PSYLLID ECOLOGY AND BIODIVERSITY IN THE PACIFIC NORTHWEST

By

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Many thanks to the entomology faculty, staff and employees of Washington State University and the University of Idaho who have had the chance to help, interact and give kind advice and support. Special thanks to my laboratory mates and the entomology graduate students for their sweet friendship, all have a special place in my heart.

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PSYLLID ECOLOGY AND BIODIVERSITY IN THE PACIFIC NORTHWEST

ABSTRACT

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Potato is the fourth-most-valuable commodity in Washington State. The emergence of zebra chip disease ("ZC") has triggered economic losses in the U.S. Pacific Northwest ("PNW") with a ca. 7% increment in the total cost of potato production, endangering the economic viability of the region. The vector of the ZC pathogen, *Candidatus* Liberibacter solanacearum ("Lso") is the potato psyllid ("PP"), *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae). The overall aim of my research was to study the PP ecology on a non-crop host, bittersweet nightshade (*Solanum dulcamara* L.) ("BN"), and determine presence of Lso. Chapter 1 is an introduction of the importance of ZC. Chapter 2 presents the population dynamics of PPs living BN in Eastern

Washington in 2012-2013. A high population and reproduction of PPs was found on BN, higher

than the numbers usually found in potato fields. Molecular analyses found an apparent absence of Lso in these PPs and plants, while revealing that all PP collected were of the Northwestern COI-haplotype. Other agricultural pests were found living on BN. Chapter 3 describes the predator community found (> 40 species), which could be reducing PP. The dominant taxa were Araneae (> 70% of all predators) and predator mites (Anystidae) (> 15% of all predators found). Observations of predator activity revealed PP egg consumption by the tiny coccinellid *Stethorus punctillum* Weise and attack of PP nymphs by the parasitoid *Tamarixia triozae* (Burks). Chapter 4 presents a checklist of the Psylloidea superfamily found in the PNW. To know the diversity of psyllid species in this region I compile the published registers and the specimens housed in PNW entomological museums. The list presents 124 species from 25 genera; 35 species are new reports in the PNW. Chapter 5 contains a scientific note about the thrips species found during my sampling of BN patches; I report these species that could have been living on BN or on the surrounded plants. Overall, my results show a complex community of PP, other agricultural pests, and predators living on BN, alongside the apparent absence of the Lso bacterium in PP and BN.

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Dedication

A mis padres Julio y Carmen, por su amor incondicional y buen ejemplo de honestidad y responsabilidad, y a mi hermano Iván, Nora y Santi, por su ayuda constante, apoyo y cariño. Sin todos ustedes, nada de esto podría haber sido posible.

To my parents Julio and Carmen, for their unconditional love and good example of hard work and honesty, and to my brother Iván, Nora and Santi, for their constant help, support and love. Without all of you, nothing of this would have been possible.

To Peter Markowski, for making my life happier.

CHAPTER ONE: INTRODUCTION

Around the world, potato is the third most important food crop for human consumption after rice and wheat (CIP 2015). For the Pacific Northwest (PNW) U.S. states of Idaho, Oregon and Washington, potato is the number one dollar-value vegetable crop representing more than 50% of the total production in the United States (NASS 2015). This region possess adequate growing conditions for mechanized potato production such as moderate flat topography, soil physical and chemical composition, mild temperatures and a long light period during summer, and constant irrigation water. Those are key elements for producing the highest yields in the world (Strand 2006). PNW potato production and processing not only provides for the domestic market, but also reaches international markets in Asia and other continents. The economic impact of potato in this region is estimated to exceed 9 billion dollars per year (ID, OR and WA Potato Commissions). One of the most important factors reducing crop yields is plant pathogens and insect pests (Gaunt 1995).

Insect vectors of plant pathogens cause serious problems in agriculture at least two ways: (1) by direct feeding on the host plant and (2) by transmitting pathogens such as viruses, phytoplasmas and bacteria causing yield losses and plant death (Radcliffe 1982). The potato psyllid, *Bactericera cockerelli* (Šulc), today causes economic harm primarily as the vector of a detrimental plant pathogen in potato crops that causes so-called zebra chip disease (ZC). This pest insect has, however, historically been reported as causing substantial losses through direct plant feeding (Wallis 1955). For example, potato psyllid outbreaks in Colorado, Wyoming, Nebraska and Montana around the 1930's caused losses in potato production in the millions of

dollars by triggering so-called “psyllid yellows” (Pletsch 1947), a plant disorder caused by psyllid feeding on the plant (Sengoda et al. 2010). Romney (1939) and Wallis (1955) mentioned psyllid movement from southern breeding areas in Texas and Arizona to upper states during the potato-growing season (Figure 1-1 A). Wallis (1955) mentioned that Washington and Oregon were free of psyllids, and that Idaho had only reports of psyllid yellows but no psyllids collected. Even with intensive monitoring of this insect in potato fields, potato psyllids are found in low quantities in this region (Wenninger et al. 2012).

Vector-borne plant pathogens, such as the newly-molecularly-detected *Candidatus* Liberibacter bacteria (*C*L), have caused severe economic losses in agriculture around the world (Haapalainen 2014). Citrus Huanglongbing or citrus greening is caused by *Candidatus* Liberibacter americanus in Brazil, *C*L asiaticus in Asia and North America (Florida), and *C*L africanus in South Africa (Fig. 2). Huanglongbing caused death of 8 million of citrus trees and reduced 69% of area planted in the Philippines in some outbreaks. In some provinces of Thailand, 95% of tree fields were severely affected. In Indonesia, 3 million of trees died in several outbreaks (Da Graça 1991). *C*L solanacearum in potatoes, which causes ZC, has been detrimental as well in potatoes and other solanaceous crops in Central and North America, and in New Zealand (Trumble 2009, Rosson et al. 2006, Munyanez et al. 2007, Liefting et al. 2009). *C*L solanacearum is close related to *C*L asiaticus according to their genome sequences, and *C*L solanacearum genetic diversity is probably much larger than previously thought (Lin et al. 2011). The category *Candidatus* was accepted by the International Code of Nomenclature of Bacteria to name prokaryote organisms that cannot be maintained in laboratories as pure cultures for characterization and can only be described in limited terms (Murray and Stackebrandt 1995).

Candidatus Liberibacter solanacearum (Lso) is a Gram-negative bacterium adapted to live in the oxygen-limited vascular cells of the phloem (De Boer 2007, Secor and Rivera-Vargas 2004). This adaptation is consistent with the limited capacity of Lso for aerobic respiration due to the deficit of key enzymes involved in oxidative phosphorylation (Lin and Gudmestad 2013). Lso presents five different haplotypes related to geographical ranges and based on polygenetic analysis of 16S rRNA (Nelson et al. 2011). Haplotype A and B are transmitted by *B. cockerelli* in potatoes in North America (Fig. 2A). Arizona and California have Lso haplotype A, which is the same as in other countries such as Guatemala, Honduras, western Mexico and New Zealand. The PNW has the Lso haplotype B, the same as in eastern Mexico (Haapalainen 2014). Haplotype B is more virulent than haplotype A (Thompson et al. 2015). Texas, Kansas and Nebraska have both Lso haplotypes present (Fig. 2B). Haplotype C is transmitted by the psyllid *Dyspersa apicalis* in carrots (*Daucus carota* L.) in Finland, while haplotypes D and E are transmitted by the psyllids *Bactericera trigonica*, *B. trembayi* and *B. nigricornis* in celery and carrots in Spain (Fig. 2) (Haapalainen 2014). Haplotypes D and E are also found in Morocco (Tahzima et al. 2014). The first cultured Liberibacter pathogen is *L. crescens* from a Caricacea fruit in Puerto Rico (Fagen et al. 2014a, b). Higher diversity of a pathogen entails higher risk of overcoming host resistance and disease control measures (Lin and Gudmestad 2013).

ZC was first detected in Mexico in 1994, in Texas in 2000 and in California by 2007 (Fig. 1B) (Secor and Rivera-Varas 2004, Munyaneza et al. 2007). Initial outbreaks of ZC in central states were estimated to have reduced by 30% the potato acreage, with losses over 25 million dollars annually (Rosson et al. 2006). ZC was assumed to be a southern US problem and received little attention from potato growers in the PNW until 2011, when an outbreak of this

disease was first reported in this region causing alarm to the entire industry (Fig. 1B) (Hamm et al. 2011, Nolte et al. 2011, Crosslin et al. 2012). Control practices have increased the use of insecticides, likely weakening biological control and encouraging the development of insecticide resistance. The added psyllid costs have led to a 5 to 7% jump in total costs of production in some regions (Patterson 2012). In Idaho, the use of pesticides from 2007 to 2012 suffered an increment range of 56% to 129% depending on the region (Patterson 2012). A recent economic analysis suggests that these rising expenses jeopardize growers' economic viability (Greenway 2013). Comparing estimated percentages of total insecticide expenditure in potatoes in the U. S. in 2000 and 2013, the potato psyllid uses > 20% of the budget (Greenway et al. 2014). Insecticide resistance by the potato psyllids to imidacloprid has been observed in Texas and initial development of resistance to spirotetramat may be occurring there (Trumble 2014). Resistance to imidacloprid and spinosad in populations of the potato psyllid has been found in California (Liu and Trumble 2007). In the PNW no insecticide resistance has been observed (Trumble 2014).

Different haplotypes of potato psyllids in North America, based on genetic differences of the Cytochrome Oxidase I gene (COI), have been identified (Liu et al. 2006, Swisher et al. 2012, 2014). Texas, Wyoming, Nebraska and Kansas have only the Central psyllid COI-haplotype (Fig. 1C). New Mexico has a combination of Central, Western and Southwestern COI-haplotypes; Colorado has both the Central and Southwestern haplotypes; and California has the Central and Western haplotypes. Interestingly, Idaho, Oregon and Washington have three haplotypes, the Northwestern, Central and Western (Fig. 1C), perhaps making the epidemiology of zebra chip in the PNW complicated (Liu and Trumble 2007, Mustafa et al. 2015). Psyllids

with different haplotypes vary in biological traits such as fecundity, host preferences, and endosymbionts (Liu and Trumble 2007, Mustafa et al. 2015, Cooper et al. 2014). The Northwestern haplotype has not been collected outside of the PNW, strongly implying it is a locally-evolved resident of this region (Swisher et al. 2013).

Epidemiology and etiology of diseases vectored by insects are often complex, and ZC is not an exception (Lin and Gudmestad 2013). Acquisition of the pathogen by the vector, followed by inoculation, determines vector efficiency in transmitting and spreading the pathogen to new plants (Buchman et al. 2011). When psyllids have access to the whole plant, a higher pathogen acquisition success occurs (Buchman et al. 2011, Rashed et al. 2012). The rate of success in inoculation increases proportionally with the number of insects, but disease progress is not affected by psyllid numbers (Rashed et al. 2012). Plants inoculated with higher number of psyllids had higher bacterial titer but disease progress was not affected by bacterial quantity (Rashed et al. 2012). A single psyllid adult can transmit the pathogen to a potato plant in six hours of exposure, and if more psyllids are present, transmission time reduces to one hour (Buchman et al. 2011). Psyllid adults are highly efficient vectors of Lso, more so than psyllid nymphs (Buchman et al. 2011b). All of the development stages of the potato crop are susceptible to ZC infection but early infections are most damaging (Gao et al. 2009). Lso has a range from 17^{o}C to 32^{o}C for good development in the field (Cranshaw 2001), adjusted with its vector's range of an optimal of 27^{o}C to a top of 32-35^{o}C (List 1939, Lin and Gudmestad 2013). The pathogen can move from the foliage to the tubers within two days but symptoms are not visible and the pathogen is not detectable from the tubers at that time (Rush et al. 2015). In tubers from plants infected less than one week before harvest, or a few days before the vines are killed,

symptoms were not observed (Rush et al. 2015). Nevertheless, the pathogen continues developing in the infected tubers during storage (Rush et al 2015). In tubers, pathogen titer reached detectable levels from 7 to 14 days after infestation (Rashed et al. 2014). Lso can be detected in leaf tissue after 3-4 weeks (Rashed et al. 2014). Yield reduction is significant when vectors transmit the pathogen to early-development stages of the plants (Rashed et al. 2014). After storage of infected seed potato, all tubers failed to sprout (Rashed et al. 2015). Tubers for consumption, from plants infected one week before harvest, did not exhibit any disease symptoms or tested positive for Lso at the moment of harvest but after cold storage, 38% of the tubers tested positive for Lso (Rashed et al. 2015).

Lso infection disrupts the carbohydrate flow in the phloem through the plant to the roots. Lack of carbohydrates will initiate plant decline (Buchman et al. 2011a, Lin and Gudmestad 2013). Lso infection of plants five weeks before harvest, produces higher levels of phenolics, peroxidases, polyphenol oxidases, reducing sugars, amino acids, and defense proteins than later-infected plants (Rashed et al. 2013). Levels of the reducing sugars glucose and sucrose are elevated in infected tubers (Buchman et al. 2011a). These amounts of sugars react with amino acids at high temperatures developing the Maillard reaction (Gao et al. 2009) and producing the characteristic dark burnt vascular stains of Lso-infected fried potato chips (Munyaneza et al. 2009). Lso infection also disrupts biochemical composition at the level of leaves and stems (Wallis et al. 2015), and significantly reduces net photosynthetic rate (Gao et al. 2009). Lower levels of asparagine, aspartic acid, glutamine, fructose, glucose, sucrose, a ferulic acid derivative and quinic acid were found in leaves from Lso-infected potato plants (Wallis et al. 2015). Leaf starch accumulation was higher in leaves from infected plants (Gao et al. 2009), as were levels of

proline, serine, four phenolic compounds and terpenoids (Wallis et al. 2015). In stems, asparagine, aspartic acid, ellagitannins, monoterpenoids and sesquiterpenoids were grater in Lso-infected plants, suggesting defense-related terpenoid compounds might increase in infected plants (Wallis et al. 2015).

The insect *Bactericera cockerelli* (Šulc, 1909) (Hemiptera: Triozidae), also called the potato or tomato psyllid, has a length of 1.4mm, is light to dark brown with a head and thorax black with white lines and spots (Essig 1926). Psyllids can complete a generation in less than a month, with usually 3 or more generations per year (Hodkinson 2009). Once the potato psyllid acquires Lso, the pathogen can be transmitted constantly. An Lso-circulative and persistent mode of transmission was defined for *B. cockerelli* due to the presence of Lso in all organs, mainly in salivary glands and in the gut, hemolymph, bacteriocytes and reproductive organs (Cooper et al. 2014); Lso is vertically heritable (Hansen et al. 2008). Lso has negative effects in its host psyllid, altering fecundity and nymphal survival (Nachappa et al. 2012) when the bacterial titer increases (Nachappa et al. 2014). Most psyllid species are specific feeders attacking only a narrow range of host plants (Hodkinson 1974). Only a few psyllid species are polyphagous, e.g. *B. nigricornis* and *B. cockerelli* (Hodkinson 1974). According to Knowlton and Thomas (1934), *B. cockerelli* can complete its nymphal development and emerge as a normal adult upon the following 36 species of the family **Solanacea**: *Atropa belladonna* (deadly nightshade), *Datura metel* (devil's trumpet), *D. innoxia* (downy thornapple), *D. stramonium* (jimsonweed), *Hyoscyamus albus*, *H. niger* (black henbane), *Solanum pimpinellifolium* (currant tomato), *Lycium halimifolium* (boxthorn, matrimony vine), *Nicandra physalodes* (shoo-fly plant, apple of Peru), *Nicotiana glutinosa*, *N. tabacum* (common tobacco), *N. texana*, *Physalis angulata*,

P. franchetti (Japanese lantern), *P. heterophylla*, *P. peruviana* (golden berry), *P. pubescens*, *Salpiglossis* sp. (painted tongue), *Solanum aviculare*, *S. ballisii*, *S. capsicastrum* (false Jerusalem cherry), *S. carolinense* (horsenettle), *S. citrullifolium* (watermelon nightshade), *S. gracile*, *S. ledorodorsum*, *S. mexicanum*, *S. nigrum* (black nightshade), *S. phasianium*, *S. pyracanthum* (porcupine tomato), *S. racemigerum*, *S. sanitwongsei*, *S. sisymbriifolium* (sticky nightshade), *S. triflorum* (cutleaf nightshade, wild tomato), *S. tuberosum* (potato), *S. villosum* (woolly nightshade) and *S. melongena* (eggplant); one from **Convolvulaceae** family: *Convolvulus arvensis* (field bindweed) and one from **Menthaceae** family: *Micromeria chamissonis* (Knowlton and Thomas 1934, for common names: Uniprot, NPGS).

Additionally, according to Wallis (1955), potato psyllids can breed on the following 22 **Solanaceae** species: *Capsicum frutescens* (var. *conoides* and *grossum*) (chili pepper), *Lycium andersonii* (water jacket), *L. exsertum* (Arizona desert-thorn), *L. fremontii* (Fremont's desert-thorn), *L. pallidum* (pale desert-thorn), *L. parishii*, *L. torreyi* (Torrey wolfberry), *Nicotiana affinis* (flowering tobacco), *N. glauca* (tree tobacco), *Nierembergia hippomanica* (cup flower), *Physalis comata* (wild ground-cherry), *P. ixocarpa* (tomatillo, cultivated ground cherry), *P. lanceolata* (prairie ground-cherry), *P. lobata* (purple ground-cherry), *P. longifolia* (wild tomatillo, longleaf ground-cherry), *P. mollis* (field or longleaf ground-cherry), *P. pruinosa* (husk tomato), *P. rotundata* (round leaved ground-cherry), *Solanum elaeagnifolium* (silverleaf nightshade, white horse-nettle), *S. jamesii* (wild potato), *S. lycopersicum* (tomato), *S. rostratum* (buffalo-bur), and 2 **Colvolvulaceae**: *Ipomoea batatas* (sweet potato) and *I. purpurea* (morning glory) (Wallis 1955, for common names: Uniprot, USDA, NPGS).

Recently, other species have been included in the long list of hosts of *B. cocekerellli*, including *Capsicum annuum* (Chilli pepper) according to Vargas-Madriz et al. (2011) and *Physalis philadelphica* (Mexican ground-cherry) according to Crespo-Herrera et al. (2012). In Thinakaran et al. (2015a, b) studied another perennial plants, *Lycium barbarum* (common matrimony vine) and *Solanum elaeagnifolium* (Silver leaf nightshade) as a host of potato psyllids. This *L. barbarum* is distributed throughout the PNW growing region and supports large numbers of psyllids of the Western and Northwestern COI-haplotypes (Thinakaran et al. 2015a). The actual source of psyllids in PNW potato fields is unclear (Horton et al. 2015). Realization that potato psyllid is resident in the PNW led to a search for a host plant that could be used by the insect to bridge those seasonal intervals when the potato crop is unavailable (Horton et al. 2015).

A perennial nightshade, bittersweet nightshade (*S. dulcamara*), was found to support the psyllid year-round (Jensen et al. 2012, Murphy et al. 2013, Horton et al. 2015). Interestingly, the psyllids on *S. dulcamara* were found to be almost entirely of the Northwestern haplotype (Swisher et al. 2013b), despite the presence of the other two haplotypes in PNW potato fields (Swisher et al. 2013a). Bittersweet nightshade is also called climbing nightshade, blue bindweed, fellenwort, dogwood, woody nightshade, poison flower, poison berry, snake berry, or scarlet berry (Britton and Brown 1913). *S. dulcamara*, is a Eurasian species, widely introduced in thickets, clearings and open woods (Hitchcock and Cronquist 1973), and in wetlands (Waggy 2009). It often occurs on disturbed sites with other non-native species or in waste places (Waggy 2009, Britton and Brown 1913). This plant is somewhat woody below vines that grow branched (Hitchcock and Cronquist 1973). The vines tend to climb or scramble to 1-3 m (Hitchcock and

Cronquist 1973) when growing associated with, most commonly, cottonwood (*Populus* spp.), willow (*Salix* spp.), alder (*Alnus* spp.), Russian-olive (*Elaeagnus angustifolia*) or other common vegetation of riparian areas (Dixon and Carter 1999).

