

Host and Non-host ‘Whistle Stops’ for Psyllids: Molecular Gut Content Analysis by High-Throughput Sequencing Reveals Landscape-Level Movements of Psylloidea (Hemiptera)

W. Rodney Cooper,^{1,9,✉} David R. Horton,¹ Mark R. Wildung,² Andrew S. Jensen,³ Jenita Thinakaran,¹ Dalila Rendon,⁴ Louis B. Nottingham,^{5,✉} Elizabeth H. Beers,⁵ Carrie H. Wohleb,⁶ David G. Hall,⁷ and Lukasz L. Stelinski⁸

¹Yakima Agricultural Research Laboratory, USDA-ARS, 5230 Konnowac Pass Road, Wapato, WA 98951, ²Laboratory for Biotechnology and Bioanalysis, Washington State University, Pullman, WA 99164, ³Northwest Potato Research Consortium, 95873 E. Goldmohr Ln, Lakeview, OR 97630, ⁴Mid-Columbia Agricultural Research and Extension Center, Oregon State University, Hood River, OR 97031, ⁵Tree Fruit Research and Extension Center, Washington State University, 110 N. Western Avenue, Wenatchee, WA 98801, ⁶Grant-Adams County Extension, Washington State University, 1525 E. Wheeler Road, Moses Lake, WA 98837, ⁷US Horticultural Research Laboratory, USDA-ARS, 2001 South Rock Road, Fort Pierce, FL 34945, ⁸Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, and ⁹Corresponding author, e-mail: rodney.cooper@ars.usda.gov

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Abstract

Psyllids (Hemiptera: Psylloidea) are phloem-feeding insects that tend to be highly specific in their host plants. Some species are well-known agricultural pests, often as vectors of plant pathogens. Many pest psyllids colonize agricultural fields from non-crop reproductive hosts or from non-host transitory and winter shelter plants. Uncertainty about which non-crop species serve as sources of psyllids hinders efforts to predict which fields or orchards are at greater risk of being colonized by psyllids. High-throughput sequencing of *trnL*, *trnF*, and ITS was used to examine the dietary histories of three pest and two non-pest psyllid species encompassing a diversity of lifecycles: *Cacopsylla pyricola* (Förster) (Psyllidae), *Bactericera cockerelli* (Sulc) (Triozidae), *Diaphorina citri* Kuwayama (Liviidae), *Aphalara loca* Caldwell (Aphalaridae), and a *Cacopsylla* species complex associated with *Salix* (Malphighiales: Salicaceae). Results revealed an unexpectedly high level of feeding on non-host species by all five psyllid species. The identification of the dietary history of the psyllids allowed us to infer their landscape-scale movements prior to capture. Our study demonstrates a novel use for gut content analysis—to provide insight into landscape-scale movements of psyllids—thus providing a means to pinpoint the non-crop sources of pest psyllids colonizing agricultural crops. We observed previously unknown psyllid behaviors during our efforts to develop this method and discuss new research directions for the study of psyllid ecology.

Key words: pear psylla, potato psyllid, citrus psyllid, gut content analysis, landscape ecology

There are approximately 3,800 described species of psyllids (Hemiptera: Psylloidea), of which about 45 are pests of agricultural crops or ornamental plantings (Ouvrard et al. 2015). Several of the pest species are vectors of plant pathogens, especially the pathogen groups of ‘*Candidatus Liberibacter*’ and ‘*Candidatus Phytoplasma*’. One feature that is common to many psyllid species is that their respective lifecycles must be considered at a landscape

scale. In addition to the part of the lifecycle spent on the reproductive host, many psyllid species regularly occur on plant species which fail to support reproduction and development (Hodkinson 2009, Burckhardt et al. 2014). These ‘shelter’ plants provide important refuges and feeding resources for adult psyllids at times of the year or in geographic locations that the reproductive host is not available or not conducive to psyllid development and may

thus help psyllids to bridge these segments of the life cycle. Shelter plants, especially those used in winter, sometimes occur outside the geographic range of the reproductive host species. Only by understanding the role of these feeding hosts for psyllids, including what species are preferred and how extensively those species are utilized under natural field conditions, will we be able to correctly interpret the landscape-scale movements and lifecycles of psyllids.

A primary challenge in studying landscape-scale processes of psyllids is in monitoring movement of the insects through the landscape. Intensive sampling of different habitats and plant species for presence of a targeted insect species can reveal a snapshot of the insect's distribution among different habitats and may also allow limited inferences about the movement through the landscape. Tracking movements between habitats require other approaches, generally involving a marking procedure (Southwood 1978, Hagler and Jackson 2001). Marking approaches have proven to be valuable in addressing movement by insects within or between habitats at modest geographic scales, but they are not always logistically feasible at landscape scales due to expense, properties of the markers, and difficulties in applying markers at the geographic scale required to address region-wide movements of insects (Hagler and Jackson 2001).

In this report, we show how molecular gut content analysis can be used to infer movements by insect herbivores at a landscape level. Gut content analysis allows us to identify what plant species psyllids had previously colonized and fed upon before being captured. Our initial proof-of-concept approach (Cooper et al. 2016) relied upon amplification of plant DNA using universal polymerase chain reaction (PCR) primers, cloning the amplicons into bacterial vectors, and sequencing amplicon clones using Sanger-based technology. Results of that study demonstrated that plant DNA could indeed be amplified from phloem-feeding insects such as psyllids (Cooper et al. 2016). Results additionally showed that plant DNA could be detected even after maintaining the psyllids for 2 wk at 25°C or for several months in winter on a plant species different from the original (Cooper et al. 2016, Cooper 2018). The length of time that plant DNA can be detected from psyllids suggests that gut content analysis could be used to describe the dietary histories of psyllids moving through the environment.

The methods developed by Cooper et al. (2016) worked well for small sample sizes and for psyllids which had fed upon a limited number of plant species but would be cost-prohibitive for large sample sizes and for psyllids which may have sampled many plant species. The ability to identify DNA from a large number of plant taxa would be required to allow use of gut content analysis to examine psyllid movements and feeding across a landscape-scale environment. In this study, we show that a direct sequencing approach and use of a PacBio SMRT platform allows us to describe the dietary histories of field-collected psyllids. This high-throughput approach, frequently used to identify bacterial 16S rRNA in environmental or biological samples (Schloss et al. 2016), has been adapted here to examine gut contents of field-collected psyllids that may have fed on multiple plant species during their movements through the environment.

For this demonstration, we targeted five psyllid species in four families that move extensively through the landscape in search of host plants or in search for wintering shelter plants but differ substantially in host plants and lifecycles (Table 1). Two objectives were addressed. First, we developed the technology and confirmed that we could detect and identify plant DNA in field-collected psyllids by examining specimens collected directly from host and shelter plants, and therefore with a partially known dietary history. In our second objective, we examined whether direct sequencing can be used to infer possible landscape-level movements of psyllids. This objective

included an examination of psyllids whose dietary history was partially known, as well as specimens for which we had no knowledge of recent feeding history. We revealed previously unrecognized psyllid behaviors and plant associations during our efforts to develop this method and discuss these unexpected findings in relation to life history of psyllids.

