SEASONAL DYNAMICS OF AN URBAN LEAFHOPPER COMMUNITY IN METCHOSIN, BRITISH COLUMBIA: INVESTIGATING DIVERSITY TRENDS, PARASITOID (HYMENOPTERA: DRYINIDAE) RELATIONSHIPS, AND THE POTENTIAL FOR A DEFENSIVE SYMBIOSIS

by

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Abstract

Descriptions of leafhopper (Auchenorrhyncha: Cicadellidae) communities in non-agricultural urban environments are lacking, despite leafhoppers being diverse and abundant members of cosmopolitan trophic webs. As abundant herbivores, leafhoppers have the potential to form associations with defensive endosymbionts that mediate trophic interactions with their natural enemies. This study provides the first description of leafhopper diversity and their associations with dryinid parasitoids (Hymenoptera: Dryinidae) in Metchosin, British Columbia. Using morphospecies sorting and barcoding of mitochondrial gene cytochrome c oxidase I, 23 taxa were shown to form a community dominated by generalist leafhoppers *Dikraneura mali* and *Ribautiana debilis* (Typhlocybinae). Seasonal trends, including periods of highest abundance, species richness, and parasitism, were largely consistent over two years of collection, peaking in late September, late June, and August/September, respectively. The largest difference between years was found in leafhopper abundance and rates of occurrence of dryinid parasitoids. Parasitism rates suggest dryinids may be an important natural enemy of *R. debilis* in this community and a previously unknown association was revealed between hosts *R. debilis* and *D. mali* and their unidentified dryinid parasitoid. Examining the 16S rRNA amplicon libraries of *D. mali* and *R. debilis* revealed a *Rickettsia bellii*-like endosymbiont with 100% infection frequency in a subset of *D. mali* and a *Wolbachia* endosymbiont in *R. debilis*. Trophic interactions of leafhoppers, dryinids, and symbionts in this community deserve further investigation.

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This thesis is dedicated to my mom, Hope Hickli.

Introduction

Leafhopper ecology and life history

Leafhoppers (Auchenorrhyncha: Cicadellidae) are phytophagous insects that contribute significantly to trophic webs, forming an important food source for invertebrates and vertebrates alike, from hymenopterans to bats (Vulinec *et al.*, 2017; Ardanuy Gabarra, 2017). They are among the largest of insect families, with over 22,000 species described worldwide, around 1,800 of which are found in Canada (Maw *et al.*, 2000; Forero, 2008).

Leafhoppers are abundant and diverse members of nearly all terrestrial ecosystems, however, because of close associations with important agricultural crops (including maize, tobacco, cereals, and grapes), leafhoppers are often studied in the context of agriculture (Saguez *et al.*, 2014; Moya-Raygoza, 2020). Leafhoppers are prominent in agricultural systems both as herbivores and vectors of plant pathogens, making them economically important pests (Olivier *et al.*, 2009, 2014, 2012). Leafhopper-transmitted plant pathogens (e.g. *Xylella fastidiosa*, causative agent of Pierce's disease transmitted by *Graphocephala atropunctata*) can devastate agricultural crop yields (Redak *et al.*, 2004). Research on leafhopper abundance and diversity has focused heavily on the generalist species that exploit agricultural systems (Moya-Raygoza, 2020). Little is known about the distribution, diversity, and abundance of leafhoppers in other anthropogenic environments.

Leafhoppers depend upon host plants for food and habitat. In wild systems, leafhoppers span the range of generalists, which are often widespread and migratory, to mono- or oligophagous specialists that depend on native flora. In an assessment of anthropogenic risk factors involved in insect declines, Sánchez-Bayo and Wyckhuys (2019) identify one of the main quantifiable factors as the expansion of intensive agriculture, systems which are often monocultures that stifle natural complexity and diversity. Keene *et al.* (2020) examined leafhopper diversity and abundance in native prairies and those with a history of agricultural production and showed widespread domination of native remnant areas by generalist leafhoppers. With evidence that insects are being altered by urbanization and that insect abundance overall is declining due to anthropogenic influence (Sanchez-Bayo and Wyckhuys, 2019; Hallmann *et al.*, 2017; Schmitt and Burghardt, 2021), the diversity and ecological interactions of essential arthropod species like leafhoppers merit further study.

Leafhoppers are often the most abundant arthropods in grass-dominated ecosystems, where they are considered keystone species in their roles as herbivores and prey (Hamilton, 2014; Chisté *et al.*, 2017). Ninety percent of known leafhopper genera are endemic. In the Pacific Northwest, grassland-endemic leafhoppers comprise a high percentage of leafhopper fauna, despite this being a region where grasslands contribute a relatively small percent of land cover (Nielson and Knight, 2000; Hamilton, 2002). Hamilton (2014) identified 91 endemic leafhopper species in the Pacific Northwest, including 43 in Southern Vancouver Island and the adjacent mainland of Vancouver. Greater Victoria (including adjacent communities such as the nearby Metchosin) is identified as an important area for endemic leafhoppers but these habitats are under high pressure due to land conversion and ongoing urbanization (Hamilton, 2014).

