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Research

Directed Sequencing of Plant Specific DNA Identifies the Dietary History of Four Species of Auchenorrhyncha (Hemiptera)

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Abstract

Auchenorrhyncha (Hemiptera) includes several families of sap-feeding insects that tend to feed on a wide-range of host plants. Some species within Auchenorrhyncha are major agricultural pests that transmit plant pathogens or cause direct feeding damage. Nearly all pest Auchenorrhyncha are highly polyphagous, have mobile nymphs, and colonize crops from noncrop habitats. We examined whether methods for gut content analysis developed for more specialized Hemiptera identify dietary history of polyphagous Auchenorrhyncha. We used high-throughput sequencing of the plant genes *trnF* and ITS to examine the dietary history of *Circulifer tenellus* (Baker) (Cicadellidae), *Colladonus geminatus* (Van Duzee) (Cicadellidae), *Colladonus montanus reductus* (Van Duzee) (Cicadellidae), and *Lycorma delicatula* (White) (Fulgoridae). *C. tenellus* is a vector of the vegetable pathogens *Candidatus* Phytoplasma trifolii and beat curly top virus. Both *Colladonus* species are vectors of *Ca.* Phytoplasma pruni, the pathogen associated with X-disease of stone fruits. *Lycorma delicatula* is an invasive pest of grape and ornamentals in the eastern United States. Results showed spring hosts for *C. tenellus* included Brassicaceae, especially *Sisymbrium* sp. (tumble mustard), and spring hosts for both *Colladonus* species included *Taraxacum* sp. (dandelion). Gut content analysis also detected a decrease in host-breadth by *L. delicatula* from early to late instars. Results demonstrate that directed sequencing of plant DNA identified the dietary history of leafhopper and planthopper pests. Expanded use of gut content analysis will help identify the noncrop sources of phytoplasma-infected *C. tenellus* and *Colladonus,* and to examine seasonal changes in host shifts by *L. delicatula.*

Key words: X-disease, little cherry disease, beet leafhopper, spotted lanternfly, potato purple top

There are over 3,000 species of Auchenorrhyncha (Hemiptera), the suborder that includes leafhoppers (Cicadellidae) and planthoppers (Fulgoridae), in North America (Dietrich 2005, McKamey 2005). Some of these species are major pests of fruit and vegetable crops that damage plants by direct feeding or by transmitting plant pathogens (Nielson 1968). Many leafhoppers and planthoppers are highly polyphagous and can develop on many unrelated hosts (Novotny 1994). These insects are also highly mobile, and regularly disperse

between crop and noncrop habitats (Waloff 1973, Novotny 1994). This polyphagy and high mobility challenge efforts to manage leafhopper and planthopper pests. Noncrop hosts of agricultural pests have long been thought to contribute to pest pressure and crop damage (Bianchi et al. 2006). Documentation of such noncrop hosts could aid in the areawide management of these pests and of the plant pathogens they transmit.

Molecular gut content analysis can identify the dietary history of herbivores and infer their landscape movements prior to capture. This procedure is often performed using species-specific PCR primers (Wang et al. 2017), Sanger-based sequencing of PCR products (Cooper et al. 2016, Avanesyan and Lamp 2020, Gonella et al. 2020, Avanesyan et al. 2021a), or direct sequencing of barcoding genes (Hereward and Walter 2012, Cooper et al. 2019, Barthel et al. 2020, Hepler et al. 2021, Reyes Corral et al. 2021). Dietary plant DNA can be detected from species of Auchenorrhyncha using Sanger-based sequencing, but this method cannot detect multiple plant taxa from individual samples (Avanesyan and Lamp 2020, Gonella et al. 2020, Avanesyan et al. 2021a). High-throughput directed sequencing provides a more complete dietary record by sequencing multiple plant DNA targets (Avanesyan et al. 2021b) and has been useful for tracking landscape-scale movements of psyllids (Hemiptera: Sternorrhyncha: Psylloidea) (Cooper et al. 2019, Barthel et al. 2020, Reyes Corral et al. 2021).

The quality of data obtained and the plant signal longevity of high-throughput sequencing for gut content analysis may be influenced by differences in insect feeding behaviors, breadth of host or food plant use, and gut morphology. Since feeding behaviors vary considerably among phytophagous Hemiptera (Backus 1988), it is unknown whether the high-throughput sequencing approach developed for psyllids (Cooper et al. 2019, Barthel et al. 2020) is suitable for identifying the dietary history of Auchenorrhyncha. Here, we show that molecular gut content analysis can be used to infer movements of three leafhopper species and one species of an invasive planthopper. We used directed sequencing to identify dietary history of *Circulifer tenellus* (Baker) (Cicadellidae), *Colladonus geminatus* (Van Duzee) (Cicadellidae), *Colladonus montanus reductus* (Van Duzee) (Cicadellidae) (hereafter *Co. reductus*), and *Lycorma delicatula* (White) (Fulgoridae). *C. tenellus* (beet leafhopper) is a major pest of vegetable crops as a vector of beet curly top virus (Chen and Gilbertson 2016), *Candidatus* Phytoplasma trifolii (16SrVI; beet leafhopper transmitted viresence agent) (Munyaneza et al. 2006), and *Spiroplasma citri* (Liu et al. 1983). *Co. geminatus* and *Co. reductus* are important vectors of *Candidatus* Phytoplasma pruni (16SrIII), the pathogen that causes X-disease of stone fruits. *L. delicatula* (spotted lanternfly) recently became established in the eastern United States where it threatens commercial grape and ornamentals (Parra et al. 2017). All four Hemipteran species extensively use noncrop plants and disperse into crops from noncrop sources. The methods for gut content analysis described in this report will help identify (1) from which plants vectors acquire phytoplasmas and (2) the noncrop food sources of insects that colonize crops.