S. dulcamara hosts other agricultural pest such as the Colorado potato beetle (*Leptinotarsa decemlineata*) (Hare 1990), aphids (Semtner et al.1998, Flynn et al. 2006), and other insect plant feeders (Viswanathan et al. 2005). This nightshade has also been reported to house diseases such as the wilt-causing bacterium *Ralstonia solanacearum* (Elphinstone et al. 1998, Secor and Rivera-Varas 2004), and viruses (Smith 1931, Osmond et al. 1990).

Presence or impacts of predators or parasitoids on *B. cockerelli* collected from bittersweet nightshade has not previously been examined, but the effect of natural enemies on psyllids was observed in two host plants, American nightshade (*S. americanum* Miller) and potato crops (Butler 2011). Within that study, field experiments found significant reduction of potato psyllids exposed to natural enemies on both host plants, compared to the relatively high survivorship seen on caged plants that predators could not access (Butler 2011). This same research identified as key natural enemies of the potato psyllid in potato, tomato and pepper crops, the insect predators *Orius tristicolor*, *Geocoris pallens* Stal and *Hypodamia convergens* (Butler 2011). In solanaceous crops, predation of the potato psyllid has been studied for several species of hemipterans, coleopterans, neuropterans and mites (Knowlton 1933a,b, Knowlton 1994, Pletsch 1947, Al-Jabr 1999, Xu and Zhang 2015). In Utah, Knowlton (1933a) observed larvae of Chrysopidae preying upon psyllid nymphs in potato fields, and reported active predation of the potato psyllid by the big-eyed bug, *Geocoris decoratus* Uhler in laboratory experiments (1994). This predator is generally distributed and commonly occurs upon potatoes, and was also found to

be an important enemy of the beet leafhopper, nymphs of chinch bugs, and other small insects (Knowlton 1934). Several species of the genus *Chrysoperla* (e. g. *C. carnea* and *C. rufilabris*) have been observed consuming potato psyllids in different stages in the field and in laboratory trials (Pletsch 1947, Al-Jabr 1999). The ladybird beetle *Hippomania convergens* Guerin is an active predator of *B. cockerelli* nymphs (Knowlton 1933b).

The parasitoids *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) and *Metaphycus psyllidis* Compere (Hymenoptera: Encyrtidae) are included in the list of natural enemies of the potato psyllid (Romney 1939, Compare 1943). Pletsch (1947) described the parasitoid *T. triozae* attacking psyllid nymphs of the fourth and fifth development stage. Low parasitism of *T. triozae* was observed in the field and in the laboratory by Johnson (1957) in Colorado, and Butler and Trumble (2011) in California, due to low synchronization with the insect host and hyperparasitism by *Encarsia pergandiella* Howard and *E. peltata* (Clckerell) (Hymenoptera: Aphelinidae). Further studies qualify this parasitoid as a potential biological control agent of the potato psyllid living in natural areas (Butler and Trumble 2011) and a promising natural enemy of *B. cockerelli* in New Zealand (Workman and Whiteman 2009). Some of the most common insecticides (e. g. Abamectin, imidacloprid-cyfluthrin, and spinetoram) used in potato crops were found to reduce survival of *T. triozae*, suggesting that these chemicals are incompatible with biocontrol by this parasitoid within conventional potato production (Liu et al. 2012).

S. dulcamara also has been reported as a source of the entomopathogenic fungus *Beauveria bassiana* (Hare 1990), but there are no reports of naturally-occurring entomopathogenic fungi associated with *B. cockerelli* (Lacey et al. 2011). Some reports of the use of *B. bassiana* show significant mortality of psyllid nymphs in laboratory trials (Al-Jabr

1990). Isolates of *B. bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea* were studied in laboratory conditions against *B. cockerelli* adults and nymphs. In this study, all isolates except *B. bassiana* had high mortality 4 days after application (Lacey et al. 2009). In field trials in Texas, commercial formulations of *M. anisopliae* and *I. fumosorosea* demonstrated control of psyllid in all development stages at the same level as the control provided by the insecticide abamectin (Lacey et al 2011). Improvements in biological control of *B. cockerelli* can be expected with location and testing of additional species and isolates of entomopathogenic fungi (Lacey et al. 2011).

I explored the cultivated area of Eastern Washington to find bittersweet nightshade patches and determine if they were source of potato psyllids and Lso pathogen. While doing this sampling for psyllids, I also identified other pests and the predator community living on these plants. Furthermore, a list of the psyllid species found in entomological museums in the PNW and published records was completed as a starting point for researchers investigating the ecology of future, emerging pests and plant-pathogen insect vectors. Finally, a short scientific note was developed reporting the thrips species found while collecting potato psyllids from bittersweet nightshade.

In chapter two, I focus on the detection of the Lso pathogen in psyllids and plants, and haplotype identification of the collected psyllids. Other pests of potato and other crops were additionally identified. This chapter is written under the format of the scientific journal 'Environmental Entomology' of the Entomological Society of Entomology. The coauthors for this publication are Zhen Fu, Andrew S. Jensen and William E. Snyder.

In chapter three, I present the diversity of predators and natural enemies found in the same sampling effort made for psyllids in Chapter Two. This chapter is written under the format of the scientific journal 'Environmental Entomology' of the Entomological Society of Entomology. The coauthors for this publication are Zhen Fu, Andrew S. Jensen and William E. Snyder.

Chapter four presents a checklist of the superfamily Psylloidea present in the Pacific Northwest. There was no list previously presented for this region. I visited the entomological museums of the three main universities located in this region: Oregon State University, University of Idaho and Washington State University, and USDA laboratories in WA and OR, to build the list based on the psyllids present in the collections (other diverse sources also contributed to this list, as detailed in the Chapter). This chapter is written under the format of the scientific journal 'Proceedings of the Entomological Society of Washington'. The coauthors for this publication are Andrew S. Jensen and William E. Snyder.

Chapter five is a scientific note written under the format for the 'Florida Entomologist' journal of the Florida Entomological Society, that report thrips species collected from *S. dulcamara* and surrounding vegetation. The coauthors of this publication are Joseph Funderburk and William E. Snyder.

The four subsequent chapters presented in this dissertation are independent from each other.

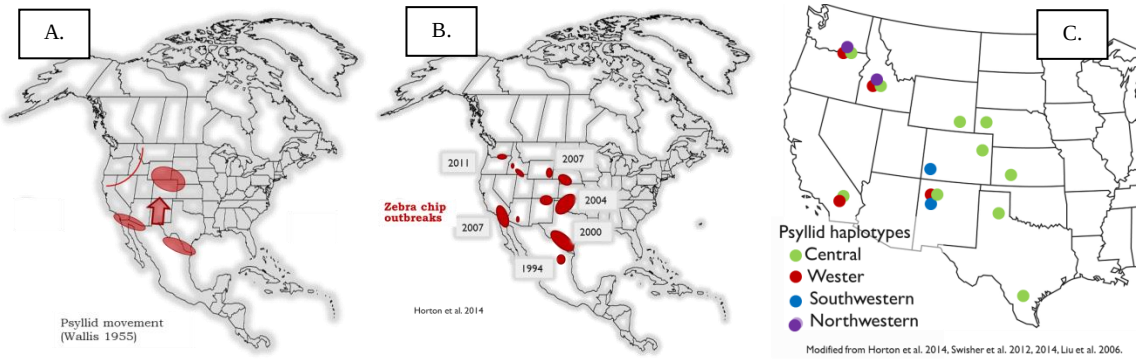

Figure 1-1. Wallis (1955) theory about psyllid movement from breeding areas to potato crop seasons up north. B. Zebra chip outbreaks in North America. C. COI psyllid haplotypes identified in the United States.

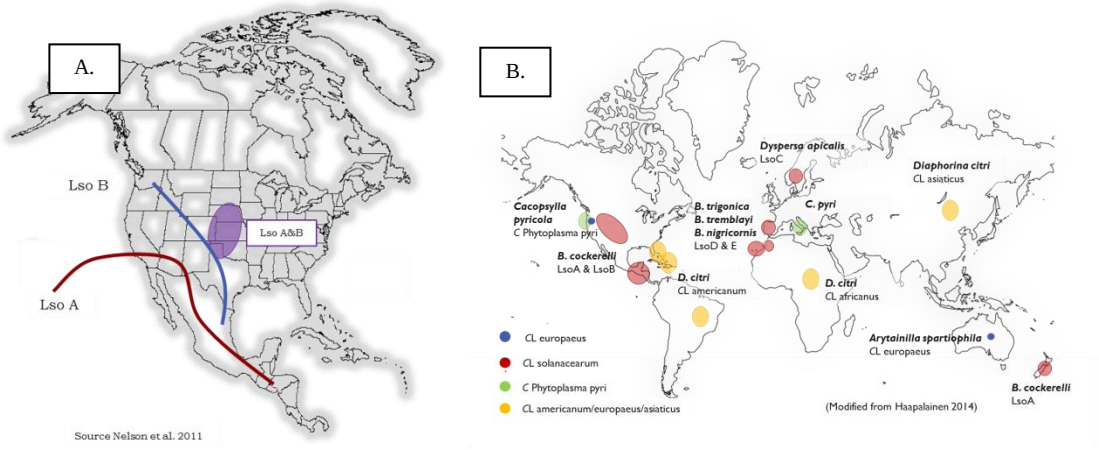

Figure 1-2. A. Haplotypes A and B of Lso in the United States. B. Some plant pathogens transmitted by psyllid species around the world.

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CHAPTER TWO: POPULATION DYNAMICS OF THE POTATO PSYLLID ON A NON-CROP HOST PLANT

Abstract

Recent outbreaks of zebra chip disease, caused by the bacterium *Candidatus* Liberibacter solanacearum and vectored by the potato psyllid (*Bactericera cockerelli* Šulc, 1909), surprised the northwestern-U. S. potato industry. Previously, the pathogen was unknown here and its vector was thought to be rare. We now know that the introduced weed bittersweet nightshade (*Solanum dulcamara* L.) houses potato psyllids in our region, although psyllid densities and reproduction have not been described and tracked on this host plant. Over two years we regularly monitored potato psyllid populations in bittersweet nightshade patches spanning the potato-growing region of eastern Washington. Psyllids occupied these plants throughout the year, at densities several orders of magnitude higher than those reported within nearby potato crops. Psyllid densities were lowest in spring and highest in fall, different than the mid-summer population peak seen in local potato fields. Egg production was observed throughout the single growing season where this was monitored, peaking in mid-summer. A subset of the psyllids that we collected was assigned to genetic groups using PCR and Sanger sequencing; all psyllids thus tested belonged to the Northwest haplotype. No psyllid or nightshade plant that we tested was found to carry the zebra chip bacterium. Aphid, beetle, and thrips pests of potato also were regularly found in nightshade patches. Altogether, our study confirms that while bittersweet

nightshade could act as a robust source of pests that eventually migrate to potatoes, in eastern Washington this plant did not appear to consistently harbor infected vectors or the zebra chip pathogen.

KEY WORDS vector ecology, zebra chip disease, bittersweet nightshade, psyllid haplotype

When zebra chip disease first emerged in potato fields of the southwestern U. S. in the 2000s, its impacts were devastating (Secor and Rivera 2004, Munyaneza et al. 2007a). Zebra chip is caused by a bacterium, *Candidatus* Liberibacter solanacearum ("Lso"), that is vectored by the potato psyllid, *Bactericera cockerelli*. The disease reduces yield and can make the crop unmarketable, causing a physiological defect in tubers that results in pronounced discoloration after cooking (especially in fries or chips; Secor et al. 2009). Yield losses in the southwest approached 50% in some years, costing tens of millions of dollars (Trumble 2009, Munyaneza et al. 2007a). While insecticide-spray regimes have now been developed to protect potato crops in the southwestern states, the development of effective integrated pest management schemes there has been thwarted, in part, by continuing uncertainty about how, why and when the insect moves among crop and non-crop host plants (Munyaneza et al. 2009, Murphy et al. 2013).

The Pacific Northwest states of Washington, Idaho and Oregon lead the U.S. in potato production in both total yields and crop value (USDA 2014). The region was initially thought to be safe from zebra chip disease, because the potato psyllid was thought to be rare in the Pacific Northwest and the pathogen absent (Wallis 1951, Cranshaw 1994, Hamm et al. 2011). The sudden appearance of zebra-chip-like symptoms during the 2011 growing season thus was a

surprise, and was soon followed by the confirmation of Lso infection and robust regional potato psyllid populations; crop losses mirrored those seen in the southwestern U. S. (Trumble 2009, Hamm et al. 2011, Crosslin et al. 2012a, b, c). Early research has indicated that the ecology of the potato psyllid in the Pacific Northwest may substantially differ, in several key respects, from what is seen in the southwestern U. S. (e.g., Liu et al. 2006, Liu and Trumble 2007). First, psyllid populations in northwestern-U. S. potato fields often are dominated by psyllids of the Northwest haplotype, distinguished by unique DNA sequences within the COI gene (Swisher et al. 2012). Northwest haplotype psyllids have never been recovered outside of our region, and exhibit behavioral and reproductive differences from the Central, Western and Southwestern haplotypes dominant in other regions of North America (Swisher et al. 2013a, Mustafa et al. 2015). Second, perennial, high-quality non-crop hosts may be particularly important for persistence of psyllids in the Pacific Northwest, being necessary to allow the insects to survive the relatively harsh winter conditions between potato-growing seasons (Jensen et al. 2012, Murphy et al. 2013). These unique characteristics of northwestern psyllid ecology have spurred research on regional psyllid genetics (Swisher et al. 2012, 2013b, Swisher and Crosslin 2014) and host-use ecology (Rondon et al. 2012, Horton et al. 2015).

Bittersweet nightshade, *Solanum dulcamara* L., has received particular attention as a non-crop host of potato psyllids in our region (Jensen et al. 2012, Murphy et al. 2013, Swisher et al. 2013b). This long-lived perennial solanaceous weed, originally introduced from Europe (Hawkes et al. 1979), is common along permanent waterbodies in the Pacific Northwest (Waggy 2009). The association with wetlands allows the plant to maintain green foliage throughout our typical summer drought, and often into early winter due to the temperature-buffering capacity of

waterbodies at lower altitudes (Jensen et al. 2012, Murphy et al. 2013). Indeed, potato psyllids have been found on bittersweet nightshade in Idaho, Oregon and Washington, at various points throughout the year (Jensen et al. 2012, Wenninger et al. 2012, Murphy et al. 2013). Most of the psyllids found on this nightshade are of the Northwestern haplotype, although Western haplotype psyllids have also been occasionally reported (Swisher et al. 2013b). Lso-infected psyllids have rarely been found on bittersweet nightshade (Swisher et al. 2013b), perhaps clouding the role of this weedy host as a driver of zebra chip problems in potato crops. We sought to expand upon these earlier findings by (1) quantifying psyllid densities on widely-dispersed nightshade patches over several growing seasons, while tracking haplotype-makeup of these insects; (2) regularly testing both psyllids and nightshade foliage for the presence of Lso through this time series; and (3) searching for potato pests other than psyllids on the nightshade plants. We also tracked natural enemy densities and biodiversity within these same bittersweet nightshade patches, but will present these data elsewhere (Castillo Carrillo et al., manuscript).

Materials and Methods

Identification of Bittersweet Nightshade Patches. As discussed above, bittersweet nightshade is associated with irrigation ditches, ponds, rivers, and other wetlands that contain year-round water (Jensen et al. 2012). Such locations are relatively uncommon in the arid conditions of Washington's Columbia Basin, much of which receives < 5 cm of precipitation during a typical summer (NOAA 2015). We identified possible sites to search for bittersweet nightshade through visual inspection of freely-available, web-published satellite images

(https://www.google.com/maps) of areas surrounding known potato-production areas. When waterbodies were identified in the satellite images, we looked for nearby public road access and visited the sites to search for nightshade patches. Sometimes, we noticed roadside patches of the weed while in transit to or from sites being surveyed. Our site-location process began in June 2012, and by October 2012 we had located 12 nightshade patches that roughly spanned the main area of potato production in Washington (Table 1, Fig. 1). One of these, the Kennewick site (Table 1), was sampled only for plant tissue (one time) due to our inability to safely scale the steep-sided drainage pond that housed the plant. A second location, our Pullman site (Table 1), was sampled only in 2012; in 2013, this location was eliminated from our sampling network because psyllids had never been found during the first year's sampling and the site is > 160 km from the nearest potato field. Therefore, by 2013 we continued to sample 10 locations in total (Table 1).

Overview of Sampling Protocol. In 2012, as bittersweet nightshade patches were located (described above) we periodically visited each until mid-December, stopping when most patches were completely defoliated, frozen and/or snow-covered in the winter in 2012. We then resumed sampling in April 2013, as new spring growth of the plants was first noted. During each visit to each nightshade location, we followed the same sampling protocol. First, we used a D-vac suction-sampling device (Rincon-Vitova Insectaries, Ventura, CA) to collect arthropods from bittersweet nightshade foliage, including but not limited to potato psyllids. Second, we collected bittersweet nightshade stems and foliage within each patch, to be tested for the presence of Lso. Finally, only during the 2013 growing season, we conducted visual counts of potato psyllid eggs every month from late March to mid-December. Details of how sampling of each type was

conducted, and of how samples were handled post-collection, follow below.

Collection and Processing of Bittersweet Nightshade Samples. Samples of *S. dulcamara* foliage were collected from each site at each sampling date (Table 1). During each visit we haphazardly identified a 30-cm section of vine for sampling, which was collected whole. Vines were placed into paper envelopes and plastic Ziploc® bags, immediately placed on dry ice, and then stored at -20^{o} C until processing. After removing plant samples from storage, we selected 2 petiole sections from each vine, one from near the apical tip and one from the basal end of the vine, to achieve ca. 100 mg of plant foliage per sample. Plant DNA extraction was performed using the DNeasy® Plant Mini Kit (Qiagen®). PCR used the specific primers OA2 and OI2 to detect Lso, which amplifies the mitochondrial 16S region of the bacterium (Liefting et al. 2009). Our Lso testing generally used the methodology described by Crosslin et al. (2011b), but modified by using Platinum PCR SuperMix as the Taq polymerase (Invitrogen, Foster City, CA). PCR products were visualized using agarose gel electrophoresis, with DNA samples displayed as predicted 1168-bp products (Crosslin et al 2011b). Positive controls were obtained from Lso-infected plant tissue, which always showed clear bright bands in the expected 1168-bp products.

Collection and Processing of Insect Samples. Insects were sampled with a backpack-mounted D-Vac suction sampler. This D-Vac model is powered by a modified lawnmower engine, and we have previously found it to effectively collect arthropods from potato foliage (e.g., Koss et al. 2005, Crowder et al. 2010). For each patch, we collected one D-vac subsample per ca. 1 m^2 of plant area. Because nightshade patches nearly always covered > 1 m^2, we collected more than one subsample from most sites at most sample dates. Each subsample consisted of placing the D-vac collection cone on one vine section, holding the cone in place

while shaking vigorously for 10 seconds. Captured insects in the mesh bag were immediately placed on dry ice for transportation to the laboratory. Once in the laboratory, insect samples were separately sorted and identified to family or, where possible, to species. Psyllid adults were visually identified as described by Jensen (2012), and their numbers tallied. Psyllids and all other arthropods were placed in 90% EtOH and stored at -20^{o} C.

