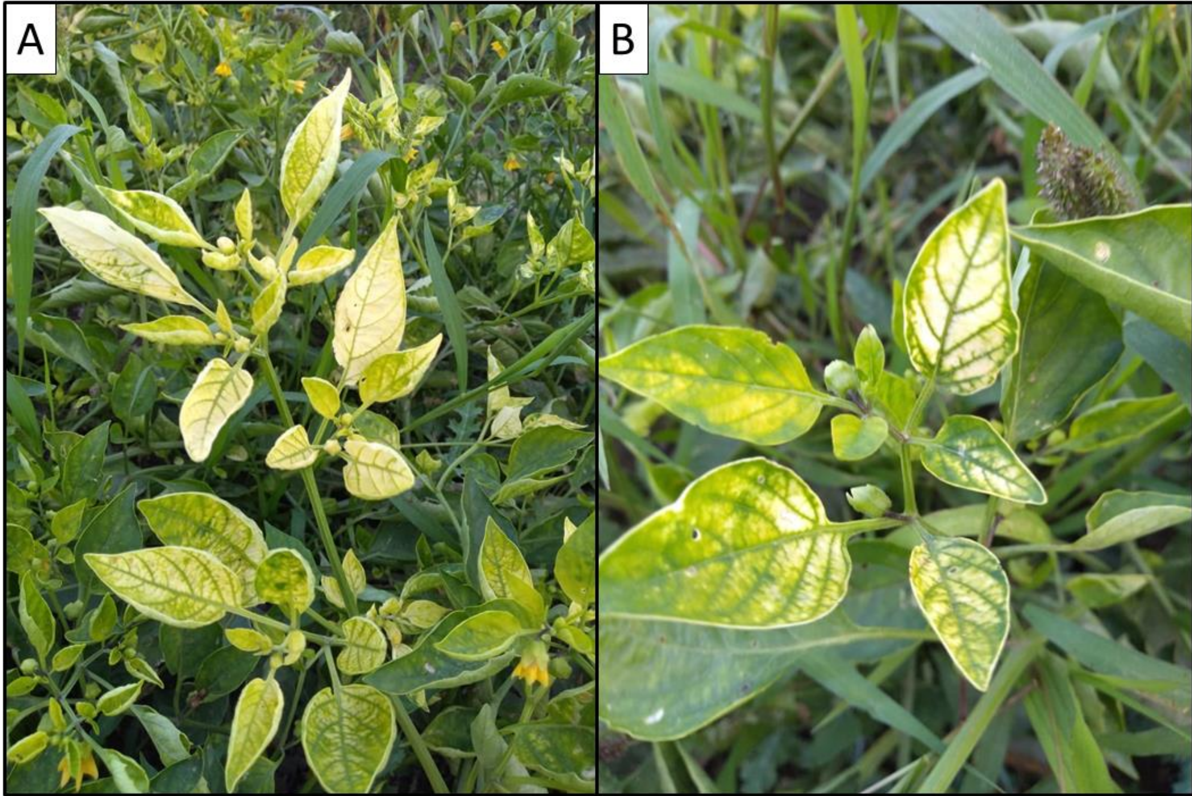

Fig. 3.1. *Physalis ixocarpa* plants in an experimental plot at the Universidad Autonoma Agraria Antonio Narro in Saltillo, Mexico during November 2019 (A). Close-up of *P. ixocarpa* exhibiting severe yellowing and curling symptoms (B).