Materials and Methods

Specimen Collection and Sample Processing

C. tenellus were captured in 3D-printed traps hung at ground level from Shepherds hooks (Horton et al. 2019, Wentz et al. 2020). These traps were chosen because they collect the insects in preservative, allowing for subsequent DNA analysis. The collection vial of each trap was filled with 10 ml of antifreeze (ethylene glycol) to preserve DNA of the captured insects. The traps were placed in uncultivated habitats at the USDA farm near Moxee, WA from 21-May to 5-June 2020. Several host plants were abundant at that time, including senescing *Sisymbrium altissimum* L. (Brassicaceae), and *Bassia scoparia* (L.) and *Kali tragus* (L.) (Amaranthaceae) seedlings. Traps were checked weekly to remove *C. tenellus*. *Co. geminatus* and *Co. reductus* were collected in a commercial cherry orchard near Wapato, WA on 22 May 2020 from herbaceous ground cover between orchard rows using a sweep net. *L. delicatula* were collected on 17 July, 12 August, and 31 October 2019 in an abandoned vineyard near Boyertown, PA and on 19 June 2019 from unmanaged woody habitats in Winchester, VA. The insects were collected randomly by hand from the grapevines and other hosts and placed directly into a 37 mL glass vial with 5 mL of 100% ethanol. All specimens were then stored at –80°C.

Because of their small size, whole leafhopper bodies were surface sterilized prior to DNA extraction (Cooper et al. 2016). Attempts were made to dissect the alimentary canal from *L. delicatula*, but prolonged storage in 100% ethanol led to dehydration and bleaching of internal organs making dissections difficult. To limit the amount of insect DNA included in gut content analysis of *L. delicatula*, we removed the legs, wings, and head/prothorax, and used the abdomen for gut content analysis as described previously for gut content analysis of predacious insects (Krey et al. 2020). DNA was extracted from each specimen using a Qiagen DNeasy blood and tissue kit® (Qiagen).

Detection of Phytoplasma

A nested PCR procedure was used to test for *Ca. Phytoplasma trifolii* in *C. tenellus* and *Ca.* Phytoplasma pruni in *Co. reductus* and *Co. geminatus*. *Ca.* Phytoplasma trifolii was amplified using primers P1 and P7 followed with species-specific primers FU5 and BLTVA-int (Table 1) (Deng and Hiruki 1991, Smart et al. 1996). *Ca. Phytoplasma pruni* was amplified using P1 and P7 followed with primers R16(III)F2 and R16(III)R1 (Table 1) (Deng and Hiruki 1991, Lee et al. 1994). For both assays, each initial reaction contained 1 µl of template DNA, Amplitaq Gold 360 master mix (Thermo Fisher), and 250 nM of each primer, with a nested step including 1:20 dilution of PCR amplicon generated from the initial step. PCR controls included DNA from a phytoplasma-infected leafhopper, no-template control, and single-primer controls. The presence or absence of PCR amplicons was observed on 1% agarose gels stained with ethidium bromide.

Molecular Gut Content Analysis

The dietary history of insects was inferred by analysis of plant-derived internal transcribed spacer (ITS) and the chloroplast *trnF* gene (Cooper et al. 2016, Cooper et al. 2019). ITS primers were ITS2F and ITS3R (Chen et al. 2010), and primers for *trnF* were B49873-e and A50272-F (Taberlet et al. 1991) (Table 1). Barcoded primers were used to label individual samples (Pacific Biosciences. 2014). Each 50 µl reaction contained Invitrogen Amplitaq Gold 360 PCR Master Mix (Invitrogen, Carlsbad, CA), 250 nM of each primer, and 5 µl of DNA template. Amplicons (400–600 bp) were observed on 1% agarose gels stained with ethidium bromide.

PCR products from each sample were combined into a single vial, with a pooled volume determined based on the brightness of the PCR amplicons on ethidium bromide-stained agarose gels. Ten microliters of PCR product were transferred if the amplicon was clearly visible on the gel, 20 µl if there was a faint band, and 30 µl if there were no visible amplicons. The pooled samples were