While all psyllids collected within our timed suction samples were quantified, the large number of psyllids collected from the many sites and dates made it logistically impractical to assign all insects to genetic haplotype, or to test each insect for the presence of Lso. Therefore, we conducted these examinations using a haphazardly-drawn subset of up to 10 psyllids collected from each collecting visit to each site. From this subset of psyllids, DNA was extracted from each individual insect following the RPEX method, which uses a buffer composed of 75 µl of 100 mM Tris-HCL (pH 8.0), 5 mM sodium ethylenediamine tetraacetic acid, and 0.5% (v/v) Tween 20 (Crosslin et al. 2013). This methodology allowed extracting DNA more efficiently compared to the methods based on the commonly-used cetyltrimethylammonium bromide method (Crosslin et al. 2006).

PCR was done using the specific primers OA2 and OI2 to detect Lso through amplification of the mitochondrial 16S region, as described above (Liefting et al. 2009, Crosslin et al. 2011b). Psyllid DNA was combined for the ≤ 10 individual insects from each location/date, as we expected that per-capita Lso infection rates would be relatively low as reported previously (Crosslin 2012b). The primer pair has been shown to have high sensitivity, as OA2/OI2c can detect Lso of DNA extraction from a composite of 1 infected psyllid among 29 uninfected psyllids diluted 1000 times (Crosslin et al. 2011b). PCR products were visualized using agarose

gel electrophoresis, with DNA samples displayed as predicted 1168-bp products (Crosslin et al 2011b). Positive controls were Lso-infected psyllids reared in a colony maintained under greenhouse conditions; in all cases, the positive-control psyllids showed clear bright bands for the expected 1168-bp products.

A subset of the same insect DNA extracted for Lso identification, was used to test for potato psyllid COI-haplotype. Because it was logistically impossible to sequence COI for all insects collected, we instead haphazardly drew a subset of psyllids (50 insects in the first year, and 49 insects in year 2), with representatives from each site/date, for haplotype determination. Molecular analysis for haplotype identification was performed by amplification of a 500 bp region of the mitochondrial cytochrome oxidase subunit I (COI) gene. PCR was conducted following protocols described by Liefting et al. (2009) and Crosslin et al. (2011b), with the *B. cockerelli*-specific primers COIF3 and COIR3 designed by Crosslin et al. (2011b). PCR products were visualized on agarose gels. Those PCR products that displayed the predicted 500 bp product were processed in downstream PCR product-cleaning using the GeneJET PCR Purification Kit (Thermo Scientific, Waltham, MA). In order to accurately locate variant sites of the COI gene of our samples compared with studies already published (Swisher et al. 2012), we conducted Sanger sequencing (Elim Biopharm, Hayward, CA) on 50 individual psyllids haphazardly selected from the 2012 samples, and 49 individual psyllids from the 2013 samples (Table 1), using the same primers as in the amplification reaction described above. Returned sequences were aligned with known COI gene sequences of the Northwestern (GenBank Accession number JQ708093), Central (GenBank Accession number JQ708094), and Western (GenBank Accession number JQ708095) psyllid COI-haplotypes (Swisher et al. 2012) using the program BioEdit v7.2.5.

Counting Psyllid Eggs. During the 2013 growing season, a vine-section was haphazardly selected within each patch, during each sampling visit, for a visual count of psyllid eggs. Psyllid eggs on that focal undisturbed portion of the plant were counted over 5 minutes using the visual-survey method described by Koss et al. (2005). Psyllid eggs are easily identified because they are attached individually by a short stalk and are bright yellow, as described by Rondon et al. (2012).

Results

Lso in Bittersweet Nightshade Samples. Altogether, we collected 159 *S. dulcamara* foliage samples across all of the nightshade sites and sampling dates (Table 1). Lso was never detected in any of our plant samples, from any site or date. Because each run included Lso positive controls (described above) that always presented clear bands indicating Lso infection (Crosslin et al 2011b), we confirm the absence of Lso in our field-collected plant sub-samples.

Densities of Psyllid Adults and Eggs on Bittersweet Nightshade through Time. Across sites, adult psyllids exhibited broadly similar population dynamics, with relatively low densities recorded in early spring and relatively high densities recorded in fall (Fig. 2, Appendix Fig. 1).

We tracked densities of psyllid eggs in the second year of our study. We started our observations when new bittersweet nightshade growth first appeared in late March; psyllid eggs were first found in April, appeared to peak in late August, and continued to be recovered in relatively low numbers until mid-December when foliage was damaged by freezing temperatures (Fig. 3).

Haplotype and Lso Status of Potato Psyllids from Bittersweet Nightshade. We did not detect Lso in any of the 947 potato psyllids drawn for pathogen testing. In all runs the positive control (described above) exhibited a clear band of the expected size. High-quality consensus sequences of 99 psyllids (Table 1) drawn for this testing demonstrated 100% identity (GenBank accession numbers of samples, MN: KR534770, CX: KR534765, ML: KR534766, PV: KR534767) with the COI gene of potato psyllids designated as the Northwest haplotype (GenBank accession JQ708093).

Other Potato Pests Collected on Bittersweet Nightshade. While our sampling efforts targeted the potato psyllid, bycatch of these efforts included a diverse community of herbivores that are major or minor pests of cultivated potato (Table 2; natural enemies were also collected and these data are reported in Castillo Carrillo et al. [manuscript]). We identified three species of aphids reported to also occur in potato crops, the green peach aphid *Myzus persicae* (Sulzer) the potato aphid *Macrosiphum euphorbiae* (Thomas) and the foxglove potato aphid or glasshouse potato aphid *Aulacorthum solani* (Kalt.). However, per-plant aphid densities were generally low, with only ca. 12 alate aphids (the only aphid stage that could be reliably assigned to species) collected at the highest-aphid-density site/month (the October sample from Moses Lake). Other herbivorous hemipterans that we collected included *Nysius* sp., *Lygus* sp. and *Circulifer tenellus* (Baker) (Fig. 4B); both *Lygus* and *Circulifer* are minor pests on potato (Munyaneza et al. 2010, Murphy et al. 2014b). We also found the thrips species *Frankliniella occidentalis* (Pergande), *Thrips tabaci* Lindeman and *Caliothrips fasciatus* (Pergande), important pests and virus-vectors in several crops (Hoddle et al. 2012, Castillo Carrillo et al. in review). The Colorado potato beetle (*Leptinotarsa decemlineata* Say) occurred at low densities, but was found at 3 sites. Other

herbivorous Coleoptera included the leaf beetle *Oulema* sp. (a mean of 0.8 adults/subsample in August in Mesa-old), the wireworm *Dalopius* sp. (2 adults/subsample in April in Mesa-new), and the flea beetle, *Psylliodes* sp. (Fig. 4D).

Discussion

The unexpected arrival of zebra chip disease in the Pacific Northwest, a region thought to be relatively inhospitable to the potato psyllid, surprised the regional potato industry (Hamm et al. 2011). Initial research uncovered the presence of individuals of the Western and Central COI-haplotypes in our region, insects that presumably migrated-in from other regions of North America (Swisher et al. 2012). However, we now know that the region also houses robust, year-round populations of a genetically-unique population of potato psyllids, which has been designated the Northwest haplotype (Swisher et al. 2012, 2013a, 2013b, Murphy et al. 2013). While previous work has reported that the solanaceous weed bittersweet nightshade, *S. dulcamara*, is likely a key non-crop host that potato psyllids use for overwintering, the work reported here is the first systematic survey of psyllid densities on this plant across several growing seasons. We found that psyllid populations on the bittersweet nightshade plants that we monitored were consistently robust at most sites (Fig. 2), with densities occasionally exceeding 500 psyllid adults per subsample (e.g., Moses Lake site in October of 2012; Appendix Fig. 1). These densities are orders of magnitude higher than are typically recorded on potato plants under commercial cultivation. For example, in our own sampling regime using roughly the same suction-sampling methods reported here, in potato fields in eastern Washington we have never

found psyllid densities higher than 3.4 insects per potato plant (Fu et al., manuscript). That relatively high densities of psyllids are maintained on bittersweet nightshade plants during the early spring, late fall, and winter when cultivated potatoes are not available as hosts (Fig. 2), further strengthens the case that bittersweet nightshade is a key non-crop host for potato psyllids (e.g., Swisher et al. 2013b). The presence of psyllids on the plants during all times when plants exhibited any green foliage (Fig. 2) reinforces the suggestion (e.g., Swisher et al. 2013b, Murphy et al. 2013) that bittersweet nightshade is a key overwintering host for the insects.

Our data support the idea that potato psyllid utilizes bittersweet nightshade for reproduction throughout the growing season. We found consistent egg production on the weeds from mid-April, shortly after green foliage first appeared on the plants, until November/December, just before green foliage was killed by frost (Fig. 3). At several locations, hundreds of eggs were recorded over the course of the growing season (e.g., the Moses Lake site; Appendix, Table 1) on the subset of vines within each patch that we surveyed. Many psyllid species use various plants as shelter hosts, i.e. hosts not used for reproduction, at various times of the year (e.g. summer in some species, winter in others; Hodkinson 2009). The multi-voltine potato psyllid is clearly using bittersweet nightshade for reproduction throughout the plant's growing season.

While psyllids can, at atypically high densities, directly reduce potato yields (e.g., Wallis 1955), the insects' key economic impact results from their acting as a vector of Lso (Munyaneza et al. 2007a, 2007b). The work reported here suggests that while bittersweet nightshade could be a key source of psyllids that eventually colonize potato fields, the weed does not appear to serve as a consistent refuge for the zebra chip pathogen in eastern Washington. Of the > 900 potato psyllids that we tested for the presence of Lso, none were found to carry the bacterium.

Likewise, of the >150 nightshade foliage samples tested for Lso, none were Lso infected. Our findings related to psyllid infection are largely consistent with a study conducted in Oregon, Washington and Idaho, where just one Lso-infected potato psyllid was recovered from > 500 insects in total that were collected from bittersweet nightshade. Interestingly, that single infected psyllid in the earlier study belonged to the Western COI-haplotype, which comprised < 3% of the psyllids collected during those sampling efforts (Swisher et al 2013b). One possibility is that Western haplotype psyllids are the key vectors bringing Lso to the Pacific Northwest; as we did not recover that genetic type in our sampling, we also did not detect Lso. In contrast to our findings, Murphy et al. (2014a) reported that 5 of 21 foliar samples of bittersweet nightshade, collected in northeast Oregon in 2012, were Lso infected. It is unclear why Lso infection of bittersweet nightshade appeared to be relatively common in the earlier Oregon survey, while never being detected in our sampling across the border in Washington. We also remain uncertain whether any other non-crop plant species commonly houses Lso in eastern Washington. Other solanaceous weeds have been shown to successfully maintain Lso infection (Henne et al. 2010, Crosslin et al. 2011a), and also appear capable of supporting potato psyllid populations (Thinakaran et al. 2015). It is possible that other weedy host plants, and perhaps also psyllids migrating-in from outside the region, could play a significant role in bringing zebra chip to northwestern potato crops each year.

Going into this study, we hypothesized that we might see a drop in psyllid densities on bittersweet nightshade plants during July and August, a time when psyllids are increasingly found in and near potato fields (C. Wohleb and T. Waters, Washington State University, personal communication). This would be consistent with a large-scale migration of psyllids from

nightshades to potato, and perhaps other annual solanaceous host plants, when the weeds would be expected to be facing drought stress during our dry summers (Thinakaran et al. 2015). While declining mid-summer psyllid densities were observed at some individual nightshade sites (e.g., Mesa-old in 2013, Mesa-new in 2012, Mattawa in both years), at several others peak densities were observed in mid-summer (e.g., Moses Lake in 2013, Mesa-old in 2012, Pasco-Kahlotus in 2013) (Appendix Fig. 1). This means that we did not observe a general abandonment of bittersweet nightshade plants at the time potato psyllids are moving into potato crops in relatively large numbers. The plants remain consistently-utilized throughout the growing season, at least into early fall, but this of course does not exclude the possibility that a proportion of adults maturing on bittersweet nightshade are migrating to potato.

Much work has focused on bittersweet nightshade's role as a host for potato psyllid, reflecting that insect's role as the vector of devastating zebra chip outbreaks (e.g., Swisher et al. 2013b). While our whole-community sampling confirmed that potato psyllid is indeed the most abundant potato pest on bittersweet nightshade plants, several other economically-important herbivores also were found. Aphids were found at most sites, including the green peach aphid (*M. persicae*) that serves as the key vector of potato leafroll and other viruses that infect potatoes (Loebenstein 2001, Radcliffe and Ragsdale 2002, Srinivasan et al. 2013). In future work, it would be worthwhile to test bittersweet nightshade foliage for the presence of potato viruses, to see if this plant is a non-crop reservoir for that suite of potato pathogens. Indeed, other species of solanaceous weeds may serve as important reservoirs for potato viruses (e.g., Srinivasan et al. 2013). Other potato pests that we recovered included Colorado potato beetles, *L. decemlineata*, albeit infrequently (Fig. 4D). The apparent inability of northwestern-U.S. potato beetle

populations to develop resistance to commonly-used insecticides, in sharp contrast to the relatively rapid development of insecticide resistance in *L. decemlineata* populations in most other parts of the world, has long puzzled entomologists (Alyokhin et al. 2015). It has been suggested that reservoirs of susceptible potato beetles on non-crop host plants may be delaying resistance development within regional potato crops (Alyokhin et al. 2015), and our work suggests that bittersweet nightshade could be providing one such refuge. Other occasional potato pests that we found on bittersweet nightshade included the thrips *Frankliniella occidentalis* (see also Castillo Carrillo et al., in review), the flea beetle *Psylliodes* sp., the wireworm beetle *Dalopius* sp., and the beat leafhopper *Circulifer tenellus*; all of these species rarely drive spray decisions within regional potato production but do cause occasional economic concerns (Guenthner et al. 1999, Vernon and van Herk 2013, Munyaneza and Henne 2013). For all of these potato pests, as with the potato psyllid, additional work is needed to determine whether the insects do indeed move back and forth between bittersweet nightshade and potato crops. The bittersweet nightshade plants that we sampled also hosted robust populations of natural enemies (Castillo Carrillo et al., manuscript), and if bittersweet nightshade is an important source of natural enemies to potato crops this may outweigh some harm the plants cause as refuges for potato pests.

In summary, we found that, in eastern Washington, bittersweet nightshade harbors large, year-round populations of potato psyllids of the Northwest haplotype (Fig. 2) with consistent egg production throughout much of the growing season (Fig. 3). Recent population-genetic analyses (Fu et al. in review) strongly suggest that potato psyllids on bittersweet nightshade and nearby potato fields form a single, regularly-interbreeding population; this in turn implies that psyllids

might be regularly moving from bittersweet nightshade into potato fields (and/or vice versa). At the same time, the role of bittersweet nightshade as a source of Lso, the causative agent of zebra chip disease, remains uncertain. Although Lso-infected bittersweet nightshade plants have been reported in other states (e.g., Swisher et al. 2013b), our systematic sampling regime uncovered no infected nightshade plants or infected psyllids on those plants in Washington's Columbia Basin. It is unclear whether there are subtle regional differences in the likelihood of Lso-infected psyllids alighting on bittersweet nightshade plants, or in the plants themselves becoming infected. Regardless, by serving as a likely source of relatively large numbers of psyllids that eventually reach potato crops, bittersweet nightshade could be serving as an important source of psyllids that spread the bacterium within already-infected potato fields. Clearly, more work is needed to determine the specific source of Lso-infected psyllids that colonize northwest-U.S. potato fields, and the degree to which bittersweet nightshade populations indirectly influence zebra chip incidence.

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Table 2-1. *By* S. dulcamara *site and date, number of psyllid adults collected, number of psyllid and plant samples tested for Lso infection, and total number of psyllids sequenced for COI-haplotype (NW=Northwestern psyllid haplotype).*

Site name	Coordinates	Sampling date				Number of psyllids		# Samples for PCR		# Samples for PCR		SEQ 2012		SEQ 2013		% Lso infection	
		2012		2013		Total per site		2012		2013		# Psyllids		# Psyllids		2012-2013	
		Psyllids	Plants	Psyllids	Plants	2012	2013	Psyllids	Plants	Psyllids	Plants	Individual	Haplotype	Individual	Haplotype	Pyllids	Plants
Mesa (old) (MO)	46°35'17.72"N, 119° 0'1.12"W	28 Jun	28 Jun	27 Apr	27 Apr	17	9	15	4	9	3	1	NW	2	NW	0	0
		17 Jul	17 Jul	24 Aug	24 Aug	156	0	30	1		3		NW	2	NW	0	0
		2 Aug	2 Aug	26 Oct	26 Oct	7	4	7	1	4	3	1	NW	1	NW	0	0
		15 Aug	15 Aug			5		5	1							0	0
		28 Aug	28 Aug			0		0	4							0	0
		11 Sep	11 Sep			0		0	5							0	0
		27 Sep	27 Sep			0		0	1							0	0
		4 Nov	4 Nov			44		26	1			2	NW			0	0
		24 Nov	24 Nov			4		4	1							0	0
			16 Dec						1								0
Mesa (new) (MN)	46°34'35.35"N, 119° 0'33.41"W	2 Aug	28 Jun	27 Apr	27 Apr	36	3	10	1		3			2	NW	0	0
		15 Aug	17 Jul	24 Aug	24 Aug	55	38	24	1	23	3					0	0
		28 Aug	2 Aug	26 Oct	26 Oct	69	280	23	1	30	3	1	NW	9	NW	0	0
		11 Sep	15 Aug			305		29	1							0	0
		27 Sep	28 Aug			250		46	2			1	NW			0	0
		4 Nov	11 Sep			1472		33	1			1	NW			0	0
		25 Nov	27 Sep			1101		63	1			1	NW			0	0
		16 Dec	4 Nov			510		47	1			1	NW			0	0
			24 Nov						2								0
			16 Dec						1								0
Colfax (CX)	46°50'51.1008"N, 117°28'43.9320"W	27 Sep	27 Jun	27 Apr	27 Apr	12	0	3	1		3	2	NW			0	0
		6 Oct	27 Jul	24 Aug	24 Aug	41	10	22	1	9	2	2	NW	3	NW	0	0
		4 Nov	27 Sep	26 Oct	26 Oct	242	239	20	1	30	2			6	NW	0	0
		25 Nov	6 Oct			210		32	1			3	NW			0	0
		16 Dec	4 Nov			9		8	1			2	NW			0	0
			25 Nov						1								0
			16 Dec						1								0
Moses Lake (ML)	46°58'53.06"N, 119°38'49.20"W;	30 Aug	30 Aug	27 Apr	3 May	59	0	22	1		4	1	NW			0	0
	46°59'34.19"N, 119°41'6.66"W;	15 Sep	15 Sep	24 Aug	24 Aug	119	100	40	2	20	4	2	NW	2	NW	0	0
	46°59'55.10"N, 119°41'5.25"W;	6 Oct	6 Oct	26 Oct	26 Oct	3458	129	44	2	45	5	1	NW	9	NW	0	0
	47° 0'7.35"N, 119°41'5.03"W	4 Nov	4 Nov			709		40	2			1	NW			0	0
		10 Nov	10 Nov			398		62	1			1	NW			0	0
		24 Nov	24 Nov			121		20	1							0	0
		16 Dec	16 Dec			21		10	1			2	NW			0	0
Caliche Lake (CL)	47° 1'53.65"N, 119°55'39.78"W	6 Oct	15 Sep	27 Apr	3 May	109	1	16	1	1	1	2	NW			0	0
		10 Nov	6 Oct	24 Aug	24 Aug	100	2	18	2	2	2	10	NW	2	NW	0	0
			10 Nov	26 Oct	26 Oct		4		1	4	2			2	NW	0	0
			25 Nov						1								0
			16 Dec						1								0
Pasco-Vineyard (PV)	46°19'38.06"N, 119° 7'13.98"W	17 Jul	17 Jul	27 Apr	27 Apr	0	0		1		2						0
		2 Aug	2 Aug	24 Aug	24 Aug	0	6		1	6	2			1	NW	0	0
		8 Aug	28 Aug	26 Oct	26 Oct	0	7		1	7	1			2	NW	0	0
		28 Aug	11 Sep			0			1								0
		11 Sep	27 Sep			0			1								0
		27 Sep	4 Nov			5		2	1			3	NW			0	0
		4 Nov	16 Dec			8		5	1			5	NW			0	0
Mattawa (MT)	46°42'32.33"N, 119°56'42.54"W	4 Sep	4 Sep	27 Apr	3 May	0	0		3		2						0
		20 Sep	20 Sep	24 Aug	24 Aug	0	0		5		2						0
		10 Nov	10 Nov	26 Oct	26 Oct	4	4	2	2	4	2	2	NW	1	NW	0	0
			16 Dec						1								0
Pasco-Kahlotus (PK)	46°16'38.59"N, 118°50'29.07"W	8 Aug	8 Aug	27 Apr	27 Apr	0	3		1	3	4			1	NW	0	0
		28 Aug	28 Aug	24 Aug	24 Aug	0	10		1	9	2			1	NW	0	0
		11 Sep	11 Sep	26 Oct	26 Oct	2	11	2	1	11	3	2	NW	3	NW	0	0
		27 Sep	27 Sep			0			1								0
		4 Nov	4 Nov			0			1								0
Pullman (PU)	46°43'8.50"N, 117° 9'59.37"W	16 Aug	16 Aug			0			1								0
		27 Sep	27 Sep			0			1								0
			9 Nov						1								0
			24 Nov						1								0
Sacajawea Park (SJ)	46°12'12.7692"N, 119°02'49.6644"W	4 Nov	15 Sep	27 Apr	27 Apr	0	0		1		3						0
			4 Nov	24 Aug	24 Aug		0		1		1						0
				26 Oct	26 Oct		0				1						0
Warden (WD)	46°54'44.90"N, 119° 7'26.09"W	15 Aug	15 Aug	24 Aug	24 Aug	0	0		1		1						0
		30 Aug	30 Aug	26 Oct	26 Oct	0	0		1		1						0
		14 Sep	14 Sep			0			1								0
		6 Oct	6 Oct			0			1								0
Kennewick (KN)	46°10'33.8"N, 119°17'30.4"W		7 Aug						1								0
Total						9658	860	730	89	217	70	50	50	49	49	0	0