Materials and Methods

Insect Collections

Five species of psyllids encompassing a diversity of lifecycles were examined (Table 1). Taxa included three species of agricultural pests (*C. pyricola*, *B. cockerelli*, and *D. citri*), whose landscape-scale movements substantially complicate control efforts (Horton et al. 1994a, 1995, 2015a, 2016; Halbert and Manjunath 2004), and two taxa (*A. loca* and *Salix*-feeding *Cacopsylla* spp. [hereafter 'willow psyllids']) that develop on non-agricultural plant species. All five species are collected regularly on non-host shelter plants, including two taxa (willow psyllids, *A. loca*) that may overwinter preferentially or obligatorily on coniferous species (Hodkinson 2009). Dispersal traits may be triggered by deterioration of the host plant (e.g., *B. cockerelli*) or may be an obligatory movement (e.g., univoltine *Cacopsylla* from their *Salix* host plants to coniferous shelter plants). For species that overwinter extensively or exclusively away from the host plant (*C. pyricola*, *A. loca*, willow psyllids), psyllids have a defined period of deliberate movement from the host by diapausing or diapause-destined adults, and a second period of movement as post-winter adults return to the host plant. These movements could include interim feeding on non-host species as the psyllids are looking for overwintering locations, and interim feeding on non-host species preceding locating the true host following overwintering (Mayer and Gross 2007).

Specimens were collected by three methods (Table 2). The methods differ in what inferences can be made about dietary history of the psyllid. Most specimens were collected with beating sheets directly from plants and thus were expected to harbor the DNA of the source plant species (Table 2, partially known dietary history). Detection of plant DNA in addition to that of the source species would be evidence that the psyllid had fed on other species before having colonized the source plant. We also obtained specimens of *C. pyricola*, *B. cockerelli*, and willow psyllids from sticky interception traps and by aspirating psyllids directly from the side of a house (Table 2). Because these specimens were not collected directly from plants, dietary histories for these specimens were completely unknown. Specimens collected locally were directly frozen and stored at -80°C until they were processed for molecular gut content analysis. Specimens collected by off-site coauthors were moved immediately into 70% ethanol and shipped to Cooper for storage at -80°C.

Specimens of *C. pyricola* were collected directly from this species' reproductive host (pear; *Pyrus communis* L; Rosales: Rosaceae) in Moxee, WA and Hood River, OR, and from a non-host species (Nootka cypress, *Cupressus nootkatensis* Don; Pinales: Cupressaceae) located on the grounds of the USDA-ARS laboratory in Wapato, WA (Figs. 1 and 2; Table 2). These collections were made in autumn several weeks following leaf fall in pear and thus constituted diapausing psyllids of the winterform morphotype. *Cacopsylla pyricola* with unknown feeding history were collected in the spring of 2017 from clear sticky mesh traps placed on the perimeter of pear orchards near Wenatchee, WA (Table 2). The traps were checked weekly throughout March of 2017 when post-diapause winterform psyllids

Table 1. Life history traits of psyllid species collected for gut content analysis

Species	Common name	Psyllid voltinism and diapause	Host plants	Host growth traits	Seasonality of psyllid movements	Shelter plants
<i>Cacopsylla pyricola</i> (Förster) (Psyllidae) ^a	Pear psylla	Multivoltine; winter adult diapause	<i>Pyrus</i> (pear); Rosaceae	Perennial; deciduous	Autumn movement from host; late-winter return to host	Mixture of taxa; includes conifers
<i>Bactericera cockerelli</i> (Sulc) (Triozidae) ^b	Potato psyllid	Multivoltine; no apparent diapause, winter quiescence in some regions	Solanaceae and some Convolvulaceae	Perennial, annual	Variable: governed by host availability and phenology	Mixture of taxa
<i>Diaphorina citri</i> Kawayama (Liviidae) ^c	Asian citrus psyllid	Multivoltine; continuous reproduction	Rutaceae	Perennial; evergreen	Yearlong, but increased during spring	Mixture of taxa
<i>Aphalara loca</i> Caldwell (Aphalaridae) ^d	none	Apparently univoltine; winter adult diapause	Polygonaceae	Annual	Summer/autumn movement from host; post-winter return to host	Wintering on conifers
<i>Cacopsylla</i> spp. (Psyllidae) ^e	'willow psyllids'	Univoltine; adult psyllids in reproductive diapause from June until late-winter	<i>Salix</i> (Salicaceae)	Perennial; deciduous	May/June movement from host; post-winter return to host	Wintering on conifers

^aKaloostian 1970; McMullen and Jong 1976; Fye 1983; Horton et al. 1992, 1994a,b; Horton et al. 1998.

^bKnowlton and Thomas 1934; Ptersch 1947; Munyaneza 2012; Horton et al. 2015, 2016; Thinakaran et al. 2017; Kaur et al. 2018.

^cHalbert and Manjunath 2004, Hall and Hentz 2011, Thomas and De Leon 2011, Martini et al. 2013, Setamou et al. 2016.

^dCaldwell 1937, Burchkhardt and Lauterer 1997, Hodkinson 2009, Clewley and Wright 2014.

^eJensen 1951, Ossiannilsson 1992, Hodkinson 2009.

were actively migrating from overwintering shelter plants back to pear. *Bactericera cockerelli* were collected in autumn of 2016 from a stand of *Lycium barbarum* L. (Solanales: Solanaceae) located near Kahlotus, WA (Table 2; Figs. 3 and 4). Collections were made following potato harvest and during the autumn flush of *Lycium* when psyllid populations colonize *Lycium* hosts from other sources that likely include potato (Thinakaran et al. 2017). *Bactericera cockerelli* with unknown feeding history were captured on yellow sticky traps placed in commercial potato fields in central Washington during the summer of 2017 (Table 2). *Diaphorina citri* were collected directly from citrus trees located near Lake Alfred, FL and near Fort Pierce, FL (Table 2). Post-winter *A. loca* were collected from a juniper windbreak west of Yakima, WA in spring of 2017 prior to their post-winter movements to Polygonaceae hosts (Table 2). Willow psyllids with unknown dietary history were collected from the side of a house in West Yakima, WA in spring of 2017. These specimens constituted overwintered adults that had departed their winter shelter plants (likely conifers) and were searching for their *Salix* reproductive host.

DNA Extraction

Specimens were surface sterilized by submersing psyllids in 70% ethanol for 1–2 s, sterile deionized water for 1–2 s, and 1% bleach for 60 s, then rinsing samples twice in deionized water for 2 s. We conducted two independent trials that involved two different methods for DNA extraction and different primer sets to amplify different regions of plant DNA (see the amplification and sequencing of plant barcoding genes subsection). In the first trial, DNA was extracted from four groups of five psyllids of each collection using a CTAB precipitation method and was suspended in 100 µl of water (Crosslin et al. 2011). Specimens from all collections listed in Table 1 were included in this first trial except *C. pyricola* from Hood River, OR and *B. cockerelli* from sticky traps.

In the second trial, DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden Germany) from groups of five psyllids from each collection. Willow psyllids and *D. citri* were not included in the second trial due to low numbers of specimens. *Cacopsylla pyricola* collected from Hood River, OR and *B. cockerelli* collected from sticky traps were added during the second trial. DNA from all specimens that were collected from sticky traps was extracted using the CTAB method because the sticky residue interfered with the extraction process using the commercial kit. All DNA extraction steps were performed under a UV-sterilized hood.