Table 1. PCR primers and conditions used for experiments

Gene target	PCR Primer name	Primer sequence (5' to 3')	PCR conditions
16S of Phytoplasma[1]	P1	AAG AGT TTG ATC CTG GCT CAG GAT T	35 cycles of 94C for 30 s, 50C for 30 s, and 72C for 90 s
	P7	CGT CCT TCA TCG GCT CTT	
16S of *Phytoplasma trifolii*[2]	FU5	CGG CAA TGG AGG AAA CT	35 cycles of 94C for 30 s, 50C for 30 s, and 72C for 90 s
	BLTVAint	GAT GAT TTT AGT ATA TAT AGT CC	
16S of *Phytoplasma pruni*[3]	R16(III) F2	AAG AGT GGA AAA ACT CCC	34 cycles of 94 for 30 s, 50 C for 30 s, and 72C for 60 s
	R16(III) R1	TCC GAA CTG AGA TTGA	
trnF chloroplast gene[4]	B49873-e	GGT TCA AGT CCC TCT ATC CC	39 cycles of 94°C for 30 s, 56°C and 72°C for 45 s
	A50272-f	ATT TGA ACT GGT GAC ACG AG	
ITS gene of plants[4]	ITS2F	ATG CGA TAC TTG GTG TGA AT	34 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 45 s
	ITS3R	GAC GCT TCT CCA GAC TAC AAT	

[1]Primers were used in the initial step of nested PCR targeting Phytoplasma pruni and Phytoplasma trifolii

[2]Primers were used in the second step of nested PCR targeting Phytoplasma trifolii.

[3]Primers were used in the second step of nested PCR targeting Phytoplasma pruni.

[4]Universal primers used to detect plant DNA from insects.

then processed and sequenced at the Washington State University Laboratory for Biotechnology and Bioanalysis. Amplicons were end-repaired, ligated to the hairpin SMRT bell adapters using the Express Template kit v 2.0 and the library purified using AMPureXP beads (Beckman-Coulter). Libraries were quantified and bound to polymerase using Sequel Binding kit 3.0, loaded into 1M v3 SMRT cells, and observed for 10 h on a PacBio Sequel (Pacific Biosciences). Raw movies were processed into reads, barcodes demultiplexed and high-quality circular consensus sequences called using SMRT Link version 6.0. Average subread coverage on the ccs reads was 95x. Reads were filtered to minimum inferred quality of 0.9999 (phred 40).

Sequences were filtered to exclude those less than 400 bp or more than 700 bp to eliminate sequence fragments or chimeric products. The remaining sequences were grouped in operational taxonomic units (OTUs) using the de novo assembly tool from Geneious R10 (Biomatters, Auckland, New Zealand) with custom sensitivity settings with minimum overlap identity of 95%, 1% maximum gaps per read, and 5% maximum mismatches per read. OTUs with 5 or fewer sequences were excluded from analyses to reduce low quality reads and assemblies resulting from contamination or mis-binning of sequence barcodes. Plant taxa were identified by analyzing OTUs using the Basic Local Alignment Search Tool (BLAST) function of the National Center for Biotechnology Information (NCBI) integrated with Geneious R10 software (Altschul et al. 1990).

The number of plant genera identified was compared between infected and uninfected *C. tenellus* and between *Co. geminatus* and *Co. reductus* adults using PROC GLMMIX of SAS 9.4 (SAS Institute, Cary, NC) with a negative binomial distribution (DIST=NB option of the MODEL statement). Plant taxa were not compared among *L. delicatula* life stages because they were captured in different locations.

Results

Detection of Phytoplasma

Ca. Phytoplasma trifolii was detected in 5 out of 45 (11%) *C. tenellus* that were captured. All five phytoplasma-infected leafhopper and five arbitrarily selected uninfected leafhoppers were included in gut content analysis to ensure that both infected and noninfected leafhoppers were included. *Ca.* Phytoplasma pruni was not detected in any of the *Co. reductus* ($n = 5$) or *Co. geminatus* ($n = 5$) specimens using nested PCR.

Detection of Plant DNA from *C. tenellus*

Plants from eight families were detected from *C. tenellus* adults, including three probable hosts (Table 2). *Sisymbruim loeseli* L. (Brassicales: Brassicaceae) (London Rocket or tumble mustard), was identified from 8 out 10 *C. tenellus* adults, including 4 that were infected with *Ca.* Phytoplasma trifolii. The *trnF* product was only detected in 3 of these adults, but ITS was detected in all 8. This plant species was abundant during the trapping period between late May and mid-June and was nearing the end of its lifecycle. *Descurainia sophia* (L.) (Brassicales: Brassicaceae) (flixweed) and an unidentified *Brassica* species were detected from a single *C. tenellus* adult, which was not infected with *Ca.* Phytoplasma trifolii (Table 2). We were unable to detect a host plant from the remaining *C. tenellus* adult, which was infected with phytoplasma (Table 2). Three adults had fed previously on potato, which was planted in experimental plots near the collection site.

Detection of Plant DNA from *Colladonus* spp.

Plants from twelve families were detected from *Co. geminatus* adults (Table 3), and seven families from *Co. reductus* adults (Table 4). *TrnF* and ITS products of *Taraxacum* spp. were detected from 4 out of 5 *Co. geminatus* and from all *Co. reductus*. Two widespread and common species of *Taraxacum* were identified, *Taraxacum officinale* Weber (Asterales: Asteraceae) (common dandelion) and *Taraxacum erythrospermum* Andrzejowski ex Besser (red-seeded dandelion). Both species were detected from eight of the nine specimens containing *Taraxacum*. Dandelion was abundant in the orchard from which *Co. geminatus* and *Co. reductus* were collected. *TrnF* and ITS sequences identified as Amaranthaceae were detected from the single *Co. geminatus* adult from which *Taraxacum* was not detected. Other potential hosts detected from *Colladonus* included *Malva* (common mallow), *Trifolium* (clover), and *Stellaria* (chickweed). *Prunus* was not detected in *Co. reductus* but was detected from 3 out of 5 *Co. geminatus*. *Colladonus geminatus* fed upon significantly more plant taxa (5.2 ± 1.03 plant genera) compared with *Co. reductus* (2.1 ± 0.64 plant genera) ($F = 6.7$; d.f. = 1, 8; $P = 0.032$).