Table 2-2. *Potato pests collected on bittersweet nightshade.*

Order	Family	Species	Total individuals	Highest mean per subsample	Peak month	Place
Hemiptera	Aphididae	*Myzus persicae*	278	11.8	Oct	ML
		Macrosiphum euphorbiae	105	5.6	Nov	MN
		Aulacorthum solani	51	6	Aug	WD
	Cicadellidae	*Circulifer tenellus*	17	0.7	Nov	MO
	Lygaeidae	*Nysius* sp.	72	2.3	Aug	MN
	Miridae	*Lygus* sp.	58	3.5	Sep	CX
Coleoptera	Chrysomelidae	CPB[1]	21	7	Nov	SJ
		Psylliodes sp.	143	3.5	Apr	PK
		Oulema sp.	11	0.8	Aug	MO
	Elateridae	*Dalopius* sp.	4	2	Apr	MN
Thysanoptera	Thripidae	*Frankliniella occidentalis*	7	2	Nov	SJ
		Thrips tabaci	3	1	Aug	ML
		Caliothrips fasciatus	53	12.5	Aug	MT

[1] Colorado potato beetle, adults plus larvae.

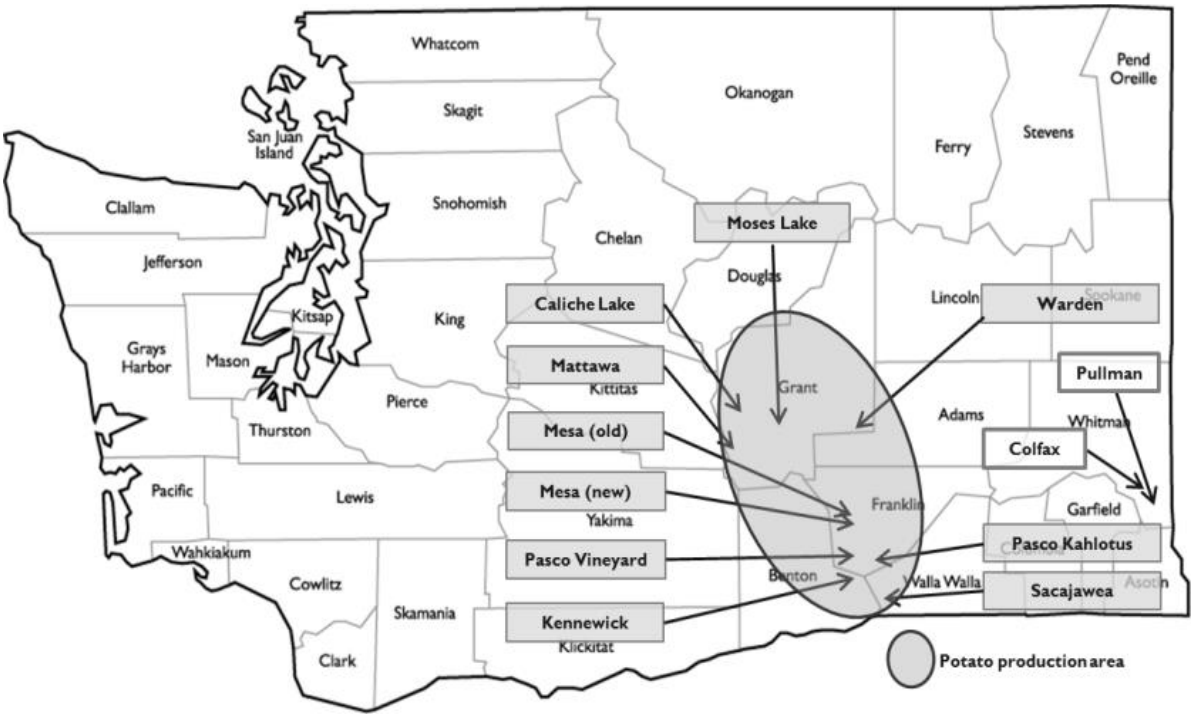

Figure 2-1. Locations of the *S. dulcamara* patches that we sampled for potato psyllids and other herbivores. The patches roughly span the potato-growing region of Washington's Columbia Basin, with the Pullman and Colfax sites lying outside where potatoes are commercially grown.

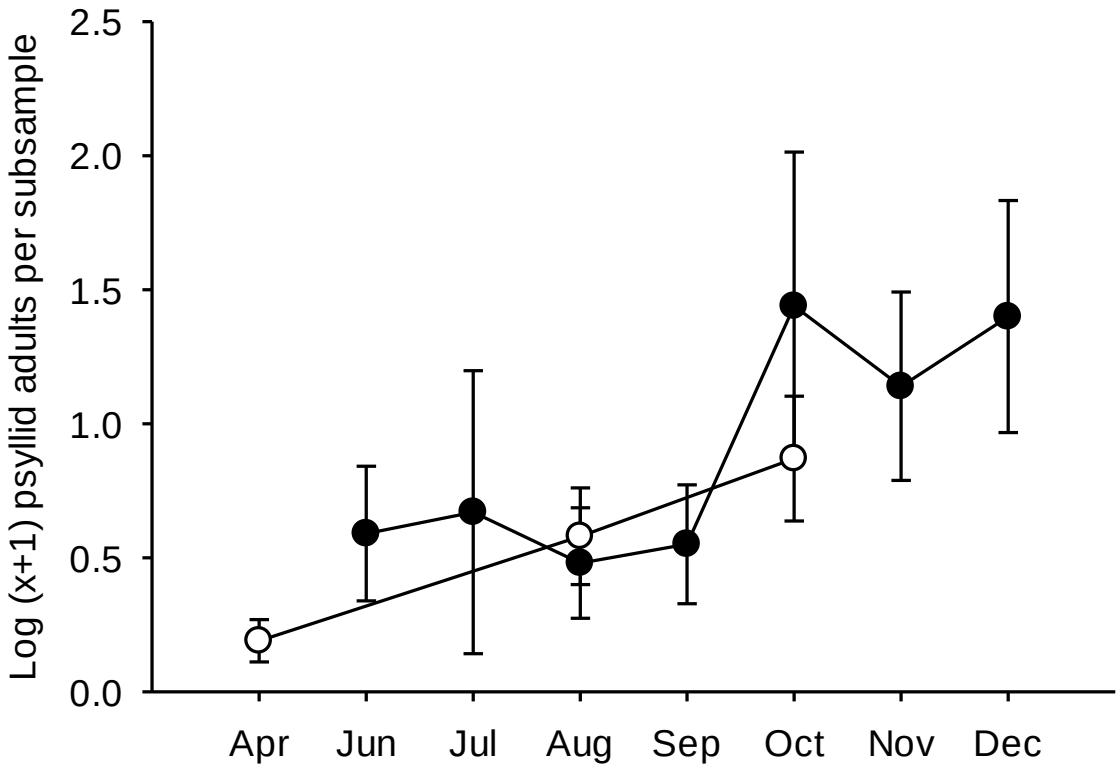

Figure 2-2. Density of potato psyllid adults suction-collected from *S. dulcamara* in Washington's Columbia Basin over two growing seasons (2012 and 2103), across ten sites (Fig. 1). Data are means ± 1 S.E.

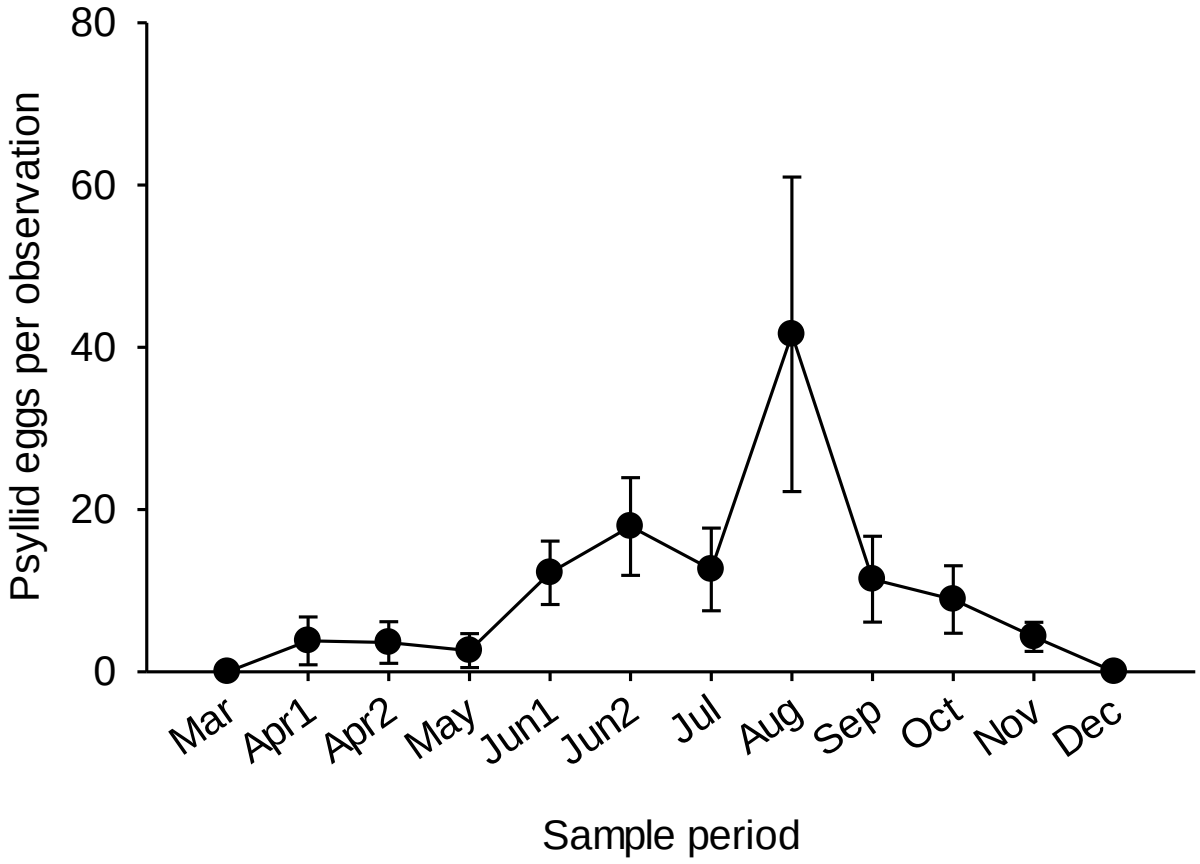

Figure 2-3. Density of potato psyllid eggs found through timed visual counts on sub-sections of *S. dulcamara* vines across multiple sites (Fig. 1). Patches were sampled first early in the month ("1") and again later in the month ("2") in April and June. Data are means ± 1 S.E.

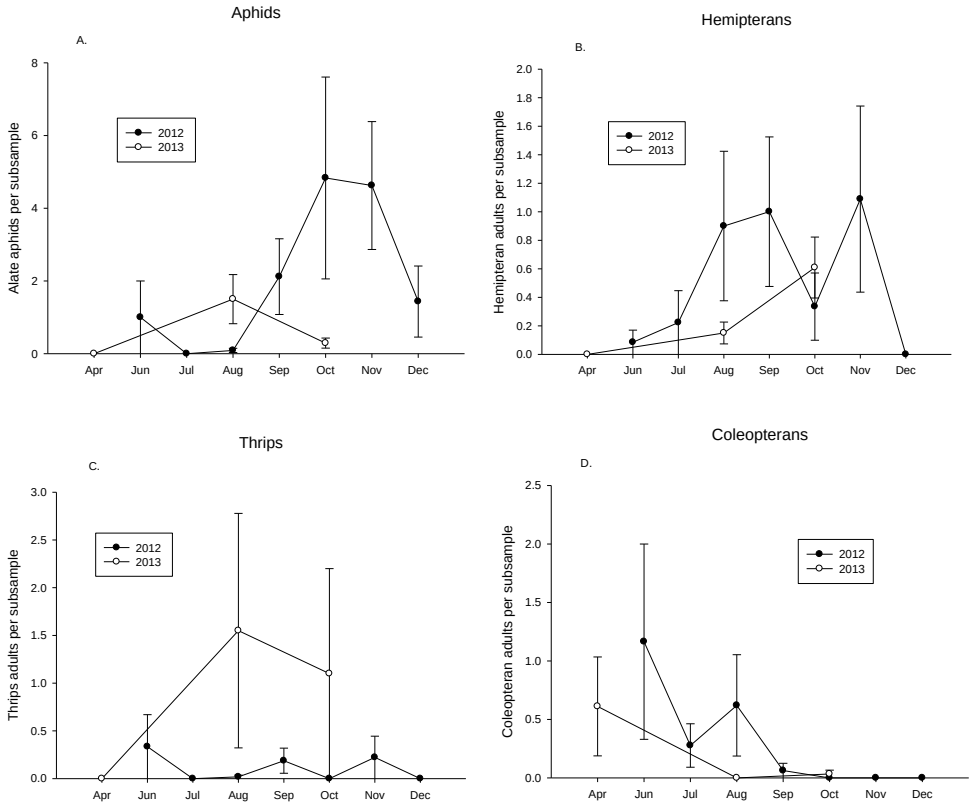

Figure 2-4. Herbivores other than potato psyllids found in *S. dulcamara* patches, that also are significant or occasional pests of cultivated potato. Possible potato pests that were found included (a) aphids, summed densities for the species *Myzus persicae*, *Macrosiphum euphorbiae*, and *Aulacorthum solani*; (b) herbivorous Hemiptera (taxa detailed in Table 2); (c) herbivorous thrips, summed across the species *Frankliniella occidentalis*, *Thrips tabaci* and *Caliothrips fasciatus*; and (d) herbivorous Coleoptera (taxa detailed in Table 2). Data are means ± 1 S.E.

CHAPTER THREE: ARTHROPOD PREDATORS ON BITTERSWEET NIGHTSHADE, A NON-CROP HOST OF THE POTATO PSYLLID

Abstract

Bittersweet nightshade (*Solanum dulcamara* L) is known to serve as a key year-round, non-crop host of the potato psyllid (*Bactericera cockerelli* Šulc, 1909) in the northwestern U.S. Recently, this solanaceous weed has been reported to also harbor a wide variety of other herbivorous insect species that attack potato (*Solanum tuberosum* L.). Here, we explore the community of predators and other natural enemies found in bittersweet nightshade patches; extensive predation at these sites could moderate the weed's role in supplying pests to nearby potato crops. Over two years, we repeatedly sampled natural enemies in bittersweet nightshade patches spanning the potato-growing region of eastern Washington State. We found robust populations of a bio-diverse community of > 40 predatory arthropod taxa. Spiders (Araneae), primarily in the Families Dictynidae and Philodromidae, made up 70% of all generalist-predator individuals collected. Other generalist predatrors included predatory mites (Prostigmata: Anystidae), Hemiptera (Anthocoridae, Nabidae and Geocoridae), and Coleoptera (Coccinellidae). The coccinellid beetle *Stethorus punctillum* Weise was observed eating psyllid eggs, while the parasitoid wasp *Tamarixia triozae* (Burks) was observed parasitizing potato psyllid nymphs. Soil beneath nightshade patches sometimes housed the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar and the entomopathogenic fungus *Beauveria bassiana*. While previous work implies that bittersweet nightshade serves as a key potential

source of pest insects that colonize potato fields, our study demonstrates that these weeds also house sizeable and species-rich communities of natural enemies. Additional work is needed to quantify predation of potato pests on these plants, and to determine whether natural enemies move from nightshade patches to potato fields.

KEY WORDS potato pests, generalist predators, parasitoids, entomopathogens, solanaceous weed

The emergence of zebra chip disease in Northwestern potato crops, caused by the bacterium *Candidatus* Liberibacter solanacearum (Lso) and vectored by the potato psyllid (*B. cockerelli*), surprised the large potato industry in pacific northwest of the U.S. (Hamm et al. 2011). Previously, it was thought the region's climate was too harsh for the psyllids to readily overwinter, and the pathogen was not known to occur here (Munyaneza et al. 2009, Hamm et al. 2011). Initial research soon revealed that a genetically-distinct psyllid population occurred exclusively in the northwestern states of Idaho, Oregon and Washington (Swisher et al. 2012). These psyllids were dubbed the Northwest haplotype, differentiated by unique sequence-coding within the Cytochrome Oxidase I gene (Swisher et al. 2012). Northwest-haplotype psyllids appear to exhibit behavioral and ecological differences from other psyllid genetic types dominant elsewhere (Swisher et al. 2013, Mustafa et al. 2015).

Northwest-haplotype psyllids are particularly abundant on a common exotic weed, bittersweet nightshade (*S. dulcamara*), native to Europe and associated in its introduced range with irrigation ditches, canals, and other permanent wetlands (Hawkes et al. 1979, Waggy 2009).

Bittersweet nightshade maintains robust densities of potato psyllids outside of the summer potato-growing season (Swisher et al. 2013), and supports growing-season-long potato psyllid reproduction (Castillo Carrillo et al., manuscript). Recent genetic evidence suggests that the psyllids found in potato crops form one frequently-interbreeding population with the potato psyllids on surrounding patches of bittersweet nightshade (Fu et al., manuscript), supporting the contention that bittersweet nightshade is a notable source of the potato psyllids migrating to potato crops each growing season (Nelson et al. 2014, Jensen et al. 2012, Swisher et al. 2013, Murphy et al. 2014). Intriguingly, Lso-infected psyllids are rare on bittersweet nightshade (Swisher et al. 2013), and few plants of this species have been found to be infected with the bacterium (Murphy et al. 2014, Castillo Carrillo et al., manuscript), rendering the plant's role in maintaining and spreading Lso unclear (Murphy et al. 2014, Castillo Carrillo et al., manuscript).