Seasonal trends in diversity and abundance are central to understanding the dynamics of a community. Leafhopper seasonality can vary due to seasonal life history (phenology) as well as abiotic factors (Wolda, 1988). In temperate zones where seasonal changes in climate are significant, insect seasonality is intimately tied to temperature (Wolda, 1988). Temperate leafhoppers are generally most active in the spring and summer, in association with warm weather and abundant plant resources (Keene *et al.*, 2020). Leafhoppers may have one generation (univoltine) or multiple generations (multivoltine) per year (Macgregor *et al.*, 2019). Some species have plastic voltinism in response to environmental cues, allowing them to take advantage of favorable weather as it occurs (Macgregor *et al.*, 2019). Temperature has a direct positive effect on the developmental rates of leafhoppers' nymphal stages (Larsen *et al.*, 1990; Taylor and Shields, 1995). Overwintering as eggs or hardy adults, leafhoppers may migrate, emerge, or oviposit at the beginning of the active season, dependent on life history and growth rate in the previous season (Larsen *et al.*, 1990).

Leafhopper symbionts and defensive symbioses

Xylem and phloem sap, the food resources of most leafhoppers, are an excellent source of sugars but very low in other nutrients, including proteins (Redak *et al.*, 2004). Like all animals, leafhoppers do not have the biosynthetic capacity to create nine of 20 essential amino acids required for constructing proteins (Douglas, 2010). Many leafhoppers have invaded the sap-feeding niche in association with obligate internal symbionts whose metabolic capabilities allow them to meet their nutritional requirements (Sudakaran *et al.*, 2017). The acquisition of these endosymbiotic microbes, often bacteria, has given leafhoppers access to the biosynthetic machinery needed to produce amino acids, an essential function that creates an obligatory symbiosis. An exception is found in the subfamily Typhlocybinae, which have reverted to feed on nutrient-rich leaf cells and who, putatively, have no obligate nutritional symbionts (Bennett and Moran, 2013).

Nutritional symbioses are only one example of novel functions that can be acquired from housing symbiotic microbes. Other symbioses confer a benefit only under certain environmental conditions, with advantages across a broad range of physiological roles and ecologically important traits. A prominent example of these facultative symbioses are those that provide a protective value to the host in the presence of certain natural enemies. Defensive symbioses can alter trophic-level interactions in significant ways. A well-studied defensive symbiont is *Hamiltonella defensa*, strains of which protect aphids against parasitic wasps by reducing successful parasitism rates by up to half (Oliver *et al.*, 2003). Defensive symbioses like these are being elucidated in nature at an ever-growing rate (Oliver *et al.*, 2014).

Leafhoppers and dryinid wasp associations

Wasps of the family Dryinidae (Hymenoptera: Dryinidae) are important natural enemies of many cosmopolitan leafhoppers as both predators and parasitoids (Guglielmino *et al.*, 2013). Dryinid-host associations have been catalogued for a minor percentage of the 1,700 known dryinid species, however, it appears most are generalist parasitoids whose hosts can be of multiple species or broader lineages (such as leafhoppers and other hemipterans) (Oman, 1949; Guglielmino *et al.*, 2013). Female dryinids are parasitoids first and predators second, prioritizing suitability of the host for oviposition over foraging (He *et al.*, 2020). Their influence on leafhopper communities can be significant, with parasitism affecting high percentages of the leafhopper community in some environments (Moya-Raygoza, 2020). Chua and Dyck (1982) observed a single female dryinid predate and parasitize 466 planthoppers over its short lifespan. In most cases, the female dryinid lays her eggs in a leafhopper near the end of its nymphal stage and parasitoid development begins internally (Oman, 1949). A single ectoparasitoid usually emerges when the leafhopper host reaches its adult stage and remains attached to the side of the host until ready to pupate. (Oman, 1949; He *et al.*, 2020). Parasitized leafhoppers can moult and develop normally while the dryinid larva grows but upon reaching maturity the larvae will consume its host (Jervis, 1980).

Because parasitism can be a major source of fatality in leafhoppers, leafhopper-dryinid relationships are a suitable place to look for a symbiont-based defense strategy (Barrett *et al.*, 1965; He *et al.*, 2020). High dryinid parasitism rates have been shown in laboratory experiments to alter the bacterial communities of leafhoppers, by reducing the presence of a *Spiroplasma* symbiont (Moya-Raygoza *et al.*, 2006). Dryinid parasitism has the potential to exert significant selective pressures that alter the facultative bacterial community of leafhoppers; however, despite this apparent ecological opportunity, no defensive symbioses have yet been found to mediate their interactions. Dryinid wasps are of particular interest as biological control agents for leafhopper pests because of their dual nature as leafhopper enemies (Shi *et al.*, 2009). Cataloguing of symbionts associated with host leafhoppers will provide further insight into how symbionts affect insect fitness and ecological relationships. Therefore, understanding this trophic relationship and its effects in natural environments has implications for both ecology and agriculture.