Amplification and Sequencing of Plant Barcoding Genes

The trnF and trnL regions of chloroplast were PCR-amplified from specimens included in the first sequencing trial. The primers for trnF were B49873-e: GGT TCA AGT CCC TCT ATC CC and A50272-F: ATT TGA ACT GGT GAC ACG AG (Taberlet et al. 1991). The primers for trnL were B49317-c: CGA AAT CGG TAG ACG CTA CG and A49855-d: GGG GAT AGA GGG ACT TGA AC (Taberlet et al. 1991). In the second trial, the trnL region of chloroplast and the internal transcribed spacer region (ITS) was amplified. Primers for ITS were ITS2F: ATG CGA TAC TTG GTG TGA AT and ITS3R: GAC GCT TCT CCA GAC TAC AAT (Chen et al. 2010). PCR conditions for reactions using all three primer pairs included an initial denaturation step of 94°C for 10 min followed by 39 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 45 s, followed by a final extension at 72°C for 10 min. Each 50 µl reaction contained Invitrogen Amplitaq Gold 360 PCR Master Mix (Invitrogen, Carlsbad, CA), 250 nM of each primer, and 5 µl of DNA template. PCR controls included negative controls,

Table 2. Psyllid collections for gut content analysis

Psyllid species	Collection	Location	Date
Partially known dietary history			
<i>Cacopsylla pyricola</i>	Pear trees, developmental host	Moxee, WA	17 Nov. 2016
	Pear trees, developmental host	Hood River, WA	27 Nov. 2017
	Pear trees, developmental host	Colony insects (known dietary history)	-
	Ornamental Nootka, shelter plant	Wapato, WA	24–27 Nov. 2016
<i>Bactericera cockerelli</i>	<i>Lycium barbarum</i> , developmental host	Kahlotus, WA	6 Oct. 2016
	<i>Solanum tuberosum</i> , developmental host	Colony insects (known dietary history)	-
<i>Diaphorina citri</i>	<i>Citrus</i> sp., developmental host	Lake Alfred, FL	16 May 2017 and 4 Mar. 2019 ^a
		Fort Pierce, FL	16 May 2017
		Colony insects	4 Mar. 2019 ^a
		Ahtanum, WA	8 Nov. 2016
<i>Aphalara loca</i>	<i>Juniperus</i> sp., shelter plant	Ahtanum, WA	8 Nov. 2016
Unknown dietary history			
<i>Cacopsylla pyricola</i>	Sticky traps	Wenatchee, WA	Spring 2017
Willow <i>Cacopsylla</i>	Side of a house	Ahtanum, WA	8 Apr. 2017
<i>Bactericera cockerelli</i>	Sticky traps	Columbia Basin of WA	Jul. and Aug. 2017

^aUsed for Sanger sequencing only

single-primer controls, and *B. cockerelli* and *C. pyricola* obtained from laboratory colonies. *Bactericera cockerelli* and *C. pyricola* colonies were reared on potato (*Solanum tuberosum* L.; Solanaceae) or pear only, respectively, at 25°C with a 16:8 (L:D) h photoperiod. Separate asymmetric-barcode forward and reverse primers (Pacific Biosciences 2014) were used for each collection. The presence of 400- to 600-bp amplicons were observed on 1% agarose gels stained with ethidium bromide.

PCR products from all specimens in each of the two trials were pooled into single samples. Twenty microliters of PCR product was transferred if amplicons were not visible, 15 µl was transferred if a faint amplicon was observed, and 10 µl of product was transferred if the amplicon was clearly visible. The combined samples were then shipped to the Washington State University Laboratory for Biotechnology and Bioanalysis and sequenced directly using the Pacific Biosciences (PacBio) sequencing platform. Pooled barcoded amplicons were ligated to the hairpin SMRT bell adapters using PacBio Template kit v 1.0, and library purified using AMPureXP beads (Beckman-Coulter). Libraries were quantified and bound to the P6 polymerase, magbead loaded into a single SMRT cell, and observed for 6 h using C4 chemistry on a PacBioRSII. Raw movies were processed into reads, reads processed into high-quality reads of interest and barcodes separated using SMRT Portal version 2.3. Average read-length was 24.6Kb, which produced an average single molecule coverage of 33x. This resulted in nearly all the data being very high quality, with phred scores between 35 and 45 (99.95 and 99.995 accuracy).

Post hoc sanger-based sequencing was performed on *D. citri* as described by Cooper et al. (2016) due to low return of sequences from PacBio. Six specimens from Fort Pierce and Lake Alfred (three per location) were chosen because PCR produced highly visible amplicons in preparation for high-throughput sequencing. Additionally, new specimens were obtained from Lake Alfred and from a laboratory colony reared on *Citrus* (Table 2). PCR was performed with all three primer pairs. Amplicons were excised from gels, purified using GenElute minus EtBr spin columns (Sigma, St. Louis, MO), and were cloned using a TOPO TA cloning kit with TOP10 *E. coli* chemically competent cells (Invitrogen, Carlsbad, CA). Plasmid DNA was extracted from 10 selected colonies per specimen using the QIAprep spin mini prep kit (Qiagen, Valencia, CA), and DNA clones were sequenced by MC Laboratories (MC Lab, San Francisco, CA) (Cooper et al. 2016).

Sequence Analysis

Sequences smaller than 400 bp and larger than 600 bp were excluded from analysis. In the first trial, this included chains of sequences caused by the binding of trnL and trnF primers after the samples were pooled. Sequences were clustered using the MAFFT alignment tool of Geneious R10.0.02 using the default settings (200PAM/Ki2 scoring matrix, gap open penalty of 1.53, and offset value of 0.123) (www.genious.com; Katoh et al. 2002, Kearse et al. 2012, Katoh and Standley 2013). Sequences with >98% identity were grouped as operational taxonomic units (OTUs), and 3 to 10 sequences from each OTU were analyzed using the BLAST function of NCBI database to putatively assign a taxonomic classification (Altschul et al. 1990). The first and last sequences in each OTU were included in the BLAST analyses to ensure dissimilar sequences (up to 2% differences in sequence identity) within each unit were included. Taxonomic classifications are reported to the family level but identified plant genera or species are discussed when applicable or when the presence of plant species' was physically confirmed. Unidentified and nontarget sequences including matches to insect or bacteria genomes and non-target regions of plant DNA were discarded. PCR contamination was monitored by the identification of plants other than *Solanum* or *Pyrus* from colony-reared psyllids.

Results

PCR Amplification and Sequencing

Visible amplicons were produced from very few specimens with DNA extracted using a CTAB precipitation method (Table 3). However, trnL and trnF plant products were identified from specimens regardless of whether amplicons were visible (Table 3). After removing bacterial and other nontarget sequences, a total of 2843 trnL and 9487 trnF sequence reads were analyzed from DNA extracted using the CTAB precipitation method. Specimens with DNA that was extracted using a commercial kit more consistently produced visible PCR amplicons, and 4729 trnL and 4741 ITS sequence reads were analyzed from these samples after removing nontarget sequences (Table 3).

Bactericera cockerelli and *C. pyricola* were reared in colonies on potato and pear, respectively, and PCR reaction from no-template controls were included in sequencing analysis to measure levels of contamination. No-template controls did not return sequencing results,



Fig. 1. *Cacopsylla pyricola* were collected in November of 2016 directly from pear trees located at the USDA experimental orchard near Moxee, WA outlined with red. This pear orchard is adjacent to an apple orchard, various deciduous and conifer windbreaks, and steppe habitats. Satellite image was obtained from Google Earth software (Google Inc., Mountain View, CA).