Table 2. Plant genera and families identified from Phytoplasma-infected and uninfected *Circulifer tenellus* by sequencing the plant genes, trnF, and ITS

	Phytoplasma-infected					Phytoplasma-uninfected				
Plant Taxa	29-May	29-May	5-June	5-June	5-June	29-May	5-June	5-June	12-June	12-June
Probable host plants										
Brassica (Brassicaceae)	–	–	–	–	–	–	–	–	+	–
Descurainia (Brassicaceae)	–	–	–	–	–	+	–	–	+ +	–
Sisymbrium (Brassicaceae)	–	+	+	+	+ +	+ +	+	+ +	–	+
Food plants										
Lactuca (Asteraceae)	–	–	+	–	–	–	–	–	–	–
Cucumis (Cucurbitaceae)	–	–	–	+	–	–	–	–	–	–
Iteadophne (Lauraceae)	–	+	–	–	+	–	–	–	–	–
Abies (Pinaceae)	–	–	–	–	–	+	–	–	–	–
Pinus (Pinaceae)	–	+	–	+	+	–	–	–	–	–
Poa (Poaceae)	+	–	–	–	–	–	–	–	–	–
Acer (Sapindaceae)	–	+	+	–	–	–	–	–	–	–
Solanum tuberosum (Solanaceae)	+	–	–	–	–	+	–	+	–	–
No. of plant taxa	2	4	3	3	3	4	1	2	2	1

[1]+ indicates that plant ITS or TrnF sequences were detected, while + + indicates that both products were detected.
[2]OTUs that were represented by fewer than 5 sequence reads were excluded.

Table 3. Plants genera and families identified from *Colladonus geminatus* by sequencing the plant genes, trnF, and ITS

Plant taxa	Adult 1	Adult 2	Adult 3	Adult 4	Adult 5
Oxybasis (Amaranthaceae)	–	–	+	–	+
Chenopodium (Amaranthaceae)	–	–	+	–	+
Taraxacum (Asteraceae)	+ +	+ +	–	+ +	+ +
Cirsium (Asteraceae)		+ +		+	+
Capsella bursa-pastoris (Brassicaceae)	–	–	–	–	+
Stellaria (Caryophyllaceae)	–	–	–	+ +	+ +
Trifolium (Fabaceae)	+	–	+	+	–
Malva (Malvaceae)	–	+	+ +	–	
Abies (Pinaceae)	–	–	–	–	+
Plantago (Plantaginaceae)	–	–	–	–	8
Polygonum (Polygonaceae)	–	+ +	–	–	–
Prunus (Rosaceae)	–	+	–	+ +	+
Salix (Saliceae)	–	–	+	–	–
Acer (Sapindaceae)	–	–	+	–	–
No. plant taxa	2	5	6	5	9

[1]+ indicates that plant ITS or TrnF sequences were detected, while + + indicates that both products were detected.
[2]OTUs that were represented by fewer than 5 sequence reads were excluded.

Detection of Plant DNA from *L. delicatula*

A total of seventeen plant families were detected from early instar (1st–3rd) *L. delicatula* nymphs collected near Winchester, VA (Table 5) with an average of 2.5 ± 0.56 plant genera per individual. Seven plant families were detected from 3rd and 4th instar *L. delicatula* nymphs from Pennsylvania, with an average of 1.9 ± 0.41 genera per individual insect (Table 6). *Vitis* was detected in all 11 late instars and was the only plant detected in 7 of 11 late instars (Table 6). Ten plant families were detected from adults captured in August of 2019 (Table 7) and 11 families were detected from adults captured in October (Table 8). *Vitis* was detected in 7 out of 8 adults collected in August (Table 7), but just 2 out of 9 adults collected in October (Table 8).

Discussion

Predicting insect life history, landscape ecology, and regional population dynamics to improve pest management relies on the availability of accurate information about the breadth of the insect's use of plant hosts. Identifying host plants of insects often involves intensive sampling of plants to detect presence and frequency of multiple life stages. Host plants—those that support nymphal development—can be relatively easy to identify for species with immobile or less mobile nymphs with narrow host ranges, such as the Sternorrhyncha. In contrast, species within the Auchenorrhyncha typically have highly mobile nymphs that feed upon many unrelated plants. These traits challenge efforts to identify their breadth of plant use or identify hosts that may be critical for development.