In this study, I examined leafhoppers collected from a single residential site on Vancouver Island, British Columbia. The sample site is part of a long-term study on temporal trends in insect biomass with the goal of revealing insect responses to climatic, anthropogenic, and biotic variables in the area (Nikel, 2019). This study represents the first analysis of trends in leafhopper diversity and abundance in the region. Leafhoppers were collected over two years; using morphospecies sorting and molecular identification, the composition, diversity, and parasitoid-host associations were catalogued in the sample community. Using 16S ribosomal RNA amplicon sequencing to analyze the symbiotic biome of host leafhoppers, this study aims to gather insight into the dynamics of a tripartite interaction of symbiont, parasitoid, and their common leafhopper host, by answering the following questions:

1. What leafhoppers compose this community and how does their diversity and abundance vary over time?
2. What is the extent and variation in dryinid parasitism on leafhopper hosts over the season?

3. Is there evidence for a defensive symbiosis in leafhopper taxa affected by parasitism?

Methods

Sample site and collection

The District of Metchosin is an oceanside municipality of Greater Victoria on Southern Vancouver Island, British Columbia that exists on the traditional lands of the Sc'ianew First Nation (Sc'ianew First Nation, 2022). Nearby agriculture is common, but not extensive, and most farms in the area are not monocultures but have diverse products (District of Metchosin, 2012). The municipality promotes a zero pesticide usage agenda (Nikel, 2019). The area is urban with a low population density, being developed as a settler colony beginning ca. 1851, and remains abundant in semi-natural and managed green spaces (Statistics Canada, 2022; Metchosin Heritage Advisory Select Committee, 2017).

The sample site is in a residential property at 48°22'14.5"N 123°31'51.3"W at a mean altitude of 17 m above sea level. The sample site contains predominately mixed grass meadow with riparian and forested areas dominated by Garry oak (*Quercus garryana*), Douglas fir (*Pseudotsuga menziesii*), and invasive Scotch broom (*Cytisus scoparius)*.

Specimens were previously collected at the sample site between May 14th and Oct 25th in 2020 and May 1st and October 15th in 2021 using an ez-Malaise Trap BT1002 (height 180 by 165 cm). Samples were collected every two weeks and preserved in 75% ethanol in the collection vial. No data was collected between July 20 to August 3, 2020 due to a damaged trap. In total, 11 samples were obtained for 2020 and 12 for 2021.

Temperature data was collected on HOBO U23 Pro v2 Temperature/Relative Humidity Data Loggers which recorded temperature every 30 minutes over the period of sample collection. In 2020, the sample site was fitted with a temperature logger. In 2021, temperature data was gathered at an adjacent site which is used as a climatic proxy for the sample site.

Specimen sorting

All following procedures were conducted in laboratories at the University of Victoria, British Columbia, Canada. Adult leafhoppers were sorted into morphospecies by exterior visual similarity under a dissecting microscope and stored in 95% ethanol. Each leafhopper sample was sorted into morphospecies by a single researcher. Leafhoppers were counted and separated by date, morphospecies, and presence or absence of dryinid parasitism. Individuals parasitized by dryinid wasps were identified as having externally visible larval tumors. Early stages of dryinid parasitism are not visible without dissection, so only parasitoid larvae in further stages of development were counted. Parasitoid larvae were then sorted into types based on size and appearance. A repetition of the morphospecies and parasitism sorting process was performed for all samples as quality control.

Molecular methods

All molecular analyses were conducted on samples collected between June and October 2021. Leafhopper DNA was extracted from pooled whole leafhoppers ($2 \leq n \leq 5$) or from single severed abdomens when practical. Leafhoppers were mechanically and chemically lysed with a bead beater and Thermo Fisher PrepMan® Ultra lysis buffer. Dryinid larvae of each visually distinct 'type' were removed from the abdomen of host leafhoppers. DNA was extracted using chloroform and phenol, precipitated with ethanol, and diluted. The mitochondrial gene cytochrome c oxidase I (COI) was amplified by polymerase chain reaction (PCR) using Applied Biological Materials Inc. MegaFi™ Fidelity polymerase and primers 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (HCO) and 5'-GGTCAACAAATCATAAAGATATTG-3' (LCO). PCR parameters for the amplification of COI were as follows: initial denaturation at 98° for 30 seconds (s), 35° cycles of 98° for 10 s, 54° for 30 s, 72° for 30 s, and final anneal at 72° for 2 minutes. PCR products were sent to Sequetech for Sanger sequencing. *Dikraneura mali*, *Ribautiana debilis*, *Balclutha rhenana*, and *Allygus mixtus* were subject to replicate (n=2) extractions and barcoding.

To search for potential defensive symbionts in the the prokaryotic community of parasitized leafhopper species, DNA was extracted from pooled (n=20) unparasitized leafhoppers from two species observed to be dryinid hosts (*Dikraneura mali* and *Ribautiana debilis*). DNA was extracted with a QIAGEN DNeasy Blood & Tissue Kit. After being precipitated with ethanol and diluted, DNA samples were sent to GENEWIZ for 16S rRNA amplicon sequencing.