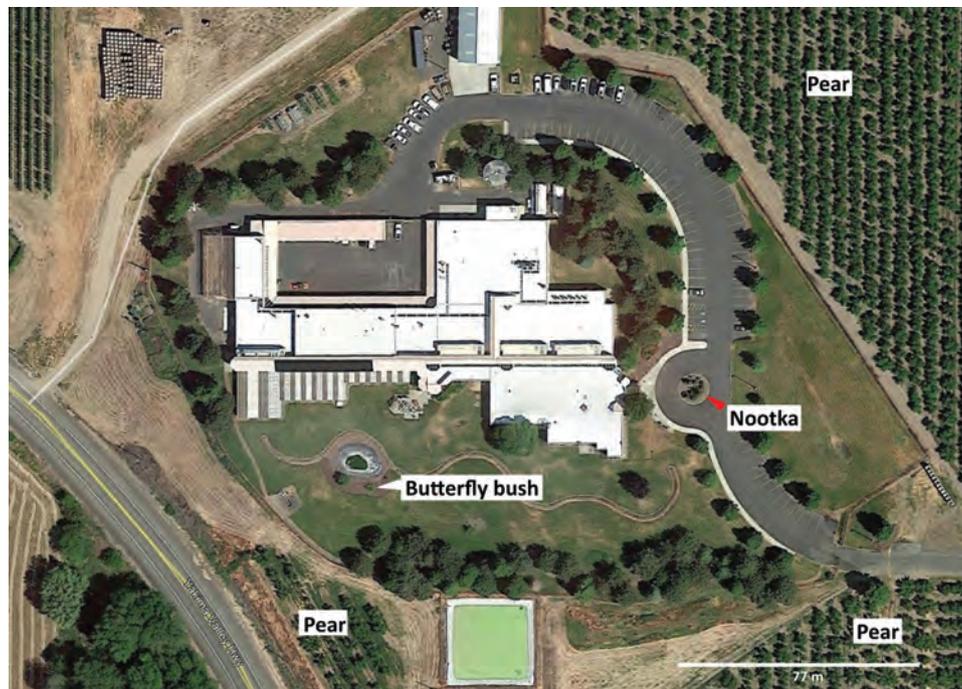


Fig. 2. *Cacopsylla pyricola* were collected in November of 2016 from an ornamental nootka cypress (red arrow) located at the USDA laboratory near Wapato, WA. The laboratory is adjacent to commercial pear orchards. Various deciduous and conifer trees and shrubs, including butterfly bush, landscape the laboratory grounds. Satellite image was obtained from Google Earth software (Google Inc., Mountain View, CA).

and nearly all sequences from colony-reared psyllids were identified as the rearing host plants (*Solanum* or *Pyrus*). Less than 1% of sequences from colony-reared specimens were identified as *Lactuca* (Asterales: Asteraceae) or *Calystegia* (Solanales: Convolvulaceae) suggesting the presence of low levels of PCR contamination or mis-binning of sequences. Samples with similarly low rates of *Lactuca* or *Calystegia* were not considered in the final analyses.

Gut Content Analysis of *Cacopsylla pyricola*

Eighteen plant families were detected from *C. pyricola* collected in November of 2016 from a pear orchard near Moxee, WA, and six plant families were detected from psylla collected in November of 2017 from a pear orchard near Hood River, OR (Table 4). These specimens were collected directly from pear trees and the majority of Rosaceae sequences were identified as *Pyrus*, but *Malus* was also



Fig. 3. *Bactericera cockerelli* were collected in October of 2016 from a stand of *Lycium barbarum* (red circle) near Kahlotus, WA. The surrounding habitat consists mostly of semi-arid steppe habitat mixed with irrigated crop circles and wetland/riparian habitats. Satellite image was obtained from Google Earth software (Google Inc., Mountain View, CA).

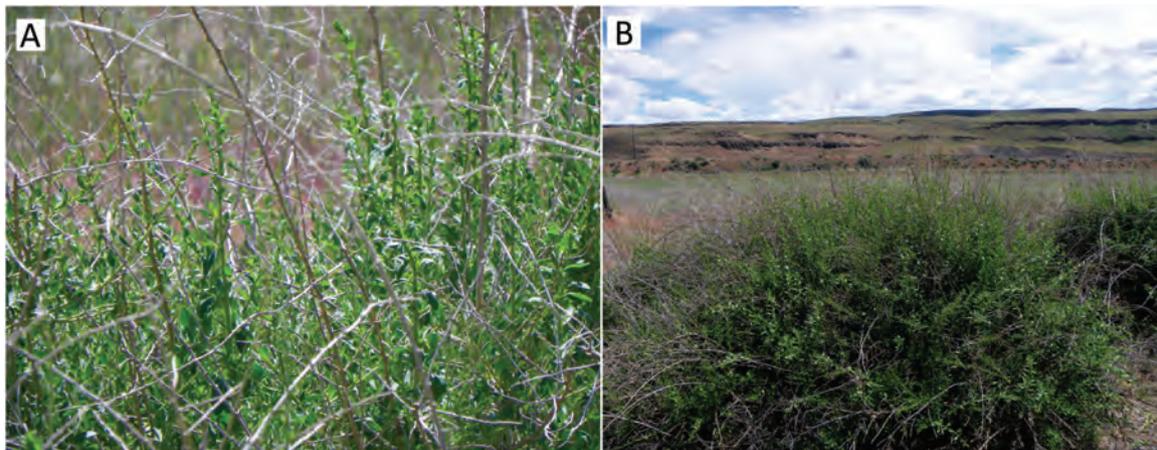


Fig. 4. *Lycium barbarum* (A) located near Kahlotus, WA. The surrounding habitats consist of semiarid steppe (B).

identified. Also identified were various wild or naturalized trees such as *Salix* (Malpighiales: Salicaceae), *Juniperus* (Cupressaceae), *Thuja* (Cupressaceae), *Pinus* (Pinaceae), annual and perennial plants such as *Artemisia* (Asteraceae) and *Convolvulus* (Convolvulaceae), and regional crops such as *Solanum* (Solanaceae), *Humulus* (Rosales: Cannabaceae), melon (Cucurbitales: Cucurbitaceae), and *Medicago* (Fabales: Fabaceae).

Nine plant families were detected from *C. pyricola* collected from Nootka Cypress (Cupressaceae) after they migrated to this overwintering shelter plant from pear orchards (Table 4). All sequences within the Rosaceae were identified as *Pyrus* (Rosaceae). Non-*Pyrus* taxa primarily included butterfly bush (*Buddlea*; Lamiales: Buddlejaceae), *Convolvulus*, *Humulus*, melon, and *Medicago*.

Ten plant families were detected from post-diapause *C. pyricola* collected from sticky traps in spring of 2017 (Table 4). Since these psyllids were captured in flight interception traps, they had

an entirely unknown feeding history. All sequences putatively identified as Rosaceae were most closely aligned with *Pyrus* and *Malus*. Non-*Pyrus* taxa identified from these specimens included conifers (Cupressaceae, especially *Thuja*) and numerous taxa with annual lifecycles that would have been present in early spring, such as *Medicago* and *Brassica*.

Gut Content Analysis of *Bactericera cockerelli*

Thirteen plant families were detected from *B. cockerelli* collected in autumn of 2016 from a stand of *L. barbarum* (Table 5). Most Solanaceae were identified as *Lycium*, but *S. tuberosum*, *Nicotiana attenuata* Torr ex S. Wats, and *S. dulcamara* L. were also identified within this family. Many sequences were identified as Asteraceae (Table 5), especially *Lactuca serriola* L. (prickly lettuce).