While the role of bittersweet nightshade as a critical host for potato psyllids, and perhaps other potato pests (e.g., Castillo Carrillo et al., in review), is well defined, relatively little is known about the community of natural enemies on these plants. If predators, parasitoids and pathogens commonly attack the potato pests seeking refuge on bittersweet nightshade, this might reduce the number of herbivores available to migrate to potato fields. Furthermore, robust populations of natural enemies in bittersweet nightshade could provide a source of beneficial arthropods to migrate to potato fields (e.g., Szendrei and Weber 2009). In both of these ways, bittersweet nightshade might be indirectly contributing to the suppression of potato pests; this could somewhat mitigate the harm bittersweet nightshade does to potato production as a source of potato pests. Here, we report an intensive survey of natural enemies on bittersweet nightshade plants scattered across the potato-growing region of eastern Washington State, U.S.A., continued

over two years. Arthropod collections were complemented by timed observations of predator activity, and also by collections of entomopathogens in soils beneath a subset of the nightshade patches.

Materials and Methods

The predator data presented here were derived from the sampling regime described in Castillo et al. (manuscript), who reported potato psyllid densities, reproduction, COI-haplotype, and Lso status through time in the same set of bittersweet nightshade patches. Details of how we initially located the nightshade-patches that we sampled are presented in that earlier manuscript, while here we focus on our characterization of the community of natural enemies collected, in part, as bycatch during our psyllid sampling efforts. Briefly, we tracked natural enemy densities and biodiversity in 10 patches of *S. dulcamara* located across the potato-growing region of eastern Washington state, U.S.A. (Table 1; see also Castillo et al., manuscript). Patches were first located in April 2012, and as new patches were discovered they were added to the sampling network until all 10 patches were in place in October of 2012 (Castillo Carrillo et al., manuscript); we then continued periodically sampling these patches through October of 2013 (Castillo Carrillo et al., manuscript). Samples were collected monthly in 2012, but we reduced our sampling intensity to 3 sample dates in 2013 (April, August and October) to avoid depleting arthropod populations from too-frequent collections (Castillo Carrillo et al., manuscript). Natural enemies were sampled using three methods: (1) suction-sampling using a D-vac device (Koss et al. 2005, Crowder et al. 2010); (2) visual observations during timed observations of sections of

nightshade vines (Koss et al. 2005); and (3) soil collections beneath plants and placement of sentinel host insects to collect entomopathogenic nematodes and fungi (Jabbour et al. 2011). Each of these sampling efforts is described in detail below.

Suction Samples. Arthropods were collected from bittersweet nightshade foliage using a backpack-mounted D-Vac suction sampler (http://www.rinconvitova.com/d-vac.htm). This D-Vac model is powered by a modified lawnmower engine, and we have previously found it to effectively collect predatory arthropods from potato foliage (e.g., Koss et al. 2005, Crowder et al. 2010). For each patch, we collected one D-vac subsample per ca. 1 m^2 of plant area. Because nightshade patches nearly always covered > 1 m^2, multiple subsamples were collected from most sites/dates. Each subsample consisted of placing the D-vac collection cone on one vine section, holding the cone in place while shaking vigorously for 10 seconds. Captured insects in the mesh collecting bag were immediately placed on dry ice for transportation to the laboratory where they were stored at -20^o C. Later, in the laboratory, insect samples were sorted to family, genus or species. After sorting the field-collected samples, all arthropods were placed in 90% EtOH and stored at -20^o C.

Morphological predator identifications, for most taxa, were made by the lead author. Spiders were identified using a variety of published keys (i.e., Bradley 2013, Kaston 1978 and Ubick et al. 2005), with our identifications verified by Dr. Rod Crawford (Curator of Arachnids, Burke Museum, University of Washington). Predatory bugs were identified using published keys (i.e., Tamaki 1972, Harris 1928, Lewis and Horton 2010) and confirmed by Dr. David Horton (USDA-YARL, Wapato, Washington, USA). Predatory beetles were identified using the published keys of Triplehorn and Johnson (2005), Marshall (2006) and Gordon (1985), and

through comparison with specimens housed in the M. T. James Museum (Department of Entomology, Washington State University, Pullman, WA, USA); the coccinellid *S. punctillum* was identified by Dr. Steve Heydon (Senior Museum Scientist, Bohart Museum of Entomology, University of California – Davis). The predatory mites *Anystis agilis* (Banks) and *Bdella* sp. were identified by Drs. Cal Welbourn and Ron Ochoa (Florida Department of Agriculture and Consumer Services, Florida; USDA Beltsville, Beltsville, MD, USA). The psyllid nymph parasitoid *T. triozae* was identified using the published description (Burks 1943), confirmed by identified by Dr. Robert L. Zuparko (Essig Museum of Entomology, University of California – Berkeley, Berkeley, CA, USA). A large group of other parasitoid Hymenoptera and Diptera was collected alongside the generalist predators and *T. triozae*, but identification of these many, often minute, insects was beyond the logistical scope of this project.

Observation of Predator Activity. Monthly during the 2013 growing season, from early spring (March) to the beginning of winter (December), a vine-section was haphazardly selected within each bittersweet nightshade patch, during each sampling visit, for a visual survey of predator activity. Natural enemies were counted over 5 minutes using the visual-survey method described by Koss et al. (2005). Two timed observations were performed per patch and per visit when nightshade patches were relatively small (i.e., the Caliche Lake, Pasco-Vineyard, Warden, Mattawa, Sacajawea sites), and three timed observations were conducted during each visit to the remaining sites where nightshade patches were relatively large.

Soil Sampling for Entomopathogens. We took soil samples from three sites (Pasco-Kahlotus, Mesa-new and Moses Lake; Table 1) to isolate soil-dwelling entomopathogenic nematodes and fungi. We chose these sites because (1) they roughly bridged the northern and

southern limits of potato production in eastern Washington, and (2) soil beneath the plants at these sites was readily accessible (often bittersweet nightshade grows on steep banks or from among large rocks where soil collections would be difficult). In August 2012, we collected a 10 cm-thick and 20 cm-deep layer of soil from directly beneath the basal stems of two *S. dulcamara* plants at each site. The soil was then placed in a plastic bag in a cooler on ice to be returned to the laboratory for processing.

In the laboratory, we followed protocols detailed by Lacey (2012) for extracting entomopathogenic nematodes and fungi from the soil. Briefly, each of the six soil subsamples (described above) was placed in a separate 1-liter plastic container, to each of which we added five *Galleria mellonella* (L) larvae. Larvae were placed on the soil surface and then the container inverted to allow the larvae be buried in, and travel through, the soil. From days 3 to 5 thereafter, we collected larval cadavers that presented symptoms of entomopathogen attack; infection by nematodes is indicated when larvae turned brownish or reddish, while infection by fungi is indicated by mycelium growth around the dead larvae (Lacey 2012).

Larvae suspected of nematode infection were placed in modified White traps (White 1927, Lacey 2012), which capture migrant infective nematode juveniles into water. When nematodes were trapped into the water, we transferred the worms, in 1 ml of water solution, to a Petri dish containing filter paper and 5 *G. mellonella* larvae; this allowed us to verify that the nematodes could infect this second group of insects. Nematode isolates, collected from this second group of successfully-infected *G. mellonella* larvae, were thereafter stored in water containing a drop of Triton x100® (Sigma-Aldrich) at 4^{o}C.

Larvae suspected of fungal infection were placed on glass slides in a moist Petri dish

chamber, which spurs fungal growth and sporulation; these larvae were incubated at 23^{o}C for three days until sporulation was observed. Following sporulation, larvae were moved to a laminar flow hood, washed using a 2-3% sodium hypochlorite solution, dried on sterile paper, and placed on potato dextrose agar medium contained in Petri dishes, and incubated at 23^{o}C until fungal growth was observed. Colonies of these fungi were purified with repeated isolations and maintained at 4^{o}C in the refrigerator. Infectivity was confirmed by exposing potato psyllid adults to the fungi, and then re-isolating fungi from potato psyllids using the methods described above.

Molecular Identification of Natural Enemies. Several natural enemy taxa were not readily identified using the morphological keys described above. In order to confirm our initial identifications (and/or identifications made by our cooperating taxonomic specialists, described above), we used molecular means for species verification. The species ultimately identified by molecular means were the predators *A. agilis* and *S. punctillum,* the parasitoid *T. triozae*, and the entomopathogens *B. bassiana* and *H. bacteriophora*.

For the arthropods, we first extracted DNA from randomly-picked subsamples of 16 *S. punctillum* beetles, 16 *T. triozae* wasps and 10 *A. agilis* mites from all nightshade locations and a subset of sample dates. Individual insects were processed using the DNA extraction protocols developed for the DNeasy® blood and tissue kit (Qiagen). For the entomopathogenic nematodes, approximately 50-100 worms (infective juvenile stage) from each of entomopathogenic nematode isolate were washed in sterilized distilled water, then processed for DNA extraction following the same protocol used to extract arthropod DNA. For the entomopathogenic fungi, we conducted DNA extraction for the 3 different isolates, collected as described above, following instructions of the DNeasy® plant mini kit (Qiagen) after collecting mycelium and spores

cultured on potato dextrose agar.

For molecular identification of all of the above-listed natural enemy species, we used 2 µl of the DNA in a 50 µl PCR reaction which was composed of 45 µl Platinum® PCR SuperMix (Thermo Fisher Scientific, Waltham, MA), 0.5 µl each of 10 uM forward and reverse primer (Table 2) and 2 µl of nuclease-free water. For *T. triozae*, the entomopathogenic fungus and the entomopathogenic nematode, the PCR reactions were conducted with the following condition: 2 mins at 94 °C for initial denaturation, followed by 35 cycles of 30 s at 95 °C for denaturation, 30 s at 51 °C for annealing, 1 min at 72 °C for extension, and 5 mins at 72 °C for the final extension. For *S. punctillum* PCR reactions, the PCR condition was modified to the following program: 2 mins at 94 °C for initial denaturation, followed by 35 cycles of 30 s at 95 °C for denaturation, 30 s at 46 °C for annealing, 80 s at 72 °C for extension, and 5 mins at 72 °C for the final extension. For the PCR amplification of the predator mite *A. agilis,* we used a lower annealing temperature (40 °C) by following the protocol of Folmer et al. (1994). The rest of the parameters for the thermal cycler were the same as described for amplification of *S. punctillum*. The rationale of using low annealing temperatures to amplify *S. punctillum* and *A. agilis* was these primers were designed for more general taxa (Hendrich et al. 2015, Folmer et al. 1994), therefor we decreased the annealing temperature to reduce the PCR stringency. All PCR products were examined by electrophoresis in 1.5 % agarose gel. The PCR products that displayed single bright bands with expected size were further purified by using the GeneJET PCR Purification Kit (Thermo Fisher Scientific, Waltham, MA).

Sanger sequencing was conducted by Elim Biopharm (Hayward, CA) on purified PCR samples with the same primers used in the PCR reactions (Table 2). Returned sequences were

cleaned by removing low quality bases, and consensus sequence was generated by aligning forward and reverse sequences in program BioEdit v7.2.5. We then queried these consensus sequences of predators and natural enemies to nucleotide collection databases employing the BLASTN algorithm in the BLAST program (Altschul et al. 1990). Sequences were deposited into GenBank (Table 2).

Results

We sampled predators using D-vac suction sampling and through timed visual observations, and entomopathogens by baiting soil samples with sentinel *G. mellonella* larvae.

Generalist Predators Collected Through Suction Sampling. Our suction samples revealed a predator community dominated by spiders (Araneae), representing ca. 70% of all predator individuals collected. Overall spider densities peaked in August and September in 2012, but remained at similar densities across the three 2013 sampling dates (Fig. 1). Most abundant were members of the Philodromidae (primarily in the genus *Philodromus*), which represented > 24% of all predatory arthropods that we collected (Fig. 1b, Table 3). Members of the Dictynidae, primarily the species *Dictyna bostoniensis* Emerton, were the second-most-abundant spider taxa making up > 14% of all predatory arthropods collected (Fig 1c, Table 3). Other hunting spiders we collected included members of the Salticidae, Oxyopidae and Lycosidae (Fig. 1d, Table 3). Long-jawed spiders (Tetragnathidae) were the most abundant web builders, with *Tetragnatha laboriosa* Hentz being the most-common species-representative of this Family (Fig. 1f, Table 3). Other common web-building spiders including members of the Theridiidae (Fig. 1e),

Linyphiidae, and Agelenidae. Predatory mites were also abundant in our samples, together comprising > 15% of all generalist predators that we collected. These fell into two genera, *Anystis* and *Bdella*. The first of these, and the most common, appeared to be a single species, identified as *Anystis agilis* using molecular methods (described above and below).

Predatory insects that we collected through suction sampling were dominated by predatory Hemipetera, in particular the species *Orius tristicolor* (White) (Hemiptera: Anthocoridae) which made up > 5% of all generalist predators that we collected (Fig. 2b; Table 3). Members of the Geocoridae and Nabidae were less common, but widely distributed, predatory hemipterans on bittersweet nightshade (Table 3). Predatory beetles (Coleoptera) were also found, with one species, the small coccinellid *Stethorus punctillum*, making up > 4% of the overall predator catch (Table 3); identification of this species was confirmed using molecular means (described above and below). Primarily-aphidophagous coccinellids in the genera *Hippodamia* and *Coccinella* were also found, although at lower densities than were recorded for *S. punctillum* (Table 3). Members of the Anthicidae and Carabidae were minor components of the predatory-beetle population (Table 3). Less-common predatory insects included four species of Chrysopidae and 3 of Hemrobiidae (Neouroptera), the earwig *Forficula auricularia* L., and the predator thrips *Aeolothrips fasciatus* (L.) (Table 3).

Our suction samples produced a large number of parasitoid Hymenoptera and Diptera with undetermined host associations; identifying these many species was beyond the logistical reach of our project. However, we did identify and count individuals of the parasitoid *T. triozae* (Hymenoptera: Eulophidae). This species is a known parasitoid of the potato psyllid, and was consistently recorded at the bittersweet nightshade patches that also housed robust potato-psyllid

populations (i.e., the Mesa, Moses Lake, Caliche Lake and Pasco-Kahlotus, WA, sites; however, none were found at our Colfax, WA, site) (Castillo Carrillo et al. manuscript). Wasp densities appeared to peak in October (Fig. 4), concurrent with peak densities of potato psyllids at these same sites (Castillo Carrillo et al. manuscript).

Visual Observations of Predator Activity. Because we did not collect predators during our visual observations, our visual surveys provided predator-community descriptions only at a relatively course level of taxonomic resolution. Nonetheless, the visual observations largely confirmed the predator community structure provided from our suction samples. In total, we recorded the presence of 140 predator individuals during our timed visual counts. The most-commonly-visually-observed predator taxa were spiders (Arachnida), followed by coccinellid beetles, including *S. punctillum*. Other less-commonly-observed predators included lacewings, earwigs, dragonflies, mantids, and hemipterans (mainly Anthocoridae and Geocoridae) (Table 3, Fig 5).

Fortuitously, while observing the activity of generalist predators during our visual surveys we also observed, on a few occasions, attacks on potato psyllid nymphs or eggs. Psyllid nymph mummies, parasitized by the wasp *T. triozae,* were observed in Moses Lake in September. We did not observe direct consumption of psyllids by spiders and other predators, but we did observed the tiny (1mm) coccinellid *S. punctillum* eating potato psyllid eggs at the Pasco-Kahlotus site on April, 27 of 2013.

Entomopathogens in Soil Samples. We collected soil beneath three bittersweet nightshade patches (Mesa-new, Pasco-Kahlotus and Moses Lake; Table 1) in August of 2012. Entomopathogenic nematodes were recovered from just one of these sites (Mesa-new; five

different nematode isolates were found within this single site), while entomopathogenic fungi were isolated from all three of the nightshade patches that were sampled for entomopathogens.

Molecular Identifications. We conducted PCR and sequencing techniques to provide more comprehensive molecular information of the parasitoid *T. triozae* and the predator coccinellid *S. punctillum* already identified taxonomically by experts. Two sequences of *T. triozae* (KT961708, KT961710 in Table 2) demonstrated 98% and 91% identity, respectively, to a known sequences of a *T. triozae* rRNA gene (Accession number: GQ912303.1). There were a handful of insertions/deletions and substitutions between these two sequences which are presented in Figure 6, which was coincident with the taxonomic divergence of this species into a 'complex B species', mentioned by Dr. Zuparko and now presented with the barcode KT961710. These analyzed wasps were collected from the Moses Lake, WA, *S. dulcamara* patches. While comparing sequence KT961708 (non-complex B *T. triozae* from our samples) to GQ912303.1 collected in Texas, we observed a few substitution and insertions along the sequence alignment (Fig. 6), which might reflect the geographic variation. Our sequence of the predatory beetle *S. punctillum* (accession KT961711, 661-bp) showed 99% identity to a COI gene sequence of *S. punctillum* in the GenBank (KM448697.1 Hendrich et al. 2015), agreeing with the identification based on the taxonomic keys made by Dr. Heydon. Lastly, we identified predatory mites based on a set of universal primers designed to amplify a wide range of taxa (Folmer et al. 1994), as attempts of PCR amplification using more specific primers did not succeed. Sequences amplified from general primers LCO1490 and HCO2198 ranged from 590 bp to 625 bp after cleaning the low quality sequences and ambiguous bases. These sequences (Accession No. KT998454 and KT998455) demonstrated 95% to 99% to a sequence belongs to Anystidae (accession No.

KT603428.1, Fig. 7). Our sequences were the first nucleotide sequence deposited in GenBank for *Anystis agilis*.

Molecular identification revealed that all five entomopathogenic nematode isolates were *H. bacteriophora*, while all three entompathogenic fungal isolates were *B. bassiana*. For *H. bacteriophora*; the 859-bp fragment we sequenced demonstrated 100% identity to several *H. bacteriophora* sequences in the GenBank. The sequences of entomopathogenic fungi ranged from 511 bp - 541 bp after removing the ambiguous bases and low quality sequences. They showed 99% - 100% identity to *B. bassiana* sequences in GenBank.

Discussion

Through most of two growing seasons, using a combination of suction sampling and direct visual observation, we tracked densities and biodiversity of the generalist predators found in patches of bittersweet nightshade. This revealed a diverse group of > 40 predator species, with robust predator densities recorded from early in the spring, just after green foliage was first apparent, until just before vine-kill by frost in the early winter (Fig. 1-4). Spiders, in particular hunting spiders, were the numerically-dominant members of the generalist predator community (Fig. 1a-d; Table 3). Both hunting spiders and web-building spiders were consistently found at high densities relative to the predatory insects we collected (Fig. 1e,f; Table 3). Abundant predatory insects included members of the Hemiptera and Coleoptera, e.g. *O. tristicolor*, *S. punctillum* and *H. tredecimpunctata* (L.). Less-common species included several Hemiptera, e.g. *Geocoris* spp., *Nabis* spp., along with *Coccinella* spp, *Chrysopa* spp. and *Hemerobius* spp. (Fig.

2; Table 3). Small predatory mites in the genus *Anystis* were observed consistently across sites and sample dates (Fig. 2; Table 3). Many of these taxa exhibited relatively constant densities from our early-season samples in March, until our late-season samples in December. In summary, a diverse community of generalist predators inhabited our bittersweet nightshade patches, throughout the growing season.