To survey *Rickettsia* frequency in a sample of *D. mali*, individual *D. mali* with no visible ectoparasitoids (n=15) underwent PCR amplification, as described above, with primers that bind a segment of the 16S ribosomal RNA gene: 5’-CGGCTTTCAAAACTACTAATCTA-3’ (RSSUF1) 5’-GAAAGCATCTCTGCGATCCG-3’ (RSSUR1) and 5’-GCTTTCAAAACTACTAATCTA-3’ (RSSUF2) 5’-AAAAGCATCTCTGCGATCCG-3’ (RSSUR2).

Data analysis

Partial COI sequences were quality screened by examining the strength of chromatograms and the rationality of translated sequences. Leafhopper and dryinid COI sequences were trimmed and input into the National Center for Biotechnology Information BLAST and/or Centre for Biodiversity Genomics BOLD Systems databases for identification. Database matches were screened for quality by investigating sequence similarity in amino acid alignment as well as morphologically assessing species-level identifications. Alignments were performed with MUSCLE alignment software (Madeira *et al*., 2022).

Amplicon sequences from the microbiomes of *D. mali* and *R. debilis* were examined using Geneious Prime 2022.1 (https://www.geneious.com) and compared to sequence databases. BLAST searches of the 16S amplicon datasets were performed to search for obligate symbionts, including *Nasuia deltocephalinicola*, *Zinderia insecticola*, *Baumannia cicadellinicola*, *Sulcia muelleri*, and facultative symbionts in the genera *Rickettsia*, *Wolbachia*, *Symbiopectobacterium*, *Sodalis*, *Cardinium*, *Arsenophonus*, *Spiroplasma*, and *Rickettsiella*.

Photographs of leafhoppers and larval dryinids were taken with a Wild Makroskop M420 compound microscope equipped with a Diagnostic Instruments Spot Flex camera (Model # 15.2 64 Mp Shifting Pixel) and computer equipped with version 7.4.7 SPOT software.

All statistical analyses and data visualization was performed in R v. 4.1.2 (R Core Development Team, 2022). The Jaccard index was used to infer temporal variation in species richness of roughly contemporaneous samples between years. Because of the slight offset in start/end dates between years, 3 sample dates were excluded from the similarity analysis. The association between temperature and diversity measures was determined using Pearson correlation models.

Results:

Leafhopper species identity

Morphological grouping of leafhopper samples from May through October at a single urban site in Metchosin, British Columbia (BC) in 2020 and 2021 revealed 23 morphospecies. Length of preservation in ethanol altered the appearance of leafhoppers, specifically the degree of visible pigmentation. Twenty-two morphospecies were confidently and consistently identified by exterior morphology (Fig. 1A; Fig. 1B). Morphospecies B includes representatives that could not be consistently and confidently identified between and within years (Fig. 1B). This group is composed of pale, medium-sized leafhoppers that had no discernable coloration or markings in 2020, but where similar individuals showed novel coloration in newer samples from 2021. Morphospecies B may include individuals from multiple unidentified morphospecies for whom preservation has removed identifiable characteristics. For these reasons, morphospecies B was not included in further analyses requiring species identification, including DNA barcoding and 16S analysis.

Partial COI sequences were successfully retrieved from 13 of 23 leafhopper morphospecies (Table 1). When sequences were interrogated with a BLASTN search, eight were confidently identified at the species level, two to genus, and three to the leafhopper family Cicadellidae. Of the ten leafhoppers identified to at least the subfamily level, four were from Deltocephalinae, three from Typhlocybinae, two from Cicadellinae, and one from Aphrodinae (Table 1). Barcoding of *Erythroneura* sp. and *Graphocephala atropunctata* produced low fidelity sequences, but distinctive visual similarity supports the molecular identity for these taxa.

All reported matches to leafhoppers in our dataset were sequenced from leafhoppers that were also collected in BC. Cicadellidae sp.[1] was previously collected in Sidney, BC (approximately 34 km from the sample site) in 2012, Cicadellidae sp.[2] was collected in Burnaby, BC in 2015, and Cicadellidae sp.[3] was collected in Kelowna, BC in 2014.

Community composition and seasonal trends

A total of 5,801 adult leafhoppers were collected between May and October, with 2,664 from 2020 and 3,137 from 2021. This community exhibits high unevenness, with a dominant contributor, *Dikraneura mali* (Typhlocybinae), which comprised 68.6% of all samples (Figure 2). The second most abundant species overall was *Ribautiana debilis* (Typhlocybinae), who comprised 12.6% of samples. Morphospecies B had third highest abundance, comprising 9.8% of all samples. The 20 other catalogued taxa individually represented between 0.02-2.3% of all leafhoppers sampled (Figure 2).

During their respective maximum seasonal abundances, both *D. mali* and *R. debilis* are the most abundant leafhoppers in the sample community (Figure 3). In samples outside of these abundance peaks, *D. mali* remains the predominate contributor to abundance (except at the beginning of its emergence in early May, when it is represented by a handful individuals). *Dikraneura mali* and morphospecies B were present in all samples collected, while *R. debilis* was absent in three samples (one in 2020 and two in 2021), all of which occur at the beginning of the sample period in May.