Table 3. Proportion of visible amplicons per PCR reaction (group of five psyllids) and number of returned plant sequences from gut content analysis of psyllids using universal PCR primers for trnL, trnF, and ITS

Sample	Trial 1				Trial 2			
	trnL		trnF		trnL		ITS	
	Visible amplicons	No. sequences						
<i>Cacopsylla pyricola</i>								
Pear, Moxee, WA	0/20	29	1/20	277	4/4	963	4/4	335
Pear, Hood River, OR	-	-	-	-	0/4	4	4/4	243
Nootka cypress	0/4	2	0/4	58	4/4	578	4/4	270
Clear mesh sticky traps	0/4	1	0/4	14	-	-	-	-
<i>Bactericera cockerelli</i>								
<i>Lycium barbarum</i>	1/4	2,711	1/4	8,100	4/4	1,775	4/4	1378
Yellow sticky traps	-	-	-	-	13/20	772	13/20	2,380
<i>Diaphorina citri</i>	0/8	86	1/8	165	-	-	-	-
<i>Aphalara</i>	0/4	5	4/4	19	3/3	637	3/3	135
Willow <i>Cacopsylla</i>	0/4	9	1/4	854	-	-	-	-

Eleven plant families were identified from *B. cockerelli* captured on sticky traps during the summer of 2017. These traps were located along the margins of fields of potato and detected Solanaceae were identified as *Solanum*. Other annual plants and crops present during spring and early summer were also detected, including Brassicaceae, Amaranthaceae, Fabaceae, and Convolvulaceae.

Gut Content Analysis of *Diaphorina citri*

We obtained few sequence reads from *D. citri* relative to other psyllid species examined using gut content analysis. Rutaceae (*Citrus*) was surprisingly not identified from this psyllid using high-throughput sequencing. Psyllids were collected directly from *Citrus* trees from two separate locations, and similar results (low numbers of read and absence of *Citrus*) were obtained from both locations (data not shown). However, *Citrus* was identified from all specimens using Sanger-based sequencing of cloned PCR products as described by Cooper et al. (2016). All three primer pairs (ITS, trnL, and trnF) amplified *Citrus* DNA from these specimens. In addition to *Citrus*, ITS sequences putatively identified as *Glycosmis* spp (Rutaceae; 98.8% similar) were detected in a single psyllid collected in Fort Pierce, FL.

Gut Content Analysis of *Aphalara loca*

Sequences belonging to Cupressaceae and nine other plant families—but not Polygonaceae—were detected from *Aphalara* collected directly from cypress in November of 2016 (Table 6). This univoltine psyllid develops on Polygonaceae but departs its host plant during summer months before these specimens were collected from juniper. As with other psyllid species, trees and other plants typical for the region were also identified from *A. loca*.

Gut Content Analysis of Willow Psyllids

The diversity of plant sequences detected in willow psyllids was low relative to other species examined, including only six plant families (Table 6). These psyllids were collected in April of 2017 from the side of a co-author's house and had an entirely unknown feeding history. Cupressaceae (*Juniperus*), which is an overwinter shelter host of willow psyllids (Jensen 1951, Ossiannilsson 1992, Hodkinson 2009) was detected in these specimens. However, nearly all sequences identified from these psyllids belonged to Convolvulaceae (Table 6), and mostly included the genera *Ipomoea* and *Calystegia* (Fig. 5).

Discussion

Molecular gut content analysis is commonly used to examine trophic links between insect predators and prey (Sheppard and Harwood 2005). Gut content analysis has also been used to identify the dietary history of insect herbivores (Fumanal et al. 2005, Matheson et al. 2008, Hereward and Walter 2012, Kitson et al. 2013, Wallinger et al. 2015), but the use of gut content analysis of herbivores has been mostly limited to insects that feed by chewing mechanisms or that ingest fluids from macerated plant tissues. Cooper et al. (2016) demonstrated that plant DNA could be amplified from *B. cockerelli*, and that sequence analysis of those amplicons could successfully identify whether the psyllids were reared on potato or bittersweet nightshade. The primary goals of our study were to first examine whether high-throughput direct sequencing could successfully detect and identify plant DNA in field-collected psyllids, and to then examine a novel use of gut content analysis to study the landscape-level movements of psyllids. Results of our study demonstrate that high-throughput sequencing of plant barcoding genes amplified from psyllids can provide valuable clues on the previous landscape-level movements of multiple psyllid species with diverse life history traits. Furthermore, our efforts to develop this method and novel use of gut content analysis led us to discover previously unrecognized psyllid behaviors.

Detection and Identification of Dietary History of Psyllids

Our first objective was to determine whether plant sequences can be detected from field-collected psyllids representing a diversity of taxa and lifecycles. High-throughput sequencing of plant DNA allowed us, in most cases, to detect the presence of the host or shelter plant DNA in our samples; *Pyrus* sequences were identified in *C. pyricola* collected from pear, Cupressaceae sequences were detected in *C. pyricola* collected from Nootka cypress and from *A. loca* collected from a juniper windbreak, and *Lycium* sequences were detected in *B. cockerelli* collected from *L. barbarum*. The exception was *D. citri*, from which we did not detect sequences of *Citrus* by high-throughput direct sequencing. However, *Citrus* was the primary sequence detected in a follow-up analysis using Sanger-based sequencing of cloned PCR products. Because very few plant sequences were detected by high-throughput sequencing, we suspect that human error during processing of *D. citri* samples was responsible for the failure

Table 4. Plant families putatively identified from *Cacopsylla pyricola* collected from pear trees (Rosaceae) near Moxee, WA in November 2016 and Hood River, OR in November 2017, collected from a Nootka cypress tree (Cupressaceae) near Wapato, WA, or captured on sticky traps near Quincy, WA in spring of 2017

Plant family	Pear, Moxee, WA			Pear, Hood River, OR		Nootka, Wapato, WA			Sticky traps, Quincy, WA	
	trnF	trnL	ITS	ITS	trnL	trnF	trnL	ITS	trnL	ITS
Total No. OTUs ^a	16	7, 8 ^b	11	2	9	3	1, 4 ^b	5	5	9
Amaranthaceae	+		+							
Anacardiaceae		+								
Asteraceae	+	+	+							
Betulaceae										+
Brassicaceae										+
Buddlejaceae						+				
Cannabaceae		+				+	+		+	
Caryophyllaceae	+									
Convolvulaceae		+						+	+	
Cucurbitaceae			+		+			+		
Cupressaceae		+	+		+		+		+	
Elaeagnaceae									+	
Euphorbiaceae		+				+				
Fabaceae		+	+	+	+		+		+	+
Geraniaceae					+					
Malvaceae										+
Montiaceae			+							
Pinaceae	+									
Poaceae	+				+					
Polygonaceae			+							
Rosaceae	+	+	+	+	+		+	+		+
Salicaceae	+									
Sapindaceae		+	+				+			
Solanaceae	+	+								+

^aNo. of OTUs is often greater than the number of identified plant families because multiple genera or species within a family were occasionally identified.

^bFirst number refers to OTUs identified from specimens when DNA was extracted using the CTAB method, and the second number refers to OTUs identified from specimens when DNA was extracted using a commercial kit.

to detect Rutaceae by direct sequencing. Overall, the detection of plant species that specimens were collected from confirms that detection of plant DNA from psyllids can infer the previous dietary history of a diversity of psyllid species. Both methods for DNA extraction (CTAB precipitation and the commercial kit) were suitable, but the commercial kit removes more salts and other PCR inhibitors compared with the CTAB method and produced visible amplicons were more consistently in our study.

In addition to detecting the host or shelter plant sequences, many sequences detected in psyllids were identified as plants that do not support nymphal development and are not considered overwinter shelter plants. Although diligent care was taken to avoid contamination, we cannot completely rule out contamination as a source for some of these taxa. However, most of the identified taxa were abundant near the collection sites or would have been available to psyllids at or before the time of collection. We were able to identify from the sequences what plant taxa were present at the study sites. Specific examples are provided in the preceding subsections.