Previously, we have reported that these same bittersweet nightshade patches also housed robust populations of potato psyllids, along with more-modest numbers of aphid and beetle herbivores, that commonly attack cultivated potatoes (*S. tuberosum*). This suggests the possibility that the predators we found on bittersweet nightshade could be regularly encountering, and perhaps also consuming, potato psyllids and other herbivorous potato pests. In two cases, we directly observed predation of potato psyllids. During our sampling, at one site/date we observed the parasitoid *T. triozae* attacking potato psyllid nymphs (Moses Lake, September, 2012). This parasitoid is considered an efficient biological control of potato psyllids (Yang et al. 2013, Rojas et al. 2014) and a potential control agent of psyllids in noncrop habitats (Butler and Trumble 2012a). Indeed, this parasitoid has been reported to compose half of the total natural enemy community attacking *B. cockerelli* in tomato and bell pepper crops in California (Butler and Trumble 2012a). For this reason *T. triozae* is commercially available in some countries for biological control of *B. cockerelli* (Webber 2013). We also observed the coccinellid *S. punctillum* eating psyllid eggs at one site, on one date. The genus *Stethorus* has been reported as a predator almost exclusively of spider mites (Gordon 1985, Plaut 1965), thus it was perhaps surprising that we observed this lady beetle species eating psyllid eggs. It is notable that, despite their relatively high abundances, we did not observe any spiders feeding on potato psyllids. However, there is reason to think that spiders could be feeding on potato psyllids within

our nightshade patches. For example, in New Zealand, linyphiid spiders collected from potato fields consumed > 5 potato psyllid nymphs per day in simple laboratory arenas (MacDonald et al. 2010, Walker et al. 2011). The spiders that dominated the generalist-predator community at our site have not yet been examined for their proclivity for attacking potato psyllids, although spiders are known to be effective predators of other pests on potato (Hilbeck and Kennedy 1996). The predatory Hemiptera that we found have been widely reported to feed on aphid and beetle herbivores on potato (Webber 2013). *O. tristicolor* and *G. pallens* are purported to be key natural enemies of *B. cockerelli* in potato, tomato and bell pepper in California (Butler and Trumble 2012a). While these many studies suggest the possibility of frequent predation of potential potato pests within bittersweet nightshade patches, further work is needed to quantify the frequency and impact of any such predation by our resident species under our conditions.

The arthropod community found on bittersweet nightshade is primarily of interest because this weed is thought to serve as a key source of potato psyllids that later colonize potato fields, perhaps also vectoring the zebra chip pathogen (Jensen et al. 2012, Swisher et al. 2013, Murphy et al. 2014). Potato psyllids appear to require a perennial, non-crop host to support the pest's populations outside of the potato-growing season, and to survive the relatively harsh winters seen in eastern Washington (Jensen et al. 2012, Murphy et al. 2013). Likewise, however, our study suggests that bittersweet nightshade could also be serving as a refuge for predatory arthropods important for suppressing pests in potato fields. The predators that we found include the two generalist predators widely reported to be most inpactful in potato fields, the predatory hemipterans *Geocoris* spp. and *Nabis* spp. (Koss et al. 2005). When insecticide use is relatively infrequent, or insecticides are used that selectively target pests and not predators, densities of

these two taxa can exceed five individuals per potato plant (Koss et al. 2005). Perhaps surprisingly, however, these two predator taxa, so common in potato fields, were a relatively minor component of the predator community on bittersweet nightshade. The community of spiders found in potato crops is both less diverse and less abundant than the one we found on bittersweet nightshade. Linyphiidae, the most common spiders reported in potato fields in our region (Koss et al. 2005), were only the seventh-most-common spider family in our nightshade patches (Table 3). Intriguingly, the spider community that we recorded on bittersweet nightshade closely resembles that reported in apple orchards in our region (Horton et al. 2001, Horton et al. 2002), suggesting the possibility that our perennial weeds support a spider community more typical of perennial than annual crops.

Likely reflecting the only-recent emergence of zebra chip disease as a major threat to potato production, relatively little is known about the community of predators that attacks potato psyllids in or outside of potato fields (Butler and Trumble 2012b). In perhaps the most comprehensive single study examining potato psyllid predators, Butler and Trumble (2012a) censused predators in potato-psyllid infested potato, tomato and pepper fields in southern California over 2 years. This revealed a community dominated by a bio-diverse community of spiders, coccinellid beetles, and predatory hemipterans, as well as the parasitoids *Metaphycus psyllidis* Compere and *T. triozae* (Butler and Trumble 2012a). In laboratory feeding arenas several species of coccinellid beetles, anthocorid and mirid bugs, and *Chrysopa* spp. larvae readily ate psyllid adults, nymphs and eggs. This list closely matches reports by Knowlton (1933a, b, 1934) for predators associated with potato-psyllid-infested fields in Utah, where lacewings, predatory *Geocoris* bugs, and coccinellids all were found in laboratory arenas to feed

upon potato psyllids. Unfortunately, while Butler and Trumble reported a diverse community of hunting and web-building spiders associated with potato psyllids in their cropping fields, like us they did not have an opportunity to test the feeding proclivities of their spider species. Clearly, spiders warrant greater future study as predators of potato psyllids on both weedy and crop host plants. In New Zealand, potato psyllids appear to face particularly intense predation from a diverse community of locally-native coccinellid beetles (O' Connell et al. 2012, Pugh et al. 2015), similar to what is seen in California (Butler and Trumble 2012a), but also predatory mites (Xu and Zhang 2015) as we found as possible psyllid predators on bittersweet nightshade (Fig. 3). In the only study to-date of which we are aware in which an attempt was made to measure the impact of generalist predators on potato psyllids under field conditions, Butler and Trumble (2012a) found that potato psyllids were up to 50% more abundant on potato or American nightshade (*Solanum americanum* Mill.) plants in cages that blocked access by predators, compared to uncaged controls, which they interpreted as evidence that predators were substantially reducing psyllid survivorship in the open field.

Our entomopathogen survey was far more limited in scope than our predator survey. Nonetheless, we did record entomopathogens in soils beneath each of the bittersweet nightshade patches that we sampled. Our efforts revealed two species, the nematode *H. bacteriophora* and fungus *B. bassiana*, both known to control economically-important potato pests. These two species form an important component of the community of natural enemies attacking Colorado potato beetles in potato fields in our region, infecting beetle larvae as they burrow into the soil to pupate (Lacey et al. 1999, Ramirez and Snyder 2009, Crowder et al. 2010, Jabbour et al. 2011). Indeed, the entomopathogens strongly complement potato beetle control by predatory insects,

with predators and entomopathogens joining forces to kill more potato beetles than can either guild of natural enemies on its own (Ramirez and Snyder 2009). Field work has yet to consider the impacts of entomopathogens on the potato psyllid, but laboratory bioassays suggest that *B. bassiana* has the potential to kill >50% of psyllids that contact its spores (Lacey et al. 2009). Additional work is needed to determine how often, if ever, potato pests on bittersweet nightshade contact entomopathogens in soil beneath the plants.

Understandably, given the devastating effects zebra chip disease on northwestern-U.S. potato crops, most research has focused on bittersweet nightshade's role in harboring the potato psyllid (and possibly also the bacterium that causes zebra chip) (e.g., Jensen et al. 2012, Swisher et al. 2013, Murphy et al. 2014). We are not suggesting that the diverse community of predators that we found within bittersweet nightshade patches negates the risks that this weed poses to regional potato production. Nonetheless, there is a potential for the robust and bio-diverse community of predators, parasitoids and entomopathogens that we recorded to somewhat ameliorate this risk, by suppressing potato psyllids (and other pests) and thus limiting the number of available would-be potato migrants. A number of unresolved issues remain. First, further work is needed to determine which predator species, and how frequently, prey on potato psyllids and other pests within nightshade patches. Second, it remains unclear whether any of these predators will, like potato psyllids, leave the nightshade patches and migrate to potatoes. A third factor yet to be considered is the possibility that high predator densities trigger predator-avoidance behaviors in psyllids or other pests, inciting the herbivores to leave bittersweet nightshade plants and increasing net movement to potato crops; such predator-avoidance behaviors have been shown to influence disease dynamics in other plant-disease systems (e.g., Ramirez and Snyder 2009,). In

summary, we suggest that a community-ecology perspective may be useful for fully assessing the role of bittersweet nightshade in zebra chip disease ecology and epidemiology.

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Table 3-1. *Solanum dulcamara* site coordinates.

Site name	Coordinates
Mesa-old (MO)	46°35'17.72"N, 119° 0'1.12"W
Mesa-new (MN)	46°34'35.35"N, 119° 0'33.41"W
Colfax (CX)	46°50'51.1008"N, 117°28'43.9320"W
Moses Lake (ML)	46°58'53.06"N, 119°38'49.20"W; 46°59'34.19"N, 119°41'6.66"W; 46°59'55.10"N, 119°41'5.25"W; 47° 0'7.35"N, 119°41'5.03"W
Caliche Lake (CL)	47° 1'53.65"N, 119°55'39.78"W
Pasco-Vineyard (PV)	46°19'38.06"N, 119° 7'13.98"W
Mattawa (MT)	46°42'32.33"N, 119°56'42.54"W
Pasco-Kahlotus (PK)	46°16'38.59"N, 118°50'29.07"W
Sacajawea Park (SJ)	46°12'12.7692"N, 119°02'49.6644"W
Warden (WD)	46°54'44.90"N, 119° 7'26.09"W

Table 3-2. DNA primers used to identify natural enemy species.

Taxa	Primer name	Primer sequence (5' to 3')	Reference	GenBank accession #
Anystis agilis	LCO1490	GGT CAA CAA ATC ATA AAG ATA TTG G	Folmer et al. 1994	KT998453
(Trombidiformes: Anystidae)	HC02198	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer et al. 1994	
Stethorus punctillum	ClepFoIF	ATT CAA CCA ATC ATA AAG ATA TTG G	Hendrich et al. 2014	KT961711
(Coleopetera: Coccinellidae)	ClepFoIR	TAA ACT TCT GGA TGT CCA AAA AAT CA	Hendrich et al. 2014	
Tamarixia triozae	ITS5-Tama	GGA AGT AAA AGT CGT AAC AAG G	De leon and Setamou 2010	KT961708
(Hymenoptera: Triozidae)	RNA2-Tama	CAC GAG CCG AGT GAT CCA CCG CTA AGA GT	Chang et al. 2001; De leon and Setamou 2010	KT961710
Beauveria bassiana	Fungi-ITS1	TCC GTA GGT GAA CCT GCG G	White et al. 1990	KT443981
(Hypocreales: Clavicipitaceae)	Fungi-ITS4	TCC TCC GCT TAT TGA TAT GC	White et al. 1990	KT443982
				KT443983
Heterorhabditis bacteriophora	ITS-F-nem	TTG AAC CGG GTA AAA GTC G	Stock et al. 2001; Maneesakorn et al. 2011	KT 443980
(Rhabditida: Heterorhabditidae)	ITS-R-nem	TTA GTT TCT TTT CCT CCG CT	Stock et al. 2001; Maneesakorn et al. 2011	

Table 3-3. Predator taxa found in bittersweet nightshade patches and relative abundances.

Class	Order	Family	Species	2012	2013	Total collected	Species %	Family %	Order %
Insecta	Coleoptera	Anthicidae	Spp.*	68	2	70	1.09	1.09	5.92
		Coccinellidae	*Stethorus punctillum*	129	55	184	2.87	4.60	
			Hippodamia tredecimpunctata	62	3	65	1.01		
			Hippodamia convergens	27	0	27	0.42		
			Hyperaspis oregona	6	3	9	0.14		
			Coccinella septempunctata	4	0	4	0.06		
			Scymnus marginicollis	3	1	4	0.06		
			Coccinella novemnotata	1	0	1	0.02		
			Coccinella transversoguttata	1	0	1	0.02		
		Carabidae	*Bembidion* sp. + other Carabid species	9	6	15	0.23	0.23	
	Dermaptera	Forficulidae	*Forficula auricularia*	4	0	4	0.06	0.06	0.06
	Hemiptera	Anthocoridae	*Orius tristicolor*	307	22	329	5.13	5.13	5.96
		Geocoridae	*Geocoris bullatus /G. pallens*	15	3	18	0.28	0.28	
		Nabidae	*Nabis alternatus*	13	4	17	0.27	0.55	
			Nabis sp. (nymphs)	13	2	15	0.23		
			Nabis poss. *americoferus*	3	0	3	0.05		
	Neuroptera		Spp. larvae	17	1	18	0.28	0.28	1.43
		Chrysopidae	*Chrysoperla plorabunda/johnsoni*	16	0	16	0.25	0.37	
			Chrysopa oculada	6	0	6	0.09		
			Chrysopa coloradensis	1	0	1	0.02		
			Chrysopa nigricornis	1	0	1	0.02		
		Hemerobiidae	*Hemerobius ovalis*	38	1	39	0.61	0.78	
			Hemerobius pacificus	10	0	10	0.16		
			Hemerobius variolosus	1	0	1	0.02		
	Thysanoptera	Aeolothripidae	*Aeolothrips fasciatus*	3	0	3	0.05	0.05	0.05
Arachnida	Trombidiformes	Anystidae	*Anystis agilis*	777	193	970	15.13	15.13	15.13
		Bdellidae	*Bdella* sp.						
	Aranae	Agelenidae	*Hololena* sp.	49	0	49	0.76	0.76	71.45
		Dictynidae	*Dictyna bostoniensis*	714	205	919	14.33	16.09	
			Pityohyphantes minidoka	74	39	113	1.76		
		Linyphiidae	Spp.	234	60	294	4.59	4.59	
		Lycosidae	Spp.	35	9	44	0.69	0.69	
		Oxyopidae	*Oxyopes scalaris*	255	37	292	4.55	4.55	
		Philodromidae	*Philodromus* sp.	1071	274	1345	20.98	24.08	
			Tibellus sp.	150	9	159	2.48		
			Tibellus oblongus	40	0	40	0.62		
		Salticidae	*Pelegrina* sp.	220	33	253	3.95	8.20	
			Sassacus vitis	103	16	119	1.86		
			Phidippus audax	101	3	104	1.62		
			Salticus scenicus	36	5	41	0.64		
			Habronatus sp.	8	0	8	0.12		
			Tutelina sp.	1	0	1	0.02		
		Tetragnathidae	*Tetragnatha laboriosa*	242	71	313	4.88	6.07	
			Tetragnatha sp.	61	15	76	1.19		
		Theridiidae	Spp.	239	172	411	6.41	6.41	
Total				5168	1244	6412	100	100	100

* Species of Anthicidae: *Anthicus cervicus* LaFerte, *A. floralis* (L), *A. lecontei* Champion, *Notoxus serratus* (LeConte)

Table 3-4. Predators observed foraging on bittersweet nightshade vines.

Predator order		Total observed	Percentage
Araneae	Spiders	101	69
	Mites	6	4
Coleoptera	Lady birds	16	11
	S. punctillum	9	6
Hemiptera	*Orius*	1	1
	Geocoris	1	1
Other	Lacewings	3	2
	Mantids	0	0
	Wasps*	7	4
	Dragonfly	2	1
	Earwig	1	1
Total		147	100

*not predator

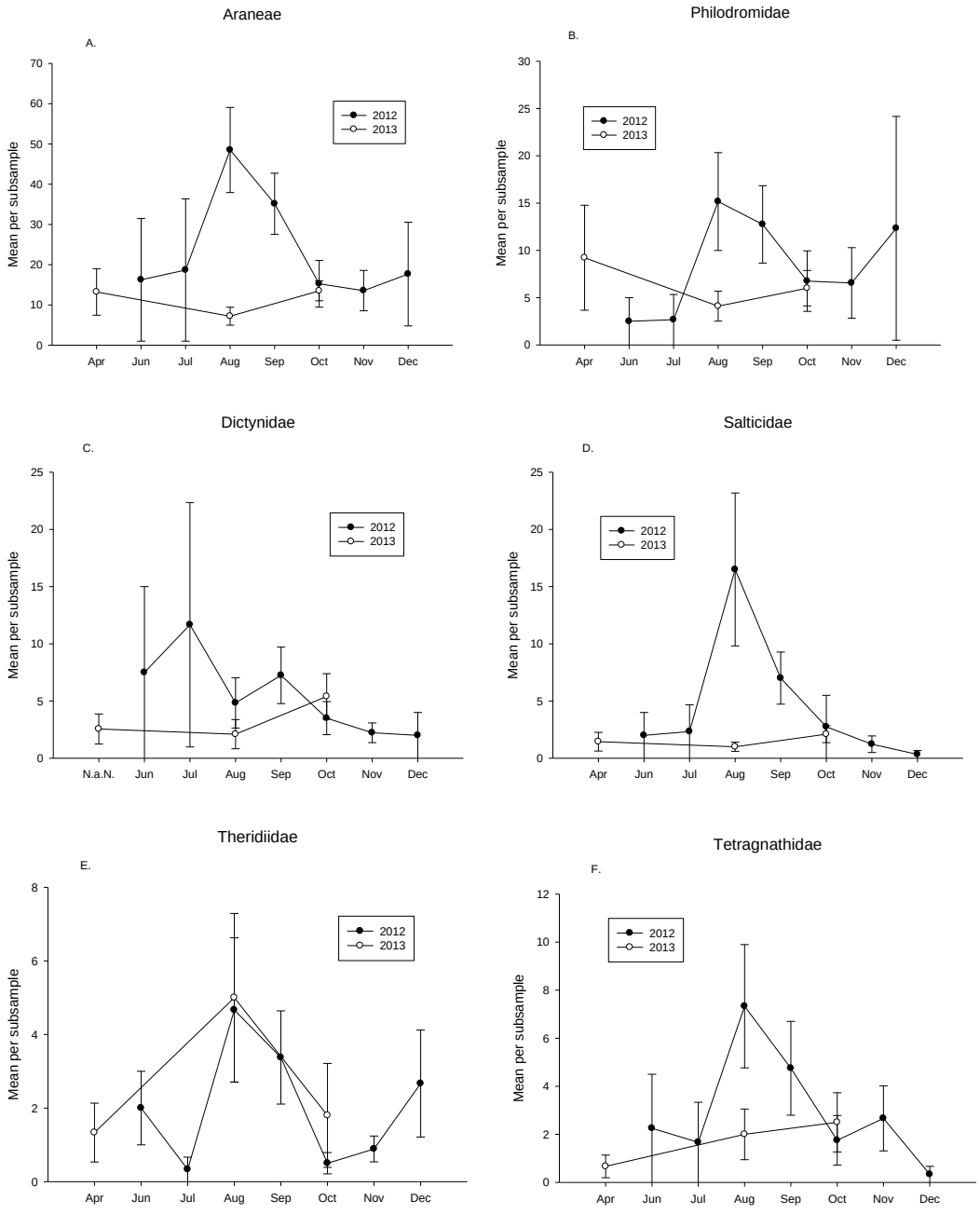

Figure 3-1. From bittersweet nightshade patches across eastern Washington, multi-year population dynamics of (a) all spiders summed across families, and for spiders in the families (b) Philodromidae, (c) Dictynidae, (d) Salticidae, (e) Theridiidae, and (f) Tetragnathidae. Data are

means ± 1 S.E. Densities of narrower taxa within these families, and of spiders less abundant than those in these families, are in Table 3.