Temporal trends in abundance showed consistency between 2020 and 2021. Both *R. debilis* and *D. mali* experience two peaks in abundance in both seasons, one in late June/early July and one in September/early October (Figure 3). *Ribautiana debilis* reaches maximum abundance within its first peak, in late June of both years. Morphospecies B has less of a distinguishable pattern, but maximum abundance is reached in late June/early July of both years. Overall abundance peaked in both years in mid-September, with similar seasonal abundance maxima of 636 and 617 leafhoppers, respectively (Figure 3). These samples are heavily affected by the abundance of *D. mali*, which represents roughly 80% of the sample community in September. *Dikraneura mali* and morphospecies B1 are the only taxa with a seasonal abundance that was greatest in the fall, although *R. debilis* and the compilation category “Other” also experience a secondary, smaller peak in this period.

In 2020, the first and last samples of the season (May 14-27 and October 12-25) show a similar abundance and equal species richness, largely diminished in comparison to mid-season samples (Figure 3, Figure 4). This range likely encompasses the main active season for leafhoppers in

Metchosin. In 2021, a final sample date only 10 days earlier represents three more species, indicating that many species cease activity between mid and late October.

Both years had highest average daytime temperatures in June (Figure 4). Species richness was significantly positively correlated to temperature over the season ($r = 0.65$, $p < 0.001$) (Figure 5). No such relationship was present with abundance, even with the removal of the dominant *D. mali*, or parasitism rate ($p > 0.05$). In both years, an upward trend through the sample period grows to peak species richness in the late June/early July sample (June 22- July 6 in 2020 and June 26 – July 9 in 2021). The maximum species found in one sample for both years was 17.

Species richness was the most consistent measure between years. Of 23 taxa, 21 were shared between 2020 and 2021. Seventeen species (out of 23 total) were found in late June and early July of both 2020 and 2021, representing a concentration of diversity near the middle of the active season. Overall, samples collected at the same time showed similarity in community composition (Figure 6). The average Jaccard similarity between years was 0.57. Highest similarity in community composition between years corresponded to the period of highest diversity, with a Jaccard index of 0.79 (Figure 6). Highest species richness and community similarity are associated with the period of highest temperature. Community similarity was lowest in August, with a Jaccard index of 0.39 between yearly samples.

Dryinid parasitism

Three leafhopper taxa had individuals that hosted the ectoparasitic stages of dryinid larvae: *R. debilis*, *D. mali*, and morphospecies B. Four morphological types of parasitoid larvae were found on the exterior of leafhopper hosts (Figure 7). Following barcoding of the COI gene, all larvae were identified to the level of family Dryinidae. Types 1, 2, 3 and 4 had 99.64, 99.84, 97.53, and 86.89% identity to sequences of unidentified Dryinidae sp. (Accession nos.: KR802165.1, KR803056.1, KR900007.1, and MF904593.1). Type 4 had a poorly resolved COI sequence. These sequences did not align significantly with known dryinid parasitoids of *Dikraneura* spp. and *Ribautiana* spp.

Alignments of sequences retrieved from the four morphological types indicate that there are two dryinids present in the sample community, one with hosts *D. mali* and *R. debilis* (Types 1-3) and one that parasitizes morphospecies B (Type 4) (Figure 7). Types 2 and 3 had partial COI sequences that were identical in alignment except at a single nucleotide which was resolved poorly in the Type 2 sequence. Type 1 was 96.47% similar to Types 2 and 3 and dissimilar nucleotides were only found in areas of poor resolution. The COI gene sequence of Type 4 was 84.31% similar in alignment to that of Type 3. Many of the nucleotide dissimilarities were found at sites that were resolved with high fidelity in the Type 4 COI sequence.

Overall, parasitism rates were higher in 2020 than in 2021. Despite differences in magnitude, the seasonal trends in parasitism remained largely consistent, appearing as a bimodal distribution in both years (Figure 8). In both years, the major peak in abundance for all taxa experiencing parasitism was in August and September. For *D. mali* and *R. debilis* in 2020 and for *R. debilis* and morphospecies B in 2021, a smaller June parasitism peak preceded the large late summer peaks.

Both *D. mali* and *R. debilis* experienced their highest parasitism rate between September 14-28, 2020 (Figure 8). The highest parasitism observed for *D. mali* was 15% of the population in the sample period. For *R. debilis* parasitism rates reached a high of 38% of the population. Morphospecies B experienced higher parasitism rates that also occurred earlier in the season, with 58% and 23% infected in 2020 and 2021, respectively. A single instance of double parasitism (two dryinid larvae on a single host) was found on *R. debilis*. The high parasitism rates observed in morphospecies B were a result of low frequencies of parasitism and leafhopper abundance. For example, at peak parasitism between August 17-31, 2020, the sample population of morphospecies B was comprised of 19 individuals, of which 11 were parasitized. At peak parasitism for *D. mali*, 77 were parasitized of 450 individuals.