Interpretation of data requires recognition of several limitations of molecular gut content analysis. First, the efficiency of PCR using the universal primers is expected to differ among primer sets and plant taxa (Taberlet et al. 1991), and may not amplify target genes from all plant taxa. We accounted for this limitation by targeting multiple gene regions with different primer sets. Another limitation is that we have incomplete information about how long the plant signal can be detected after a feeding event. We previously determined

that potato can be detected in *B. cockerelli* 2 wk after the insects were moved to *S. dulcamara* and maintained at a constant 25°C but we did not examine psyllids maintained on *S. dulcamara* for longer than 2 wk (Cooper et al. 2016). Additionally, sequences other than pear were detectable in *C. pyricola* that were collected from juniper in January and confined to pear until early April (Cooper 2018). In the current study, we were able to detect DNA of plants in some psyllids (notably *C. pyricola*, *Aphalara*, and willow psyllids) that are regionally abundant but would have only been available to psyllids weeks or months before collections. The duration of the DNA signal is likely dependent upon temperature, physiological state of the psyllid, length of the PCR product (which varies among plant taxa), and other biological and abiotic factors (Hoogendoorn and Heimpel 2001). We, therefore, do not interpret sequence abundance as estimates of time since feeding or relative importance of a plant taxa as a host or transient plant.

Gut Content Analysis to Track Landscape-Level Movements of Psyllids

A second objective of our study was to examine whether gut content analysis can infer prior landscape-level movements of psyllids. This novel use for gut content analysis will allow us to examine challenging aspects of psyllid biology and ecology and will allow us to predict which fields or orchards are at greatest risk for colonization by psyllid pests and vectors of plant pathogens. Sequencing results from *C. pyricola*, *B. cockerelli*, and willow psyllids provided convincing

Table 5. Plant families identified from *Bactericera cockerelli* collected from *Lycium barbarum* near Kahlotus, WA in October of 2016 and from sticky traps in Central Washington in July of 2017

Plant family	<i>Lycium barbarum</i> , Kahlotus			Sticky traps, Central Washington	
	trnF	trnL	ITS	trnL	ITS
Total No. OTUs ^a	11	5, 6 ^b	10	6	13
Amaranthaceae	+		+		+
Asteraceae	+	+	+		
Brassicaceae			+		+
Cannabaceae		+		+	
Convolvulaceae	+			+	+
Cucurbitaceae			+		+
Cupressaceae	+	+		+	
Fabaceae		+	+	+	+
Pinaceae		+			
Portulacaceae					+
Rosaceae	+	+	+	+	+
Salicaceae	+				
Sapindaceae					+
Solanaceae	+	+	+	+	+
Thymelaeaceae	+				

^aNo. of OTUs is often greater than the number of identified plant families because multiple genera or species within a family were occasionally identified.

^bFirst number refers to OTUs identified from specimens when DNA was extracted using the CTAB method, and the second number refers to OTUs identified from specimens when DNA was extracted using a commercial kit.

evidence that gut content analysis can indeed be used to infer previous landscape-level movements of psyllids.

Cacopsylla pyricola were collected directly from pear trees located at the USDA experimental orchard near Moxee in November of 2016. From these psyllids, we identified sequences from *Malus* (Rosaceae), *Salix* (Salicaceae), *Populus* (Salicaceae), and genera of cypress (Cupressaceae), which are all located adjacent to the pear orchard from which the psyllids were collected (Fig. 1). Autumn leaf-drop occurs later in *Malus* than in *Pyrus*, and *C. pyricola* often migrate in large numbers to the apple orchard east of the pear orchard (Fig. 1) when leaves drop from pear but are still green in apple (Horton et al. 1994a, Cruz et al. 2018). *Salix* and *Populus* species both occurred in a windbreak located to the west of the pear orchard, and a windbreak which includes cypress was located to the northwest of the orchard (Fig. 1). Winterform psyllids regularly occupy the juniper windbreak located northwest of the pear orchard in Fig. 1 (Cooper and Horton 2014). Most other taxa identified from molecular analysis of *C. pyricola* also occur at the experimental farm as either planted row crops or as natural vegetation. For example, sequences identified within the Solanaceae included *Solanum* and *Lycium*, which were both planted at the farm in 2016 and would have been present up to several weeks prior to collections (Fig. 1). In fact, yellow traps placed near the *Lycium* stand in 2016 to monitor *B. cockerelli* captured large numbers of *C. pyricola* throughout the summer and autumn (Thinakaran, unpublished data). Sequences within the Asteraceae (Asterales) included rabbitbrush, *Ericameria nauseosa* (Pall. ex Pursh), and other common natural vegetation within this family. These results indicate that some winterform psyllids present in pear orchards in November had left the orchard and subsequently returned. Alternatively, some of the psyllids could have arrived at the USDA experimental farm from a different orchard. Evidence for colonization from a different orchard is provided by the detection of hops (*Humulus lupulus* L.; Cannabaceae). Washington is the nation's leading producer of hops, and Moxee, WA is a major center for hops production within the state (NASS 2017). The USDA experimental orchard is several kilometers from

Table 6. Plant families identified from *Aphalara loca* collected from *Juniperus* (Cupressaceae) in November of 2016 and from willow *Cacopsylla* sp. having an unknown dietary history in April of 2017

Plant family	<i>Aphalara</i>			Willow <i>Cacopsylla</i>	
	trnF	trnL	ITS	trnF	trnL
Total no. OTUs ^a	3	2, 5 ^b	7	8	3
Asteraceae		+		+	
Brassicaceae		+	+		
Convolvulaceae				+	+
Cucurbitaceae		+			
Cupressaceae			+		+
Fabaceae		+	+	+	+
Pinaceae		+			
Poaceae				+	
Rosaceae	+	+	+	+	
Salicaceae	+				
Sapindaceae			+		
Solanaceae	+				

^aNo. of OTUs is often greater than the number of identified plant families because multiple genera or species within a family were occasionally identified.

^bFirst number refers to OTUs identified from specimens when DNA was extracted using the CTAB method, and the second number refers to OTUs identified from specimens when DNA was extracted using a commercial kit.

the nearest commercial hops or pear, so the detection of hops DNA in *C. pyricola* collected from the Moxee farm indicates that at least some of the collected psyllids had traveled several kilometers from a commercial pear orchard and had sampled *Humulus* during these movements.

Winterform *C. pyricola* were also collected from an ornamental coniferous shelter plant (Cupressaceae) near the USDA laboratory in Wapato, WA in November of 2016. The facility is located adjacent to commercial pear orchards (Fig. 2), and gut content sequences within