Gut content analysis by high-throughput sequencing of plant barcoding genes has been used to infer dietary history and landscape-level movements of psyllids (Cooper et al. 2019, Barthel et al. 2020, Reyes Corral et al. 2021). Although studies have shown that plant DNA can be amplified and sequenced from species of Auchenorrhyncha (Avanesyan and Lamp 2020, Gonella et al. 2020, Avanesyan et al. 2021a), a method using high-throughput sequencing

Table 4. Plant genera and families identified from *Colladonus reductus* by sequencing the plant genes, trnF, and ITS

Plant taxa	Adult 1	Adult 2	Adult 3	Adult 4	Adult 5
Taraxacum (Asteraceae)	+ +	+ +	+ +	+ +	+
Senecio (Asteraceae)	+	–	–	–	–
Betula (Betulaceae)		+	–	–	–
Capsella bursa-pastoris (Brassicaceae)	–	–	–	+	–
Stellaria (Caryophyllaceae)	–	–	–	+ +	–
Trifolium (Fabaceae)	–	–	+	–	+
Medicago (Fabaceae)	–	–	+	–	–
Fraxinus (Oleaceae)	–	+	–	–	–
Lolium (Poaceae)	+	–	–	–	–
No. plant taxa	3	2	2	2	1

[1]+ indicates that plant ITS or TrnF sequences were detected, while + + indicates that both products were detected.
[2]OTUs that were represented by fewer than 5 sequence reads were excluded.

Table 5. Plant genera and families detected from early instar *Lycorma delicatula* collected near Kearneysville, WV by sequencing the plant genes, trnF, and ITS

Plant Taxa	1st	1st	1st	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd
Arctium (Asteraceae)	–	–	–	+ +	–	+ +	–	–	+ +	–	–	–	–	+ +
Lonicera (Caprifoliaceae)	–	–	–	–	–	–	+	–	–	–	–	–	–	–
Convolvulus (Convolvulaceae)	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Medicago (Fabaceae)	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Robinia (Fabaceae)	–	+	–	–	–	–	–	–	–	–	–	–	+	–
Phaseolus (Fabaceae)	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Trifolium (Fabaceae)	–	+	–	–	+	–	–	–	–	–	–	–	–	–
Juglans (Juglandaceae)	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Nepeta (Lamiaceae)	–	–	–	+	–	–	–	–	–	–	–	–	–	–
Oenothera (Onagraceae)	–	–	–	–	–	–	+	–	–	–	–	–	–	–
Fraxinus (Oleaceae)	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Phytolacca (Phytolaccaceae)	–	–	–	–	–	–	–	–	–	–	–	+	–	+
Plantago (Plantaginaceae)	–	–	–	–	–	–	–	–	–	+ +	–	–	–	–
Triticum (Poaceae)	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Festuca (Poaceae)	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Fallopia (Polygonaceae)	–	–	+	–	–	+	–	–	–	–	–	–	–	–
Populus (Salicaceae)	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Salix (Salicaceae)	–	+	–	–	+	–	–	–	–	–	–	–	–	–
Ailanthus (Simaroubaceae)	–	–	–	–	–	+	–	–	+	–	–	–	–	–
Hyoscyamus (Solanaceae)	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Lycium (Solanaceae)	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Solanum (Solanaceae)	+	–	–	–	–	–	–	–	–	–	–	–	+	+ +
Zelkova (Ulmaceae)	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Vitis (Vitaceae)	+	+	+	–	+	+	–	+	–	–	–	–	+	–
No. plant taxa	2	4	6	2	5	4	2	1	2	1	1	2	6	3

[1]+ indicates that plant ITS or TrnF sequences were detected, while + + indicates that both products were detected.
[2]OTUs that were represented by fewer than 5 sequence reads were excluded.

is required to yield data that allows for an examination of life history traits. We demonstrate that gut content analysis by high-throughput sequencing of plant barcoding genes provides valuable insight on the dietary history, landscape-level movements, and life history traits of leafhopper and planthopper pests, despite the high mobility and breadth of host plant use by these insects.

Gut Content Analysis to Infer the Nymphal Hosts of *C. tenellus*

C. tenellus (beet leafhopper) is a major pest of vegetable crops as a vector of *Ca.* Phytoplamsa trifolii (beet leafhopper transmitted vitrescence agent, BLTVA), (Munyaneza et al. 2006), beet curly top virus, and *Spiroplasma citri* (Liu et al. 1983). All three pathogens are acquired and transmitted by *C. tenellus* when they feed on the phloem of susceptible plants. *C. tenellus* develops on Brassicaceae (Brassicales) and Amaranathaceae (Caryophyllales) (Carter 1930, Romney 1939). This insect is native to southern Europe and likely spread throughout the western United States following the introduction of invasive Mediterranean host plants (Horton et al. 2018). *C. tenellus* consistently has been a major pest of beet and radish in the western United States since the early 1900s as the vector of beet curly top virus (Carsner and Stahl 1924, Carter 1930, Horton et al. 2018). More recently, *C. tenellus* has become a major pest of potato (Solanaceae) as vector of *Ca.* Phytoplasma trifolii, which causes potato purple top disease (Crosslin et al. 2005, Munyaneza et al. 2006). Potato is not a host plant for *C. tenellus*, but this insect will feed on potato and transmit phytoplasma as it disperses among plant hosts

Table 6. Plant genera and families detected from late instar *Lycorma delicatula* by sequencing the plant genes, trnF, and ITS