Bacteria associated with Dikraneura mali and Ribautiana debilis

After comparing sequences of endosymbiotic bacteria, including those with known defensive functions against parasitoids, to the 16S amplicon datasets of *D. mali* and *R. debilis*, a symbiont was found in each leafhopper. In *D. mali*, a ~250 bp 16S rRNA sequence was found that showed 100% identity to numerous *Rickettsia* arthropod endosymbionts, including those from the whitefly *Bemisia tabaci* (Hemiptera: Sternorrhyncha) (Accession no. LN829697.2) and

Rickettsia bellii of ticks (Ixodida) (Accession no. MT006122.1). In *R. debilis*, a ~320 bp sequence was found which showed 99.06% identity to numerous endosymbiotic *Wolbachia pipientis* strains, among them those of *Aedes aegypti* (Diptera) (accession no. MH732669.1) and *Orthezia urticae* (Hemiptera: Sternorrhyncha) (accession no. MK462265.1).

No 16S rRNA sequences corresponding to categorized obligate nutritional symbionts of leafhoppers (Candidatus *Nasuia deltocephalinicola*, *Zinderia insecticola*, *Sulcia muelleri*, nor *Baumannia cicadellinicola*) were found in the 16S libraries of *D. mali* or *R. debilis*.

A survey of *Rickettsia* occurrence in a subset of the *D. mali* population returned a 100% (15/15) infection frequency. No sequence data was obtained for these products; therefore, the identity of the amplified sequences could not be supplemented by sequence alignment with known taxa.

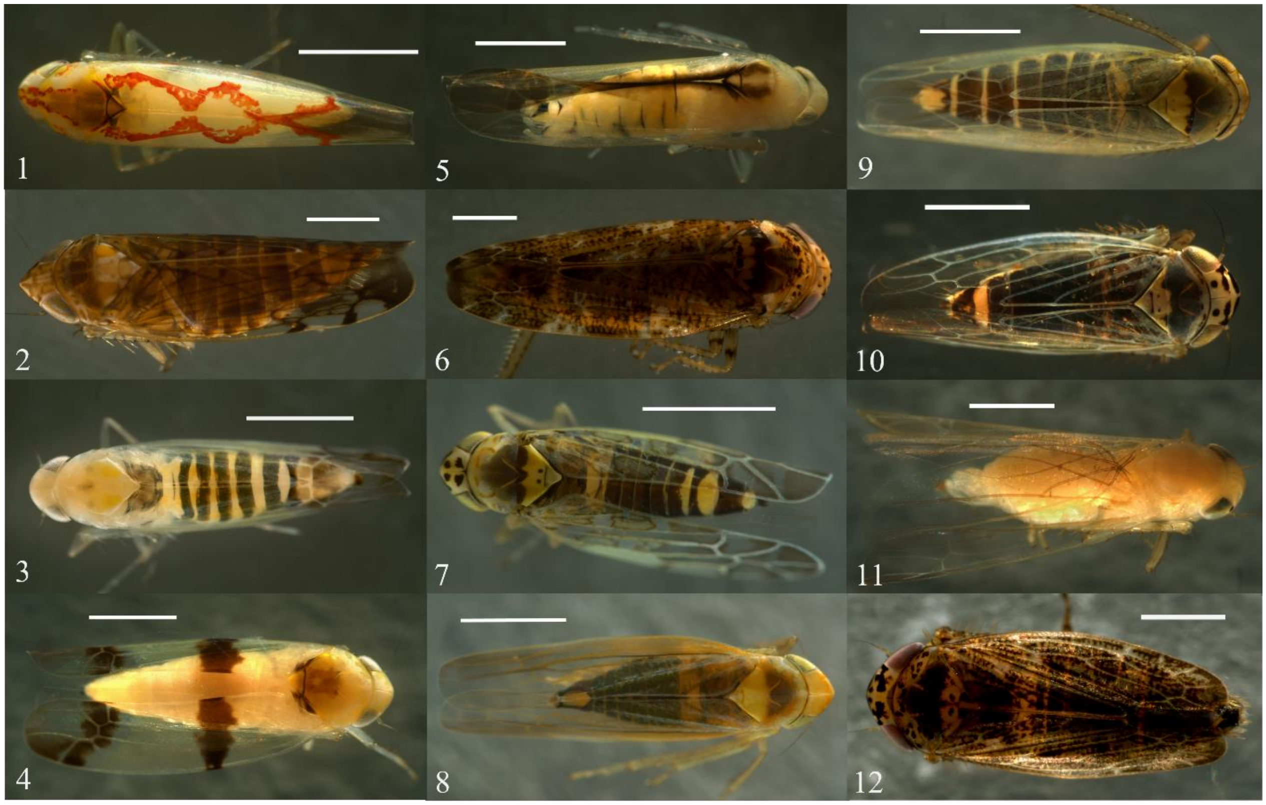

Figure 1A. Twelve of 23 leafhoppers collected in Metchosin, British Columbia, between May 2020 and October 2021. Taxa identified by molecular methods include: 2) *Osbornellus* sp.; 3) *Ribautiana debilis*; 6) *Allygus mixtus*; 8) *Dikraneura mali*; 9) *Balclutha rhenana*; 10) *Macrosteles quadrilineatus*; and 11) Cicadellidae sp.^{3}. All other leafhoppers are representatives of their unidentified morphospecies and are referenced as morphospecies: 1) B1; 4) H; 5) R; 7) T; and 12) E. Scale bar represents one millimetre.