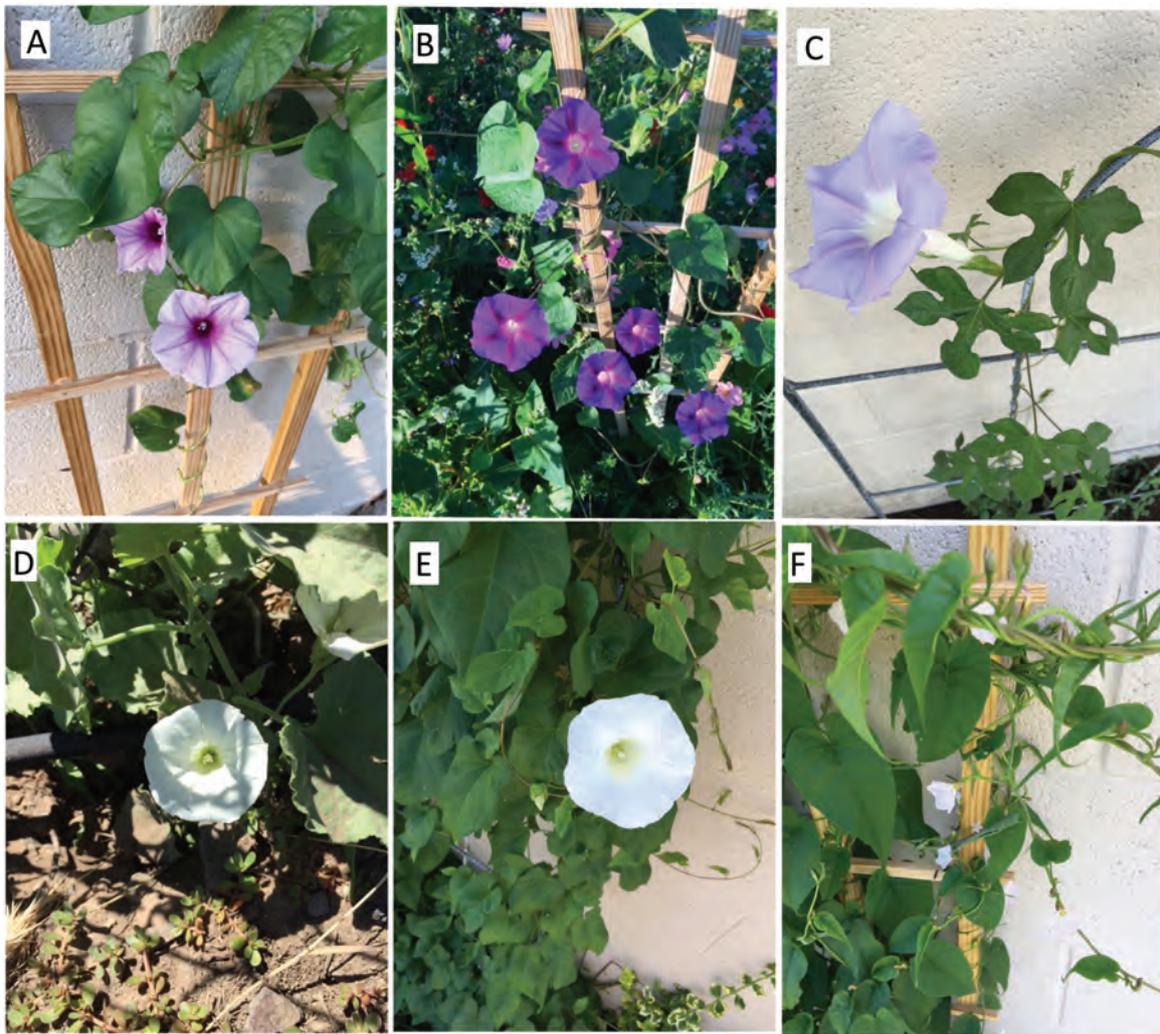


Fig. 5. Convolvulaceae planted at location where salix psyllids were collected: *Ipomoea turbinata* (A), *I. purpurea* (B), *I. lindheimeri* (C), *Calystegia macrostegia* (D), *C. sepium* (E), and *Merremia sibirica* (F).

the Rosaceae (Table 4) were composed entirely of *Pyrus*. A large number of non-*Pyrus* sequences from these psyllids were identified as *Buddleja* sp. (Lamiales: Buddlejaceae), or butterfly bush (Table 4). Exceptionally large populations of winterform psylla have been observed accumulating on a butterfly bush located on the laboratory grounds during leaf fall in pear, followed by disappearance of psyllids when leaves subsequently drop from this shrub (Horton, unpublished data). Our results indicate that at least some of the psylla collected from the Nootka cypress had arrived on this tree from butterfly bush (Fig. 2). These movements are consistent with the hypothesis that leaf fall from deciduous trees and attraction to the color of foliage triggers and guides autumn dispersal of *C. pyricola* (Horton et al. 1992, 1994a).

Bactericera cockerelli were collected directly from a stand of *L. barbarum* located near Kaholotus, WA in late fall of 2016 (Figs. 3 and 4). *Lycium barbarum* is an introduced xenophytic plant that exhibits periods of leaf flush in spring and autumn when precipitation occurs but enters a period of dormancy in response to hot and dry conditions of summer (Horton et al. 2016). Large populations of *B. cockerelli* occur on *L. barbarum* during the spring before emergence of potato and in autumn after potato harvest but are mostly absent during summer leaf fall (Thinakaran et al. 2017). Collections for gut content analysis were made after potato harvest and when

psyllids had reappeared on *L. barbarum*. The landscape surrounding the *L. barbarum* stand from which psyllids were collected includes isolated irrigated crop circles (including potato) separated by large expanses of natural steppe habitats (Figs. 3 and 4). *Solanum tuberosum* sequences were identified by gut content analysis indicating that some of the psyllids collected from this stand of *L. barbarum* had previously fed upon potato. Other plants identified from the Solanaceae included *Nicotiana attenuata* (Coyote tobacco) and *Solanum dulcamara*. *Nicotiana attenuata* is a native annual Solanaceous plant on which *B. cockerelli* can complete egg-to-adult development under laboratory conditions (Knowlton and Thomas 1934, Cooper et al. 2019). *Solanum dulcamara* grows in wetlands and riparian habitats and is a known host for *B. cockerelli* (Murphy et al. 2013, Cooper et al. 2019). The detection of *S. dulcamara* sequences was unexpected due to the semi-arid habitat surrounding the collection site (Fig. 4), but satellite imagery revealed the presence of a wetland habitat near the stand of *Lycium* (Fig. 3). Non-Solanaceae plants were also identified from *B. cockerelli* including weeds within the Asteraceae. Our results suggest that at least some of the *B. cockerelli* specimens included in our analysis had developed on Solanaceae other than *Lycium*, including potato, and then colonized *Lycium* as adults.

Bactericera cockerelli were also captured on yellow sticky traps deployed in potato fields throughout the Columbia Basin. We

unfortunately observed poor amplification and sequencing of plant DNA from psyllids that were captured on sticky traps but are currently testing whether PCR amplification is improved when psyllids are captured directly in preservative using a 3D-printed trap (Horton et al. 2019a). Because *L. barbarum* supports large populations of potato psyllid in spring and because this plant loses its leaves at the same time *B. cockerelli* begin colonizing potato (Horton et al. 2016), we expected to detect *L. barbarum* sequences from these captured psyllids. Sequences identified as Solanaceae were most closely aligned with *Solanum* (especially *S. tuberosum*) and none of the sequences aligned with *Lycium*. A large percentage of sequences were identified as Convolvulaceae including field bindweed, *Convolvulus arvensis* L. (Table 5). This famously abundant weed supports egg-to-adult development of *B. cockerelli*, although with high mortality, and provides an overwintering habitat for adults (Pletsch 1947; Wallis 1955; Horton et al. 2015a, 2017; Cooper et al. 2019; Kaur et al. 2018). Our results suggest that some of the psyllids captured on traps had likely arrived in potato from *C. arvensis*.

Although *Citrus* was the primary plant taxa identified from *D. citri*, sequences identified as *Glycosmis* sp. (Rutaceae) were detected from one specimen collected in Fort Pierce, FL. *Glycosmis parviflora* (Sims) has become naturalized in South Florida (Atlas of Florida Plants Wunderlin et al. 2019) and is apparently colonized by *D. citri* (Westbrook et al. 2011). These results demonstrate movement by *D. citri* between *Citrus* and non-*Citrus* shrubs.