Plant taxa	3rd	3rd	4th	4th	4th	4th	4th	4th	4th	4th	4th
Stellaria (Caryophyllaceae)	–	–	–	–	–	–	–	–	–	–	+ +
Ipomoea (Convolvulaceae)	+	–	–	–	–	–	–	–	–	–	–
Lycium (Solanaceae)	–	–	–	–	–	+	–	–	–	–	–
Pinus (Pinaceae)	–	–	–	–	–	–	+	–	–	–	+
Cenchrus (Poaceae)	+	–	–	–	–	–	–	–	–	–	–
Festuca (Poaceae)	–	–	–	–	–	+	–	–	–	–	–
Poa (Poaceae)	+	–	–	–	–	+	–	–	–	–	–
Prunus (Rosaceae)	–	–	–	–	–	–	+	–	–	–	–
Vitis (Vitaceae)	+	+	+ +	+	+ +	+	+	+	+	+	+
No. plant taxa	4	1	1	1	1	4	3	1	1	1	3

[1]+ indicates that plant ITS or TrnF sequences were detected, while + + indicates that both products were detected.
[2]OTUs that were represented by fewer than 5 sequence reads were excluded.

Table 7. Plant genera and families detected from adults *Lycorma delicatula* by sequencing the plant genes, trnF, and ITS

Plant taxa	Adult 1	Adult 2	Adult 3	Adult 4	Adult 5	Adult 6	Adult 7	Adult 8
Ageratina (Asteraceae)	–	–	–	–	–	+	–	–
Helianthus (Asteraceae)	–	–	–	+	–	–	–	–
Lactuca (Asteraceae)	–	–	–	–	–	+	–	–
Crassula (Crassulaceae)	–	+	–	–	–	–	–	–
Trifolium (Fabaceae)	–	–	–	–	+ +	+ +	–	+
Juglans (Juglandaceae)	–	+	–	–	–	–	–	–
Musa (Musaceae)	–	–	–	–	–	+	–	–
Plantago (Plantaginaceae)	–	–	–	–	–	–	+	–
Sporobolus (Poaceae)	–	+	–	–	–	–	–	–
Ailanthus (Simaroubaceae)	–	–	–	–	–	–	–	+
Lycium (Solanaceae)	–	–	+	–	–	–	–	–
Vitis (Vitaceae)	+	+	+	+	+	+	–	+
No. plant taxa	1	4	2	2	2	5	1	3

[1]+ indicates that plant ITS or TrnF sequences were detected, while + + indicates that both products were detected.
[2]OTUs that were represented by fewer than 5 sequence reads were excluded.

Table 8. Plant genera and families detected from adults *Lycorma delicatula* by sequencing the plant genes, trnF, and ITS

Plant taxa	Adult 1	Adult 2	Adult 3	Adult 4	Adult 5	Adult 6	Adult 7	Adult 8	Adult 9
Ambrosia (Asteraceae)	–	–	–	–	–	+	–	–	–
Daucus (Apiaceae)	–	–	–	+	–	–	–	–	–
Coriandrum (Apiaceae)	–	–	–	+	–	–	–	–	–
Lampranthus (Aizoaceae)	+	–	+	–	+	+	–	–	–
Humulus (Canabaceae)	–	–	–	–	–	–	+	–	–
Medicago (Fabaceae)	–	–	–	–	–	+	–	–	–
Trifolium (Fabaceae)	–	–	–	–	–	+	+	–	–
Juglans (Juglandaceae)	+	–	–	–	–	–	–	–	–
Liriodendron (Magnoliaaceae)	–	–	–	+	–	–	–	–	–
Morus (Moraceae)	–	–	–	–	–	–	–	+	
Ailanthus (Simaroubaceae)	–	–	–	–	–	–	–	–	+
Lycium (Solanaceae)	–	–	–	–	+	–	–	–	–
Solanum (Solanaceae)	–	–	–	+	+	–	–	+	+
Vitis (Vitaceae)	–	+	–	–	–	–	–	+	–
No. plant taxa	2	1	1	4	3	4	2	3	2

[1]+ indicates that plant ITS or TrnF sequences were detected, while + + indicates that both products were detected.
[2]OTUs that were represented by fewer than 5 sequence reads were excluded.

(Horton et al. 2018). There are no direct methods to control beet leafhopper-transmitted plant pathogens, resulting in prophylactic applications of insecticides to suppress populations of the vector (Strausbaugh et al. 2012, Rondon and Oppedisano 2020).

Our current understanding of *C. tenellus* ecology in the Pacific Northwest of the United States relies primarily from literature from the early to mid-1900s (Hills 1937). *C. tenellus* overwinter as adults on autumn-germinating non-native annual plants within the