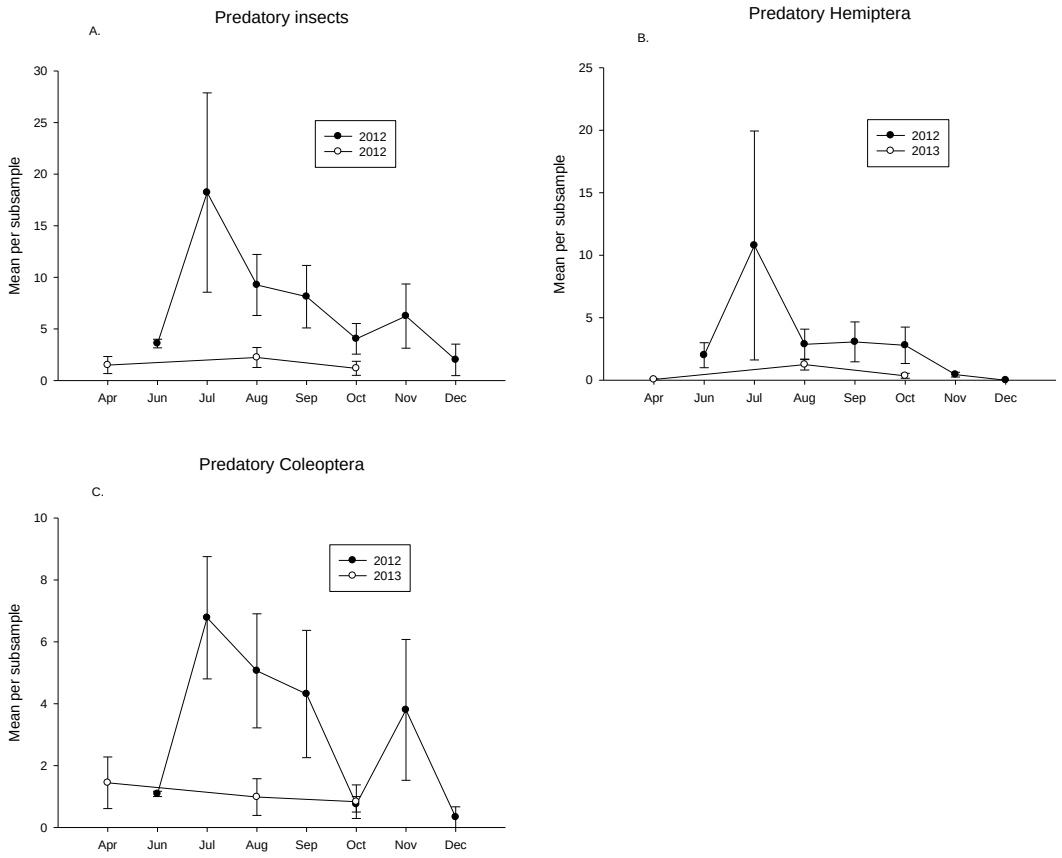

Figure 3-2. From bittersweet nightshade patches across eastern Washington, multi-year population dynamics of (a) all predatory insects summed across Orders, and (b) summed predatory Hemiptera and (c) summed predatory Coleoptera. Data are means ± 1 S.E. Densities of narrower taxa within the Orders in (b) and (c), and of predatory insects less abundant than those in these groups, are in Table 3.

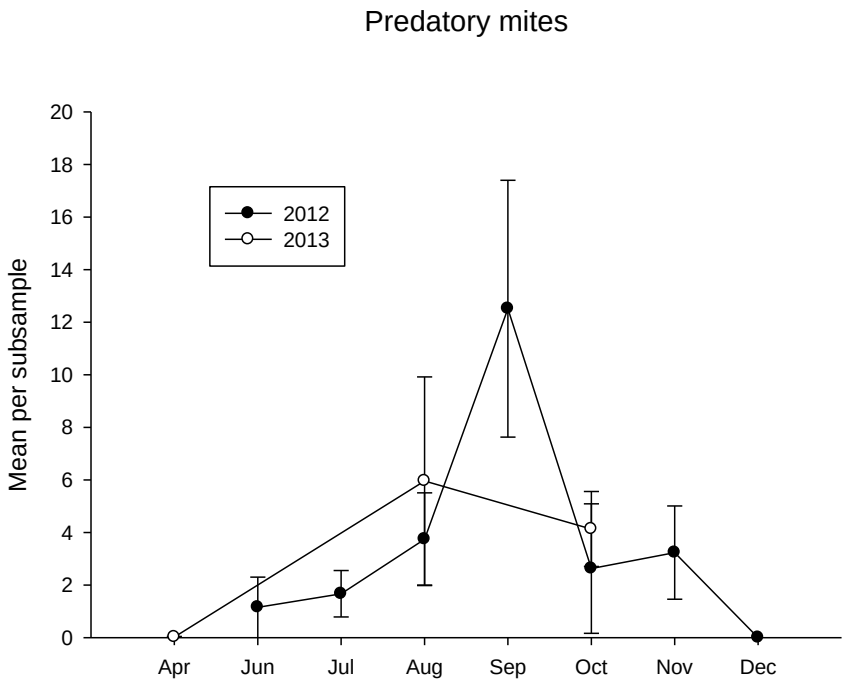

Figure 3-3. From bittersweet nightshade patches across eastern Washington, multi-year population dynamics of predatory mites. Data are means ± 1 S.E., with additional taxonomic detail provided in Table 3.

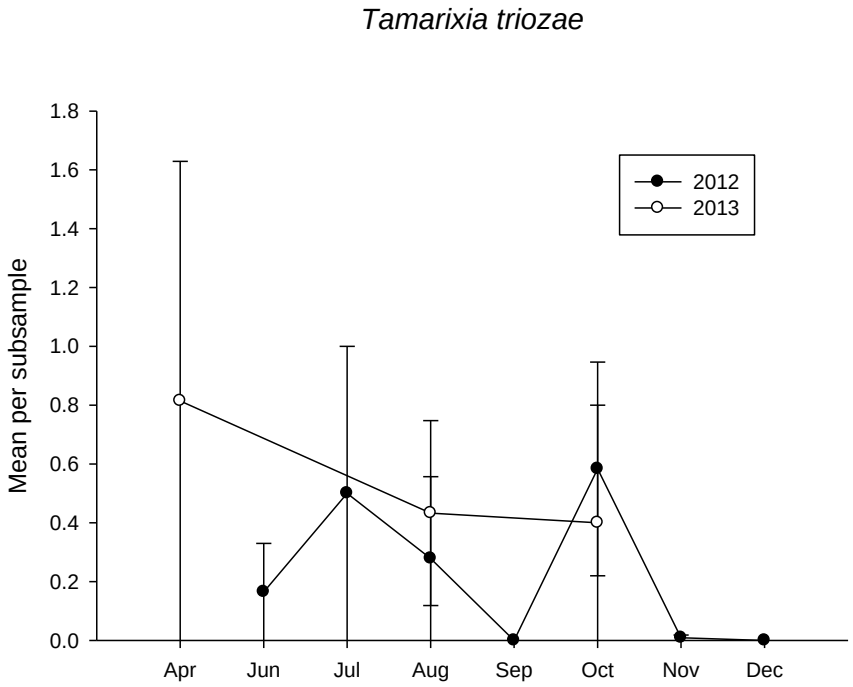

Figure 3-4. From bittersweet nightshade patches across eastern Washington, multi-year population dynamics of the potato psyllid parasitoid *Tamarixia triozae*. Data are means ± 1 S.E.

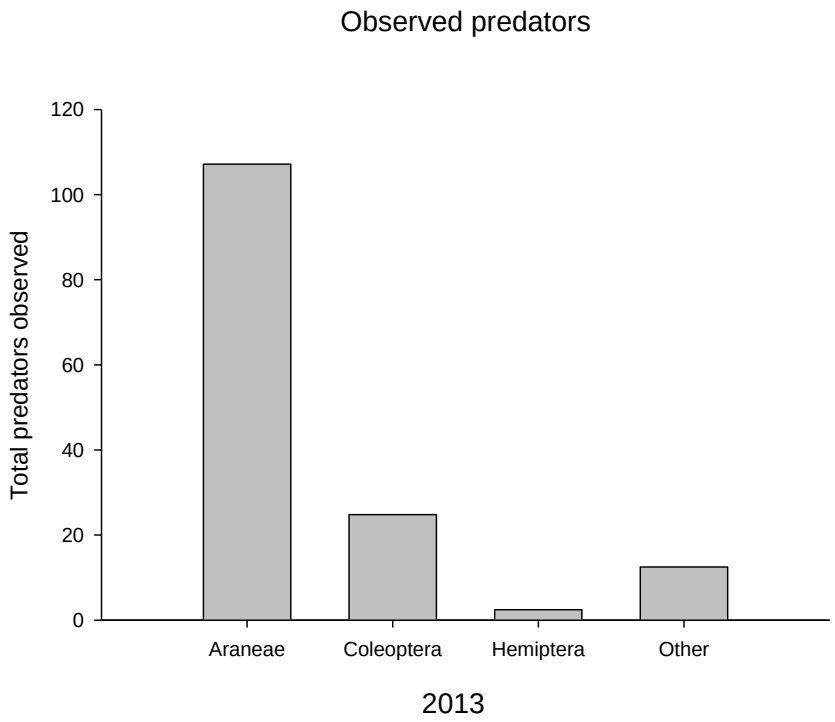

Figure 3-5. From timed visual observations of predator activity on bittersweet nightshade plants, summed number of observations of spiders (Araneae), predatory beetles (Coleoptera), predatory bugs (Hemiptera), and other predatory taxa outside of these groups (Other). Data are means ± 1 S.E. Additional taxonomic detail is provided in Table 4.

Figure 3-6. Two *Tamarixia triozae* 18S rRNA genes (KT961708 and KT961710 as GenBank accession numbers) were aligned to a 18S rRNA gene from the GenBank (Accession No.

GQ912303, De León and Sétamou 2010). Sequences were truncated to reflect the divergent region. Note that KT961710 was identified as complex B of *T. triozae* based on the taxonomic and morphological keys (described in the Results), and it demonstrated divergence, including substitution, insertion and deletion with the other two sequences from the same species.

Figure 3-7. Two *Anystis agilis* COI genes (KT998454, collected from Moses Lake, WA and KT998454, collected from Pasco, WA) were aligned to a COI gene from Anystidae in the

GenBank (Accession No. KT603428). Sequences were truncated to reflect the divergent region. Note our sequences were the first nucleotide sequence deposited in GenBank for *Anystis agilis*. Though both our mites were identified as *A. agilis* based on the taxonomic and morphological keys (described in the Results), they demonstrated ~5 % mismatches. Surprisingly, there were only substitutions rather than insertion and deletions.

CHAPTER FOUR: REVISED CHECKLIST OF PACIFIC NORTHWEST, U.S.A., PSYLLOIDEA (HEMPITERA)

Abstract

Several members of the Psylloidea vector pathogens of agricultural crops in the Pacific Northwest ("PNW") U.S. states of Idaho, Montana, Oregon and Washington. These crop-production concerns have renewed interest in the biodiversity of this superfamily in the region. We constructed a revised checklist of this group by examining published records and additional collections. This revealed 124 species of psyllids from 25 genera; 35 of these species had not been previously reported in the PNW. Our species list provides a useful starting point for entomologists investigating the ecology of emerging insect-transmitted plant pathogens in the region.

KEY WORDS: psyllid, species, biodiversity, Idaho, Montana, Oregon, Washington

The Pacific Northwest ("PNW") U.S. states of Idaho, Montana, Oregon and Washington span diverse eco-geographic zones, ranging from relatively cool and wet conditions east of the Cascade and within the Rocky Mountains, to relatively hot and dry conditions at lower elevations in the rain shadows of these mountain ranges (Raymond et al. 2014). In the last century the region has been heavily altered by agriculture (Robbins and Wolf 1994, Butler et al. 2004). The PNW leads the United States in production of apples, potatoes and wheat, while

contributing significantly to national production of other important commodities (USDA 2014, 2015, WSDA 2015, OSDA 2015). Much of this agricultural production is centered in the irrigated, arid Columbia Basin of eastern Washington and Oregon, and in valleys along the Snake River in southern Idaho (USDA 2014, 2015, WSDA 2015, OSDA 2015).

Pear (*Pyrus communis* L.) and potato (*Solanum tuberosum* L.) crops in the PNW have been harmed by plant pathogens vectored by the pear psylla, *Cacopsylla (Hepatopsylla) pyricola* (Foerster, 1848), and the potato psyllid, *Bactericera cockerelli* (Šulc, 1909), respectively. The pear psylla harms pears by vectoring the phytoplasma *Candidatus* Phytoplasma pyri (Jensen et al. 1964, Seemüller and Schneider 2004), which causes 'pear decline' (Batiste and Bulla 1980). Likewise, the potato psyllid vectors the bacterium *Candidatus* Liberibacter solanacearum, responsible for "zebra chip" disease of potatoes that degrades tuber quality in storage (Munyaneza et al. 2007; Buchman et al. 2011, Munyaneza 2012). While *B. cockerelli* is believed to be the only insect vector of the zebra chip bacterium, limited familiarity with psyllid biodiversity has restricted rigorous verification of this assumption (Crosslin et al. 2010, Haapalainen 2014). The zebra chip bacterium has been found in other *Bactericera* species, including *B. tremblayi* (Wagner, 1961) and *B. nigricornis* (Foerster, 1848) in Spain (Teresani et al. 2015), and in *Dyspersa apicalis* (Foerster, 1848) in Finland (Munyaneza et al. 2010). Uncertainty about the true community of psyllid species responsible for zebra chip transmission has heightened interest in PNW psyllid biodiversity, both within and outside of agricultural fields (e.g., Munyaneza 2010, Munyaneza and Henne 2012, Murphy et al. 2014).

Here, we construct a revised species list for Psylloidea in the U.S. Pacific Northwest. We focus on this region because of its shared ecography and importance in U.S. production of a

similar suite of agricultural commodities (USDA 2014, 2015, WSDA 2015, OSDA 2015). We build upon the valuable earlier contributions by Hodkinson (1988), who provided a checklist of Nearctic Psylloidea, by Percy et al. (2012) who provided a checklist of psyllids in California (just south of our focal area), and by Ouvrard (2015) who built an extensive online database of Psylloidea ("Psyl'list"; http://www.hemiptera-databases.org/psyllist/). Here we construct a checklist of psyllid species present in the PNW. Our list was developed using published reports and examinations of museum specimens.

Materials and methods

We constructed our species list through two main steps. First, we examined existing published species lists for the region (i.e., Hodkinson 1988, Percy et al. 2012, Ouvrard 2015), to construct an initial list of expected or known species in the region. Next, we examined Psylloidea in collections of the following key regional institutions: the W.F. Barr Museum of University of Idaho (UIDA), the Oregon State Arthropod Collection of Oregon State University (OSAC), the Oregon State Department of Agriculture (OSDA), the M.T. James Museum of Washington State University (WSU), and the USDA Yakima Agricultural Research Laboratory in Washington (YARL). We did not evaluate for accuracy the identifications in the museum collections, an undertaking that was outside the scope of our project. Additional species names were extracted from the collection list of, and two reports from, the Washington State Department of Agriculture ("WSDA"; Johansen and Brannon 1955, Beers et al. 1993) and from the online psyllid list posted by the Smithsonian National Museum of Natural History (USNM)

(http://collections.nmnh.si.edu/search/ento/). Updates of the scientific names were obtained from Ouvrard (2015), a regularly-updated world Psylloidea database. Most psyllid species have relatively narrow host-plant ranges (Hodkinson and White 1979), and while this information is not presented here it is available from Ouvrard (2015).

Results

Our compiled list contains 124 species of psyllids, belonging to 5 families and 25 genera (Table 1). In total, our list presents 35 new species-records across our four focal U.S. states (16/52 species are new records for Idaho; 2/26 species are new records for Montana; 30/76 species are new records for Oregon; and 14/60 species are new records for Washington).

Checklist.—Species of this list are classified according to Burckhardt and Ouvrard (2012). In the list we present the States in which the psyllids were collected (with underline type indicating a new record of that species in that state) and the following symbols denote the record source: ‡= OSAC, Ł= OSDA, *= Psyl'list, †= UIDA, □= USNM, Œ= WSDA, ◊= WSU, ʸ= YARL.

Family Aphalaridae

Subfamily Aphalarinae

Genus *Aphalara* Foerster, 1848.

calthae (Linné, 1761). Idaho†, Oregon Ł.

At OSDA, this species is presented as a *calthae/polygoni* complex.

curta Caldwell, 1937. Idaho†, Washington□.

loca Caldwell, 1937. Idaho†, Oregon*, Washington*□.

nubifera Patch, 1912. Washington*.

(Anomocera) parvicornis Hottes, 1958. Montana □.

(Reference for this description was not found).

rumicis Mally, 1894. Idaho*†, Oregon Ł*, Washington*.

At OSDA, this species has a question mark about identification.

simila Caldwell, 1937. Idaho*, Oregon*□, Washington*.

Genus *Craspedolepta* Enderlein, 1921.

artemisiae (Foerster, 1848). Idaho†.

americana Klimaszewski, 1979. Washington*.

angustipennis (Crawford, 1911). Idaho‡*, Montana‡*, Oregon‡*, Washington‡*.

anomola (Crawford, 1914). Oregon‡*, Washington‡*.

canadensis Journet and Vickery, 1979. Oregon‡.

constricta (Caldwell, 1936). Idaho*, Oregon‡.

flavida (Caldwell, 1938). Oregon‡*.

fumida (Caldwell, 1938). Montana*.

furcata (Caldwell, 1936). Montana‡*, Oregon‡*.

maculimagna Journet and Vickery, 1979. Oregon‡*.

magna Journet and Vickery, 1979. Idaho*, Montana *.

minutissima (Crawford, 1911). Idaho‡*†, Oregon‡ Ł*.

nebulosa (Zetterstedt, 1828). Washington*.

oregonensis Journet and Vickery, 1979. Oregon‡*.

parvula Journet and Vickery, 1979. Oregon‡*.

pinicola (Crawford, 1914). Idaho‡*†, Oregon‡*.

pulchella (Crawford, 1911). Oregon Ł.

russellae Klimaszewski, 1977. Idaho*, Oregon Ł*, Washington*.

schwarzi (Ashmead, 1904). Washington*.

smithsonia (Klimaszewski, 1979). Montana*.

vancouverensis (Klyver, 1931). Idaho*, Montana*, Oregon‡*, Washington*□.

veaziei (Patch, 1911). Oregon‡*, Washington*.

vulgaris (Journet and Vickery, 1979). Montana Ł*.

Subfamily Pachypsyllinae

Genus *Pachypsylla* Riley, 1885.

celtidisgemma Riley, 1885. Idaho†.

celtidismamma (Fletcher, 1883). Idaho*†, Oregon Ł, Washington◊.

venusta (Oste–Sacken, 1861). Idaho*†, Washington*†◊.

Subfamily Spondyliaspidinae

Genus *Cryptoneossa* Taylor, 1990

triangula Taylor, 1990. Oregon Ł.

Genus *Ctenarytaina* Ferris and Klyver, 1932.

eucalypti (Maskell, 1890). Oregon Ł.

Genus *Glycaspis* Taylor, 1960.

brimblecombei Moore, 1964. Oregon Ł.

Family Calophyidae

Subfamily Calophyinae

Genus *Calophya* Löw, 1879.

aurea Tuthill, 1942. Montana*.

dubia Crawford, 1914. Montana*.

flavida Schwarz, 1904. Oregon‡.

nigripennis Riley, 1885. Washington*.

triozomima Schwarz, 1904. Idaho*.

washingtonia (Klyver, 1931). Washington*.

Family Liviidae

Subfamily Euphyllurinae

Genus *Psyllopsis* Löw, 1879.

fraxinicola (Foerster, 1848). Idaho*, Oregon Ł.

Genus *Neophyllura* Loginova, 1973.

arbuti (Schwarz, 1904). Oregon*.

arctostaphyli (Schwarz, 1904). Montana*, Oregon‡Ł*, Washington*.

separata (Tuthill, 1943). Oregon*□.

Subfamily Liviinae

Genus *Livia* Latreille, 1802.

caricis Crawford, 1914. Idaho*, Oregon*.

vernaliforma Caldwell, 1940. Montana*.

Family Psyllidae

Subfamily Macrocorsinae

Genus *Euphalerus* Schwarz, 1904.

idahoensis Jensen, 1946. Idaho*†□.

Subfamily Psyllinae

Genus *Arytaina* Foerster, 1848.

genistae (Latreille, 1804). Oregon Ł*, Washington *y.

robusta sinuata Tuthill, 1943. Idaho□.

Genus *Arytainilla* Loginova, 1972.

spartiophila (Foerster, 1848). OregonŁ*, Washington*y.