Cacopsylla spp. associated with *Salix* were collected in early April of 2017. Because these willow psyllids were collected from the side of a house, they had an entirely unknown feeding history (Table 2). Willow psyllids are univoltine and develop within the catkins of *Salix* spp. In early April when these specimens were collected, willow psyllids had emerged from unknown overwintering locations and were presumably searching for the *Salix* host to begin egg-laying activities. Willow psyllids are often collected from conifers during winter (Jensen 1951, Ossianniilsson 1992, Hodkinson 2009). Sequences from *Juniperus* (Cupressaceae), which is located near the collection site, were identified from these specimens. However, most sequences amplified from these specimens were identified as Convolvulaceae (Table 6), especially *Ipomoea* and *Calystegia*. Some sequences identified within the Convolvulaceae could not be confidently placed within a single genus, but were distantly similar to *Ipomoea*, *Merremia*, and *Argyrea*. Although these genera are not native or naturalized in Washington, they are often planted as ornamentals. Indeed, species of *Ipomoea*, *Calystegia*, and *Merremia* were planted at the residence where the specimens were collected (Fig. 5). Results indicate that some of the psyllids collected for gut content analysis had previously fed upon these ornamental vines.

Potential Ecological Consequences of Feeding on Non-host Transitory and Shelter Hosts

Results of gut content analysis revealed an unexpectedly high rate of feeding on non-host transitory and shelter plants by all five psyllid species. These five species represent four families within the Psylloidea and represent psyllids with both univoltine and multivoltine lifecycles. Feeding on transitory and shelter plants seems obvious for univoltine psyllids such as *A. loca* and willow psyllids that spend much of the year as adults on non-host species, but the ecological significance of this behavior has largely been overlooked for dispersing multivoltine pest species such as *C. pyricola* and *B. cockerelli*. Documenting the potential role that transitory plants may have in allowing psyllid pests to survive long intervals away from host plants or to migrate over long distances is critical to understanding pest population dynamics and gene-flow among geographically isolated populations.

The published literature contains conflicting information on whether *Cacopsylla* species feed on shelter and transient plants (Hodkinson 2009). Experiments using radioactive labeling of *Pinus* did not provide evidence for winter feeding by *Cacopsylla melanoneura* (Förster) (Jackson et al. 1990), but electronic penetration graphs demonstrated that the univoltine species, *Cacopsylla pruni* (Scopoli), ingests phloem of conifers (Gallinger and Gross 2018). The published literature does not provide direct evidence for feeding on conifers by *C. pyricola*. Indirect evidence of this species feeding on shelter or transient plants is provided by the report that *C. pyricola* require a living plant to survive winter (Horton et al. 1994b), and the reports that *C. pyricola* transmits the phloem-limited bacterium, *Phytoplasma pyri*, to plant species other than *Pyrus* (Kaloostian et al. 1971, Purcell and Suslow 1984, Ullman and McLean 1988, Blomquist and Kirkpatrick 2002). Our results confirm that *C. pyricola* feed on deciduous transitory hosts and provide direct evidence that this psyllid feeds on conifer shelter plants. While we focused on behavior of winterform *C. pyricola*, summerform psyllids are also often captured outside of pear orchards (Fye 1983, Horton et al. 1995, Thinakaran, unpublished data). Dispersal behaviors of summerform psyllids have not been thoroughly examined. Gut content analysis could be used to examine feeding on transitory hosts by dispersing summerform psyllids.

Gut content analysis of *B. cockerelli* collected from *Lycium* during autumn suggested that many of the sampled psyllids had fed previously on *Lactuca serriola*, which is not a reproductive host for the psyllid. This species is a common weed that grows in a variety of habitats, including semi-arid steppe. Psyllids are strongly attracted to the color of foliage, and green *L. serriola* is abundant throughout the study region at the very time of year that psyllids move from vine-killed potatoes or dried-down annual weed hosts. These results suggest that dispersing adults commonly landed on and sampled these transitory plants. This is consistent with observations by coauthor Jensen of large populations of *B. cockerelli* occurring on rabbitbrush and bristlecone pine (*Pinus longaeva* Bailey; Pinaceae) in the Southwestern United States (Jensen, unpublished data). It is not confidently known whether psyllids acquire resources from these transitory hosts, but Knowlton (1933) reported that *B. cockerelli* adults can survive several months on certain nonreproductive plants, and the duration of adult survival was dependent upon plant species.

Horton et al. (2015b) hypothesized that perennial Solanaceae are likely required as bridge hosts for survival of *B. cockerelli* during the spring and autumn months before and after annual hosts, including potato, become available. The apparent need for bridging hosts produces somewhat of a conundrum for a genetically distinct population called the northwestern haplotype that has thus far only been collected in the Pacific Northwest (Swisher et al. 2012). There are no perennial Solanaceae or Convolvulaceae native to the inland Pacific Northwest where the northwestern haplotype has been collected (Horton et al. 2015b). This contrasts with California and Texas where the western and central haplotypes are common and where native *Lycium* spp. and *Calystegia* spp. are available when potato and other annual hosts are not (Romney 1939; Liu et al. 2006; Horton et al. 2015b, 2019b). Explanations for the existence of the Northwestern haplotype prior to the introduction of perennial Solanaceae that serve as spring and autumn bridging hosts include: 1) the Northwest haplotype colonized the Pacific Northwest from an unknown region after the introduction of perennial hosts in the late 1800s or early 1900s, or 2) the Northwest haplotype historically survived spring and autumn on a perennial host plant within the Solanaceae or Convolvulaceae that is unknown to psyllid researchers (Horton et al. 2015b). Our

finding that dispersing psyllids feed extensively on nonreproductive transitory plants, combined with collections by Jensen of *B. cockerelli* on transitory plants suggests a third possibility, namely that historical populations of *B. cockerelli* survived autumn, winter, and spring in the Pacific Northwest as adults on transitory and shelter plants until annual Solanaceae became available for oviposition and nymphal development. If this hypothesis is true, then a portion of autumn-dispersing psyllids that do not happen upon a seasonally available developmental host (*Lycium*, *S. dulcamara*, or *C. arvensis*) could survive the interval between potato harvest in autumn and emergence in spring. Validation of this hypothesis would suggest that the introduction and spread of *L. barbarum*, *S. dulcamara*, and *C. arvensis* to the Pacific Northwest likely allows a larger portion of the population to survive the bridging interval, and allows populations to increase substantially before the emergence of the potato crop.

Conclusions

We have demonstrated that direct sequencing using a PacBio platform can identify the dietary history of taxonomically distant psyllid species, and that results of gut content analysis infer the prior landscape movements of psyllids. Landscape ecology of psyllids has been studied primarily by field sampling to identify potential overwinter shelter plants or laboratory assays to identify reproductive hosts. Tools have not been available to discern landscape-level migrations of psyllids or use of transitory plant hosts during these migrations. Understanding landscape migrations and use of transitory hosts is particularly important for psyllid vectors of plant pathogens. *Cacopsylla pyricola* is a vector for ‘*Candidatus* Phytoplasma pyri’ associated with pear decline, *B. cockerelli* is a vector for ‘*Candidatus* Liberibacter solanacearum’ (‘*Candidatus* Liberibacter psyllaurosus’) associated with zebra chip disease of potato, and *D. citri* is a vector for ‘*Candidatus* Liberibacter asiaticus’ associated with Huanglongbing disease of citrus. All three of these psyllid pests spend a portion of the year on native, naturalized, or ornamental hosts or shelter plants. The movements of these psyllids between agricultural and nonagricultural plant hosts are not currently understood, and the source of psyllids carrying Phytoplasma or Liberibacter is not known. The use of gut content analysis will allow us to better understand the landscape-level movements of psyllids, to predict which fields or orchards are the most likely candidates for large influxes of psyllids from non-crop sources, and to identify non-crop plant species as likely sources of infective psyllids.

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