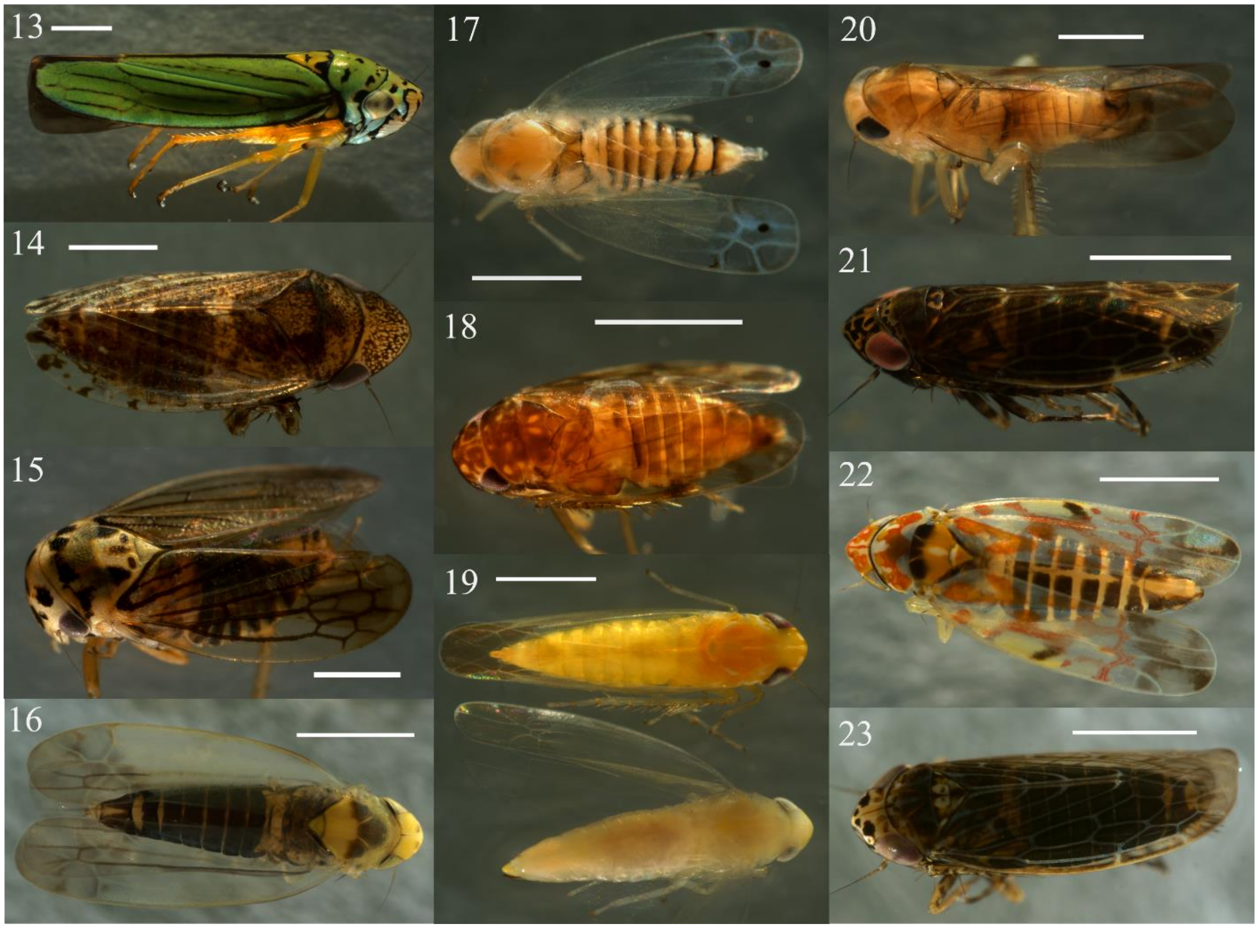

Figure 1B. Eleven of 23 leafhoppers collected in Metchosin, British Columbia, between May 2020 and October 2021. Leafhoppers identified by molecular methods include 13) *Graphocephala atropunctata*; 14) *Anoscopus serratulae*; 16) Cicadellidae sp.[1]; 17) Cicadellidae sp.[2]; 20) *Empoasca luda*; and 22) *Erythroneura* sp. All other leafhoppers are representatives of their unidentified morphospecies, whose alphabetical reference labels are: 15) X 18) F 19) two examples of the variable morphology of B 21) O and 23) N. Scale bar represents one millimetre.

Table 1. Molecular identification of leafhoppers found in Malaise trap samples from Metchosin, British Columbia in 2020 and 2021. All taxa were sorted based on morphology and identified by barcoding of mitochondrial gene cytochrome c oxidase I. Distinct leafhopper taxa identified to the leafhopper family Cicadellidae have a unique numerical superscript.