Genus *Cacopsylla* Ossiannilsson, 1970.

acuminata (Jensen, 1956). Oregony

alba (Crawford, 1914). Idaho*, Washington*.

americana (Crawford, 1914). Idaho*, OregonŁ*, Washington*Œy.

breviata (Patch, 1912). Washington†.

brevistigmata (Patch, 1912). Idaho†, Oregony

YARL's note "Questions remain about this identification."

confusa (Tuthill, 1943). Oregon‡†.

coryli (Patch, 1912). Idaho*†, Oregon*†, Washington*y.

curta (Tuthill, 1943). Oregon*.

difficilis (Tuthill, 1943). Idaho*†, Montana*[□].

fatsiae (Jensen, 1957). Washington[Œ].

fibulata (Crawford, 1914). Idaho*, Oregon*†.

hirsuta (Tuthill, 1938). Idaho*, Montana*, Oregon[Ł]*†[□y], Washington*[y].

insignita (Tuthill, 1943). Oregon[‡]†.

latiforceps (Tuthill, 1943). Oregon[□], Washington*.

magna (Crawford, 1914). Montana[‡], Oregon[‡]†[y].

YARL's note "Questions remain about this identification."

magnicauda (Crawford, 1914). Montana*.

manisi (Tuthill, 1943). Idaho*†[□].

media (Tuthill, 1943). Idaho†, Oregon[‡y].

YARL's note "Questions remain about this identification."

minor (Crawford, 1914). Oregon[‡]*[□], Washington*[◊].

minuta (Crawford, 1914). Idaho*, Oregon†, Washington†.

nordica (Jensen, 1951). Oregon*, Washington*†[□].

omani (Tuthill, 1943). Oregon†[y].

parallela (Crawford, 1914). Washington*.

pararibesiae (Jensen, 1956). Washington*[y].

peregrina (Foerster, 1848). Oregon[Ł]*, Washington*[y].

(Hepatopsylla) pyricola (Foerster, 1848). Idaho†, Washington[Ł]*†[Œ◊y].

ribesiae (Crawford, 1911). Idaho*†, Oregon*[y], Washington[y].

sinuata Crawford, 1914. Idaho*.

striata (Patch, 1911). Washington*.

usitata (Tuthill, 1943). Montana*[□].

Genus *Ceanothia* Heslop–Harrison, 1961.

ceanothi (Crawford, 1914). Montana*, Oregon[Ł], Washington*.

Genus *Euglyptoneura* Heslop–Harrison, 1961.

fuscipennis (Crawford, 1914). Idaho†[◊], Oregon[Ł]*.

robusta (Crawford, 1914). Idaho*, Montana*[□], Oregon*†, Washington*.

Genus *Nyctiphalerus* Bliven, 1955.

adustus (Tuthill, 1937). Oregon[y].

cercocarpi (Jensen, 1957). Oregon[y].

rugipennis (Crawford, 1914). Oregon[Ł]*.

tantillus (Tuthill, 1937). Idaho†.

vermiculosus (Crawford, 1914). Idaho*†, Montana*, Oregon*.

Genus *Psylla* Geoffroy, 1762.

alni (Linné, 1758). Idaho*, Oregon[Ł]*, Washington*.

astigmata (Crawford). Washington[Œ].

(Year of description not found).

buxi (Linné, 1758). Oregon[‡Ł], Washington[Œ].

caudata Crawford, 1914. Idaho*†.

floccosa Patch, 1909. Idaho†, Montana*, Oregon[Ł]*†, Washington*[◊].

galeaformis Patch, 1911. Idaho[Δ]†[◊], Oregon[Ł]*, Washington*[◊].

trimaculata Crawford, 1911. Idaho†, Washington*.

viridescens (Provancher, 1872). Idaho†.

(Reference for this description was not found).

Genus *Purshivora* Heslop–Harrison, 1961.

pubescens (Crawford, 1914). Idaho*, Oregon‡*†□y, Washingtony.

Family Triozidae

Subfamily Metatriozidinae

Genus *Bactericera* Puton, 1876.

arbolensis (Crawford, 1910). Montana*.

cockerelli (Šulc, 1909). Idaho*†, Montana*, Oregon‡*y, Washington*◊.

incerta (Tuthill, 1943). Oregon*□, Washington*□.

lobata (Crawford, 1914). OregonŁ.

maculipennis (Crawford, 1910). OregonŁ, Washington◊y.

maura (Foerster, 1848). Idaho†, WashingtonŒ◊.

minuta (Crawford, 1910). Idaho*†, Montana*, OregonŁ*y, Washington*◊.

pletschi (Tuthill, 1944). Montana*□.

pulla (Tuthill, 1939). Oregon*□, Washington□.

rubra (Tuthill, 1939). Oregon*.

salicivora (Reuter, 1876). Oregon*, Washington*.

Genus *Baeoalitriozus* Li, 2011.

diospyri (Ashmead, 1881). Montana*.

Genus *Heterotrioza* Dobreanu and Manolache, 1960.

chenopodii (Reuter, 1876). Oregon[y], Washington[y].

Genus *Lauritrioza* Conci and Tamanini, 1968.

alacris (Flor, 1861). Oregon[Ł], Washington[Œ].

Genus *Phylloplecta* Riley, 1884.

occidentalis (Tuthill, 1939). Washington*[□].

rubicola (Tuthill, 1943). Oregon[Ł], Washington*[□].

tripunctata (Fitch, 1851). Washington*.

Genus *Trioza* Foerster, 1848.

albifrons Crawford, 1910. Idaho*, Montana*, Oregon*, Washington*[◊y].

eugeniae Froggatt, 1901. Oregon[Ł].

inversa Tuthill, 1939. Oregon[Ł].

mira Tuthill, 1943. Washington*[◊].

obtusa Patch, 1911. Washington†.

quadripunctata Crawford, 1910. Montana*[□].

robusta Tuthill, 1944. Montana*.

sulcata Crawford, 1910. Oregon*.

Discussion

The Psylloidea is composed of 11 families: Aphalaridae, Calophyidae, Carsidaridae, Homotomidae, Liadopsyllidae, Liviidae, Malmopsyllidae, Neopsylloididae, Phacopteronidae, Psyllidae, and Triozidae (Ouvrard 2015). Five of these families have previously been reported as

being present in the Pacific Northwest (Ouvrard 2015), these being Aphalaridae, Calophyidae, Liviidae, Psyllidae and Triozidae. Our study confirms that these are the five families present in our region (Table 1), while updating the psyllid species present here with the addition of 35 new reports to the PNW.

The most species-rich genera are *Cacopsylla* (30 species), *Craspedolepta* (23 species) and *Bactericera* (11 species). Species of *Cacopsylla* and *Bactericera* are known as vectors of economically-important plant pathogens; the following 13 species were found in the PNW and are listed as pests by Percy (2005): *B. cockerelli* (pest of potato as mentioned above), *P. buxi* (minor pest in ornamentals), *C. pyricola* (common pear psyllid, mentioned above), *C. triangula*, *C. eucalypti* and *G. brimblecombei* (pests in *Eucalyptus* spp.; see also Brennan et al. [1999]), *P. celtidisgemma*, *P. celtidismamma* and *P. venusta* (pests in hackberry, *Celtis* spp.), *P. tripunctata* (pest in blackberries, *Rubus* spp.), *L. alacris* (pest in bay trees, *Laurus* spp.), and *T. diospyri* and *T. eugeniae* (reported as minor pests on wild persimmon, *Diospyros virginiana*, in the U.S. and myrtaceous trees such as *Eugenia* spp. in Australia). Also present in the PNW is the broom psyllid, *Arytainilla spartiophila*, which in the 1950s was released in the U.S. as a biological control agent against the invasive shrub Scotch broom (*Cytisus scoparius*) (Syrett et al. 1999); we now know that any harm to the shrub results primarily from infection by *Candidatus* Liberibacter europaeus, a bacterium vectored by this psyllid species (Thompson et al. 2013).

The PNW has periodically seen emergence of new psyllid-vectored pathogens of agricultural importance (Horton 1999, Munyaneza et al. 2007). Eruption of these pathogens is often followed by a frantic search for the causative pathogen and then its insect vector (e.g., Munyaneza et al. 2007, Munyaneza et al. 2010). At times, relative ignorance of regional psyllid biodiversity,

population dynamics, and phenology has slowed initial development of effective pathogen-vector suppression plans (Munyaneza 2010, 2012). Our revised checklist offers researchers a base line to identify possible psyllid vectors of plant pathogens as a first step to address the development of integrated pest management strategies for any future emerging pathogens known, or thought, to be psyllid-vectored. We suggest that future work be conducted to confirm museum specimen identifications, and note the need for thorough field surveys of psyllid populations, diversity, and host plant biology across seasons and habitats, in the exceptionally-diverse range of biogeographic zones in the Pacific Northwest.

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Table 4-1. Families and genera found in the PNW region. Species arrangement based on Burckhardt and Ouvrard 2012.

Family	Subfamily	Genus	Species
Aphalaridae	Aphalarinae	*Aphalara*	7
		Craspedolepta	23
	Pachypsyllinae	*Pachypsylla*	3
	Spondyliaspidinae	*Cryptoneossa*	1
		Ctenarytaina	1
		Glycaspis	1
Calophyidae	Calophyinae	*Calophya*	6
Liviidae	Euphyllurinae	*Psyllopsis*	1
		Neophyllura	3
	Liviinae	*Livia*	2
Psyllidae	Macrocorsinae	*Euphalerus*	1
	Psyllinae	*Arytaina*	2
		Arytainilla	1
		Cacopsylla	30
		Ceanothia	1
		Euglyptoneura	2
		Nyctiphalerus	5
		Psylla	8
		Purshivora	1
Triozidae	Metatriozidinae	*Bactericera*	11
		Baeoalitriozus	1
		Heterotrioza	1
		Lauritrioza	1
		Phylloplecta	3
		Trioza	8

CHAPTER FIVE: THRIPS COLLECTED FROM *SOLANUM DULCAMARA* (SOLANALES: SOLANACEA) IN WASHINGTON AND IDAHO

Bittersweet nightshade, *Solanum dulcamara* L., is native in parts of Europe and Asia. It was introduced and is established in the eastern, north-central, and Pacific Northwest regions of the USA. It is commonly found in grasslands, meadows, and occurs most frequently in riparian areas, wetlands, and deciduous forests (Waggy 2009). We report here the Thysanoptera species collected from samples of *S. dulcamara* at locations in Washington and Idaho.

Patches of *S. dulcamara* were found along irrigation canals and ponds with running water near cultivated areas. Samples were taken with a D-Vac (Model 24, Rincon-Vitova Insectaries, Inc., Ventura CA) from June to November in 2012 and in August and October in 2013 (Table 1). *Solanum dulcamara* grows using other plants for support. Seventeen species of surrounding and support plants from nine families were identified, including *Elaeagnus angustifolia* L. (Rhamnales: Elaeagnaceae), *Typha* sp. (Poales: Typhaceae), *Asclepias syriaca* L. (Gentianales: Asclepiadaceae), and *Salix* sp. (Malpighiales: Salicaceae). The D-Vac tube was placed over the plant patch for 10 to 30 sec, depending on the size of the patch. Insects collected in the mesh bag were placed on dry ice for transport to the laboratory. Thrips were extracted under stereomicroscopy in 90% ethyl alcohol. Adults were mounted in Canada balsam for identification using the keys in Hoddle et al. (2012). Vouchers of each species are located at the North Florida Research and Education Center, University of Florida, Quincy.

Eight species of thrips from three families were collected (Table 2). Species of Thripidae

included the phytophagous *Caliothrips fasciatus* (Pergande), *Chirothrips aculeatus* Bagnall, *Frankliniella occidentalis* (Pergande), *Thrips hawaiiensis* (Morgan), and *Thrips tabaci* Lindeman. Both *F. occidentalis* and *T. tabaci* are worldwide pests of many crops, and they are vectors of the serious plant viruses in the genus *Tospovirus* (Bunyaviridae) (Hoddle et al. 2012).

Two species of Phlaeothripidae were collected (Table 2). *Haplothrips verbasci* (Osborn) breeds on the stems and flowers of *Verbascum thapsus* L. (Scrophulirales: Scrophuliareae). This plant species was identified as one of the surrounding plants. *Cephalothrips monilicornis* (Reuter) breeds on the leaves of various Poaceae (Hoddle et al. 2012). Four species of Poaceae were identified growing close to *S. dulcamara*. *Aeolothrips bicolor* (L.) in the family Aeolothripidae was collected. Insects in the order Thysanoptera are mainly phytophagous or mycophagous, and obligate predation is limited to only several lineages (Mound 2005). Species of *Aeolothrips* (Aeolothripidae) are predatory on small insects including other species of thrips.

A host plant is one in which an insect breeds (Mound 2013), and more research is needed to determine which species of thrips utilize *S. dulcamara* as a host plant. Our results suggest that the plant is potentially a source of economically important thrips invading crop fields.

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Summary

Bittersweet nightshade, *Solanum dulcamara*, was sampled at numerous locations in Washington and Idaho. Eight species of thrips from three families were collected, including the worldwide plant pests, *Frankliniella occidentalis* (Pergande) and *Thrips tabaci* Lindeman, vectors of the serious plant viruses in the genus *Tospovirus*. The predator of small insects, *Aeolothrips fasciatus* (L.), also was collected.

Key Words: thrips; bittersweet nightshade; Pacific Northwest

Resumen

Un muestreo de plantas hierba mora o dulcamara, *Solanum dulcamara*, fue realizado en varias localidades de Washington y Idaho. Ocho especies de trips fueron colectadas, incluidas las especies *Frankliniella occidentalis* (Pergande) y *Thrips tabaci* Lindeman que son vectores de virus del género *Tospovirus* en cultivos agrícolas a nivel mundial. También se colectó *Aeolothrips fasciatus* (L.) que son depredadores de pequeños insectos.

Palabras Clave: trips; hierba mora; dulcamara; Noroeste del Pacífico

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Table 5-1. Sample locations and coordinates and sampling dates.

Site name	Coordinates	Sampling date	
		2012	2013
Twin Falls, ID	42°29'57.33"N, 114° 9'14.53"W;	14 Jun	
	42°29'52.13"N, 114° 9'12.60"W	10 Jul	
		8 Sep	
Mesa (old), WA	46°35'17.72"N, 119° 0'1.12"W	28 Jun	24 Aug
		17 Jul	
		16 Aug	
		4 Nov	
Mesa (new), WA	46°34'35.35"N, 119° 0'33.41"W	2 Aug	26 Oct
		4 Nov	
Colfax, WA	46°50'51.1008"N, 117°28'43.9320"W	27 Sep	
		6 Oct	
Moses Lake, WA	46°59'34.19"N, 119°41'6.66"W;		24 Aug
	46°59'55.10"N, 119°41'5.25"W;		
Mattawa, WA	46°42'32.33"N, 119°56'42.54"W	4 Sep	24 Aug
			26 Oct
Sacajawea Park, WA	46°12'12.7692"N, 119°02'49.6644"W	4 Nov	

Table 5-2. Species of thrips found sampling *S. dulcamara* patches in Idaho and Washington.

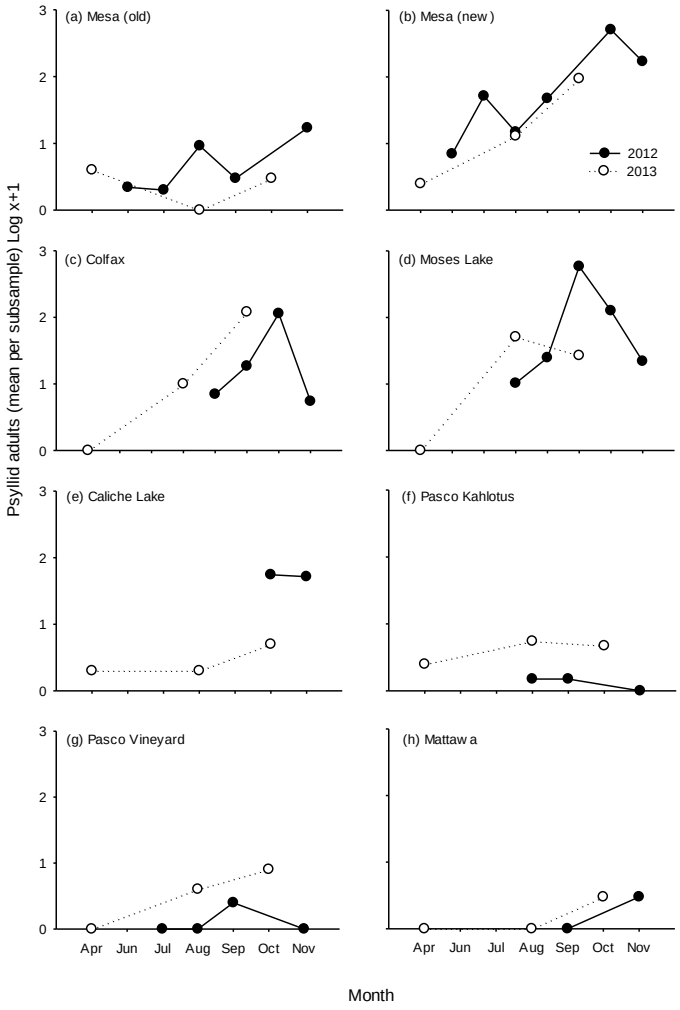
Thrips Species	Sampling places						
	TF	MO	MN	CX	ML	MT	SJ
Aeolothrips fasciatus	X	X		X			
Caliothrips fasciatus	X	X	X	X	X	X	
Cephalothrips monilicornis	X	X	X	X			X
Chirothrips aculeatus		X					
Frankliniella occidentalis	X	X				X	X
Haplothrips verbasci			X				
Thrips hawaiiensis		X					
Thrips tabaci					X	X	

Legend: TF=Twin Falls, MO=Mesa (old), MN=Mesa (new), CX=Colfax, ML=Moses Lake, MT=Mattawa, SJ=Sacajawea.

APPENDICES

Appendix 1

Online Appendix, Castillo Carrillo et al., "Psyllid Population Dynamics on Bittersweet Nightshade"



Appendix Figure 1. Population dynamics of the potato psyllid in bittersweet nightshade patches in Eastern Washington. Potato psyllids were never found at two other sites, Warden and Sacajawea.

Appendix Table 1. Date of psyllid egg counting in 10 places of Eastern Washington in 2013 (MO = Mesa (old), MN = Mesa (new), CX = Colfax, ML = Moses Lake, CL = Caliche Lake, PV = Pasco Vineyard, MT = Mattawa, PK = Pasco Kahlotus, SJ = Sacajawea Park, WD = Warden).

Number of eggs							
2013							
Site		Date	Mean/plant	Site		Date	Mean/plant
MO	29	March	0	PV	29	March	0
	27	April	2		27	April	0
	8	June	11		8	June	0
	27	June	49		27	June	11
	24	July	1		24	July	14
	25	August	1		25	August	3
	26	September	6		26	September	0
	26	October	6		26	October	0
	14	November	2		14	November	0
	19	December	0		19	December	0
MN	29	March	0	MT	12	April	0
	27	April	12		3	May	2
	8	June	14		7	June	0
	26	June	10		28	June	0
	24	July	32		23	July	0
	25	August	9		24	August	0
	26	September	15		27	September	0
	26	October	4		26	October	3
	14	November	6		15	November	0
	19	December	0		19	December	0
CX	29	March	0	PK	29	March	0
	72	April	1		27	April	4
	3	May	0		8	June	28
	27	June	6		26	June	42
	24	July	11		24	July	0
	25	August	14		25	August	10
	27	September	10		26	September	0
	26	October	33		26	October	0
	14	November	24		14	November	3
	19	December	0		19	December	0
ML	29	March	0	SJ	29	March	0
	3	May	1		27	April	0
	7	June	2		8	June	5
	28	June	2		26	June	46
	23	July	40		24	July	8
	24	August	187		25	August	0
	27	September	62		26	September	0
	26	October	25		26	October	0
	15	November	4		14	November	0
	19	December	0		19	December	0
CL	12	April	9	WD	29	March	0
	3	May	8		3	May	0
	7	june	26		7	June	0
	28	June	4		28	June	6
	23	July	9		23	July	0
	24	August	0		24	August	0
	27	September	4		27	September	0
	26	October	0		26	October	8
	15	November	1		15	November	0
	19	December	0		19	December	0