Brassicaceae, especially species of *Sisymbrium*, *Lepidium*, *Descurainia*, and *Erodium*. Overwintered females begin laying eggs on these plants in spring. Nymphs from this first generation reach adulthood at about the same time that these spring hosts begin senescing, causing the leafhoppers to disperse in search for summer hosts, which may include Russian thistle (*K. tragus*; Caryophyllales: Amaranthaceae) and kochia (*Bassia scoparia*; Caryophyllales: Amaranthaceae). While dispersing between spring and summer hosts, *C. tenellus* adults will feed upon a variety of nonhost species, including potato. It is during these movements that *C. tenellus* transmits *Ca.* Phytoplasma trifolii to potato and other hosts. Adult *C. tenellus* with phytoplasma infection rates of 10–30% typically can be collected season-long from weed habitats adjacent to potato fields in Washington (Munyaneza et al. 2008, Munyaneza et al. 2009, Murphy et al. 2012). As summer hosts senesce, adults return to autumn germinating Brassicaceae for overwintering. While the research conducted in the early 1900s (Hills 1937) documented seasonal dispersal patterns by *C. tenellus* among noncrop hosts, we do not know what role these noncrop hosts serve in epidemiology of *C. tenellus*-transmitted plant pathogens, especially *Ca.* Phytoplasma trifolii. Furthermore, it is not clear whether changes in plant communities, landscape of the Columbia Basin, and agronomic practices since the early 1900's has altered patterns in plant use and dispersal by *C. tenellus* described by Hills (1937).

We identified *Sisymbrium* sp. from 80% the *C. tenellus* adults in our study. Mature *S. altissimum* (tumble mustard) was abundant at the time of *C. tenellus* trapping, and this plant species was identified previously as an important spring host of *C. tenellus* in this area (Hills 1937). *Descurainia* sp., likely flixweed (*Descurainia sophia*) and an unidentified Brassicaceae were identified from a single specimen. Putative summer host plants within the Amaranthaceae were not identified from any of the *C. tenellus* adults, despite young *Bassia* and *Kali* plants being present in the trapping area. Only one host plant was identified from most individual leafhoppers—either *Sisymbrium* or *Descurainia*—which may have been the nymphal hosts of captured leafhoppers. In addition to the host plants within the Brassicaceae, sequences belonging to plant genera that are not known to support development of *C. tenellus* also were identified. The sequences likely represent nonhost plants that were sampled by adults after leaving the senescing hosts. Potato was planted in experimental plots within 0.5 km of the trapping site and was identified from three leafhopper specimens, including one that was infected with *Ca.* Phytoplasma trifolii.

The detection of *S. altissimum* and other annual plants that were present at the time of *C. tenellus* sampling demonstrates that gut content analysis identifies dietary history of this important insect vector. Many species of Brassicaceae are hosts for *Ca.* Phytoplasma trifolii (Golino et al. 1987, 1989), perhaps including *S. altissimum*. It seems possible that infected *C. tenellus* in our study completed development on phytoplasma-infected *S. altissimum* plants and acquired the pathogen as nymphs. More extensive use of gut content analysis of *C. tenellus* is needed to identify which plants are likely sources of Phytoplasma-infected *C. tenellus,* and to document the changes in host use by *C. tenellus* among spring, summer, and autumn populations. Doing so could allow researchers to predict when leafhoppers are likely to disperse into potato and other crops based upon when these noncrop hosts mature and senesce. Similar prediction models are being developed for *C. tenellus* in the southwestern United States (Lehnhoff and Creamer 2020).

Gut Content Analysis to Infer Nymphal Hosts of *Colladonus* spp.

Co. geminatus and *Co. reductus* are vectors of *Ca.* Phytoplasma pruni, the pathogen that causes X-disease of cherry and peach (Wolfe et al. 1950, 1951a,b, Wolfe et al. 1951, Harper et al. 2020, Prengaman 2020). Both leafhopper species are native to the western USA. Several other leafhopper species are known to transmit this pathogen, but *Co. geminatus* and *Co. reductus* appear to be the two most important vectors of *Ca.* Phytoplasma pruni in the Pacific Northwest where X-disease has recently re-emerged as a serious threat to the stone fruit industry (Jensen 1969, DuPont 2020, Harper et al. 2020). Both species can develop on a wide range of host plants within at least 14 families including Asteraceae, Apiaceae, Boraginaceae, Caryophyllaceae, Caprifoliaceae, Chenopodiaceae, Euphorbiaceae, Fabaceae, Geranicaceae, Onagraceae, Polygonaceae, Rosaceae, Solanaceae, and Umbelliferae (Severin and Frazier 1945, Severin and Klostermeyer 1950, Jensen 1953, Nielsen 1957). Previous research on *Co. geminatus* and *Co. montanus/reductus* was conducted primarily in California, so little is known about the biology of these vectors in the Pacific Northwest, including what noncrop hosts of the vectors contribute to spread of *Ca.* Phytoplasma pruni. Cherry and peach growers in this region therefore rely primarily on regular use of insecticides to reduce vector populations and culling of infected trees or entire orchards (DuPont 2020, Harper et al. 2020).

It is still unknown which noncrop host plants are sources of *Ca.* Phytoplasma-infected *Colladonus* vectors in the Pacific Northwest. Herbaceous host plants certainly have a role in X-disease epidemiology (Jensen 1970), and recent anecdotal observations suggest that dandelions (*T. officinale* or *T. erythrospermum*; Asterales: Asteraceae) may be important hosts of *Colladonus* in Washington (Harper et al. 2020). Dandelion was detected in 4 of 5 *Co. geminatus* and in 5 of 5 *Co. reductus*, suggesting that this plant was a source of *Colladonus* leafhoppers captured in the sampled orchard near Wapato, WA. Other ubiquitous broadleaf herbaceous plant species that support development of *Colladonus* also were detected, including common mallow (*Malva neglecta* Wallr.) and clover (*Trifolium* sp). Many of these herbaceous plants are potential reservoirs for *Ca.* Phytoplasma pruni (Jensen 1970). Although we cannot draw final conclusions based on our small sample size, these results do provide support for recent recommendations that growers control broadleaf weeds in orchard rows to suppress populations of *Colladonus* leafhoppers (DuPont 2020, Harper et al. 2020). The *Colladonus* specimens included in our study were collected in spring following development of the first generation and were not infected with phytoplasma. Research conducted in California showed that phytoplasma infection rates are relatively low (<5%) in the first generation of adults and increase to nearly 25% by late summer (Suslow and Purcell 1982). More extensive use of gut content analysis involving multiple seasonal generations could help identify noncrop hosts of phytoplasma-infected *Colladonus* and help determine whether *Colladonus* are colonizing orchards primarily from orchard edges or from broadleaf plants occurring within the orchard. This information is crucial for developing practical and effective recommendations for management of X-disease.

Gut Content Analysis of *L. delicatula*

L. delicatula, the spotted lanternfly, was detected in 2014 in the eastern United States where it has become established as a pest of grape and ornamental plants (Barringer et al. 2015). This insect is not known to transmit plant pathogens of grape or tree fruits (Urban 2020), but instead has caused damage by depleting host plants of phloem. This insect completes one generation a year. The nymphs are highly mobile and feed on over 100 plant taxa from at least 33 families in 17 orders, including temperate tree fruits and ornamental or forest trees (Uyi et al. 2021, Barringer and Ciafre 2020). Research in Asia suggests that the host preference of *L. delicatula* changes during

development, with a broad host range during early instars and a relatively narrow host range as they mature (Kim et al. 2011). Late instars prefer to feed on the highly invasive tree, *Ailanthus altissima* Mill (Simaroubaceae) (Kim et al. 2011, Uyi et al. 2020), which produces quassinoid defense compounds (Ohmoto et al. 1981) that *L. delicatula* may sequester making them unpalatable to birds (Song et al. 2018). Current management of *L. delicatula* is based primarily on removal of *A. altissima* (Parra et al. 2017). *A. altissima* was detected in only 9.5% of the *L. delicatula* supporting recent research suggesting that this plant is not required for development of *L. delicatula* and that additional tactics may be needed for large-scale control of *L. delicatula* in the U.S (Uyi et al. 2020).

Plants identified from gut content analysis represented taxa that are abundant in the mid-Atlantic region of the USA. Consistent with research from Asia, early instar nymphs tended to feed on a larger diversity of taxa (2.9 plant taxa per individual) than did later instars (1.9 plant taxa per individual). We detected multiple plant genera from 79% of early instars and 75% of adults but from just 36% of late instars. Grape was the most abundant taxa detected from *L. delicatula*. It was detected in most early instars and in all late instars and adults that were captured in August. Grape was detected from just 2 out of 9 adults collected in October, possibly suggesting a change in plant use as perennial hosts enter winter dormancy. This also may suggest short-term degradation of the plant material within the gut of *L. delicatula* or low persistence of plant DNA through insect molts. Since *L. delicatula* is a long-lived univoltine insect, it is important to determine how long plant DNA remains detectable after host switches, similar to work on brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) (Hepler et al. 2021).

Currently, there is limited information on which wild and cultivated host plants are required by *L. delicatula* to complete development, produce viable eggs, and maintain fitness (Uyi et al. 2020 and 2021, Nixon et al. in press), or the consistency with which the same plant taxa are fed on by *L. delicatula* in its invaded and native range. Moreover, determining plant taxa heavily fed on by *L. delicatula* may help determine plant traits that make an acceptable host, furthering insight into the feeding behavior and potential risk to new host plants as this invasive continues to expand its range. Since *L. delicatula* has emerged as a significant edge pest within vineyards (Leach and Leach 2020), additional information could be used to understand dependence on noncrop hosts and timing of movement into vineyards to improve pest management tactics. Gut content analysis will provide a useful tool to identify noncrop sources of *L. delicatula*, and to examine changes in dietary breadth that occur during development of this insect.

Conclusions

We showed that directed sequencing of plant barcoding genes identifies dietary history of leafhoppers and planthoppers. While success of high-throughput gut content analysis of leafhoppers and planthoppers was expected, it was unclear whether differences in feeding behaviors and breadth, longevity, and frequency of host plant use among insect species alters the success of gut content analysis or the quality of data collected. Using gut content analysis, we detected relatively strong signals for tumble mustard in *C. tenellus*, and dandelion in *Co. geminatus* and *Co. reductus*, and believe these consistent signals identify developmental hosts of these insects. These host plants were abundant at the time of leafhopper samplings. Gut content analysis also detected shifts in host plant use by *L. delicatula* that were documented by previous investigators. Overall, our study demonstrates the usefulness of gut content analysis for identifying the dietary history of pest leafhoppers and planthoppers. The use of gut content analysis will allow us to identify the landscape-level movements and temporal or special variations in host plant use by insects, and to identify noncrop sources of leafhopper vectors of phytoplasmas.

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