Lowest taxon identity	**Subfamily**	**Identity (%)**	**Accession no. match**	**Identity coverage (bp)**
Allygus mixtus	Deltocephalinae	100	MG401829.1	620 / 620
Balclutha rhenana	Deltocephalinae	100	MF838483.1	603 / 603
Macrosteles quadrilineatus	Deltocephalinae	99.37	KR572849.1	479 / 482
Osbornellus sp.	Deltocephalinae	100	MG405004.1	614 / 614
Dikraneura mali	Typhlocybinae	99.83	MF931485.1	642 / 643
Ribautiana debilis	Typhlocybinae	100	KR579309.1	637 / 637
Erythroneura sp.	Typhlocybinae	95.34	MG404495.1	430 / 451
Empoasca luda	Cicadellinae	99.56	MG398187.1	454 / 456
Graphocephala atropunctata	Cicadellinae	97.82	KF919492.1	402 / 411
Anoscopus serratulae	Aphrodinae	99.09	MG399552.1	543 / 548
Cicadellidae sp.^{1}	n/a	100	KR582188.1	653 / 653
Cicadellidae sp.^{2}	n/a	98.70	MG405759.1	530 / 537
Cicadellidae sp.^{3}	n/a	100	MG404222.1	624 / 624

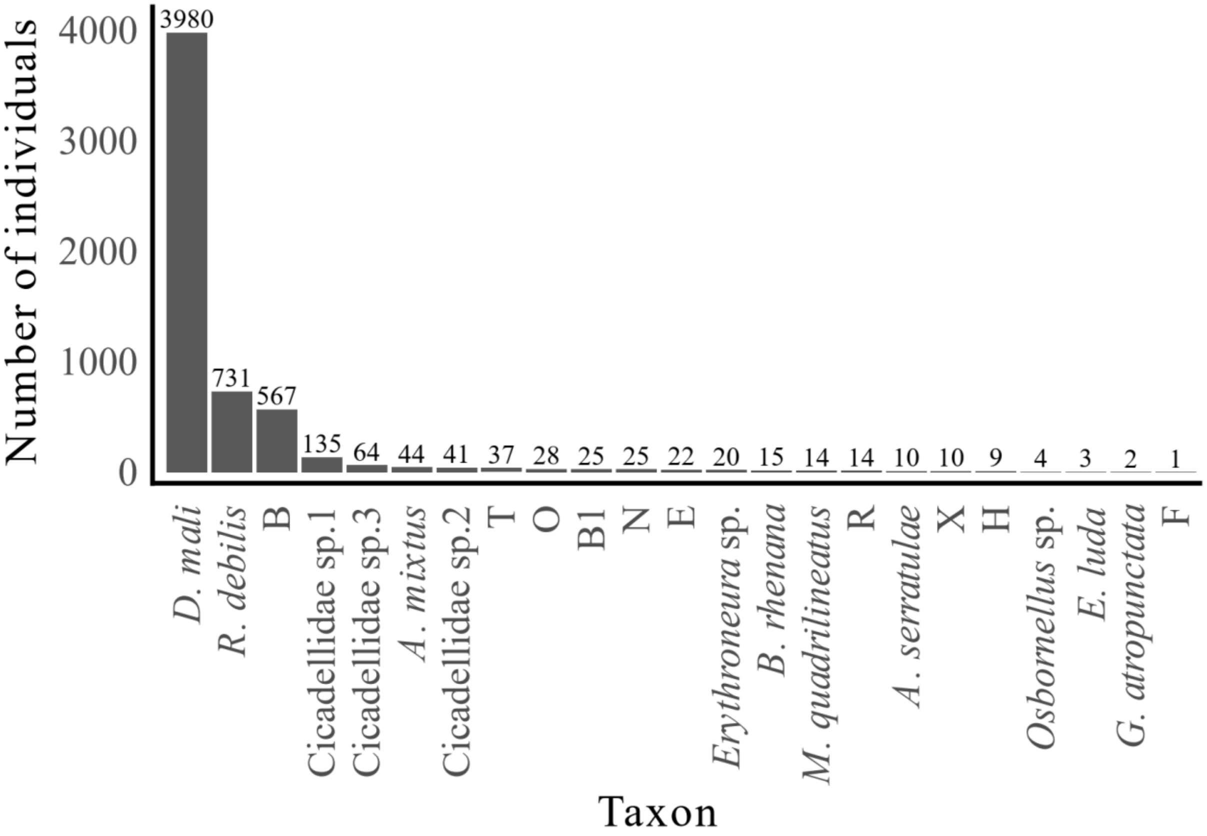

Figure 2. Total abundances of 23 leafhopper taxa collected by Malaise trap between May and October in 2020 and 2021 in Metchosin, British Columbia. Leafhoppers identified to species level include: *Dikraneura mali*, *Ribautiana debilis*, *Allygus mixtus*, *Balclutha rhenana*, *Macrosteles quadrilineatus*, *Anoscopus serratulae*, *Empoasca luda*, and *Graphocephala atropunctata.* Unidentified morphospecies are represented by their alphabetical label.

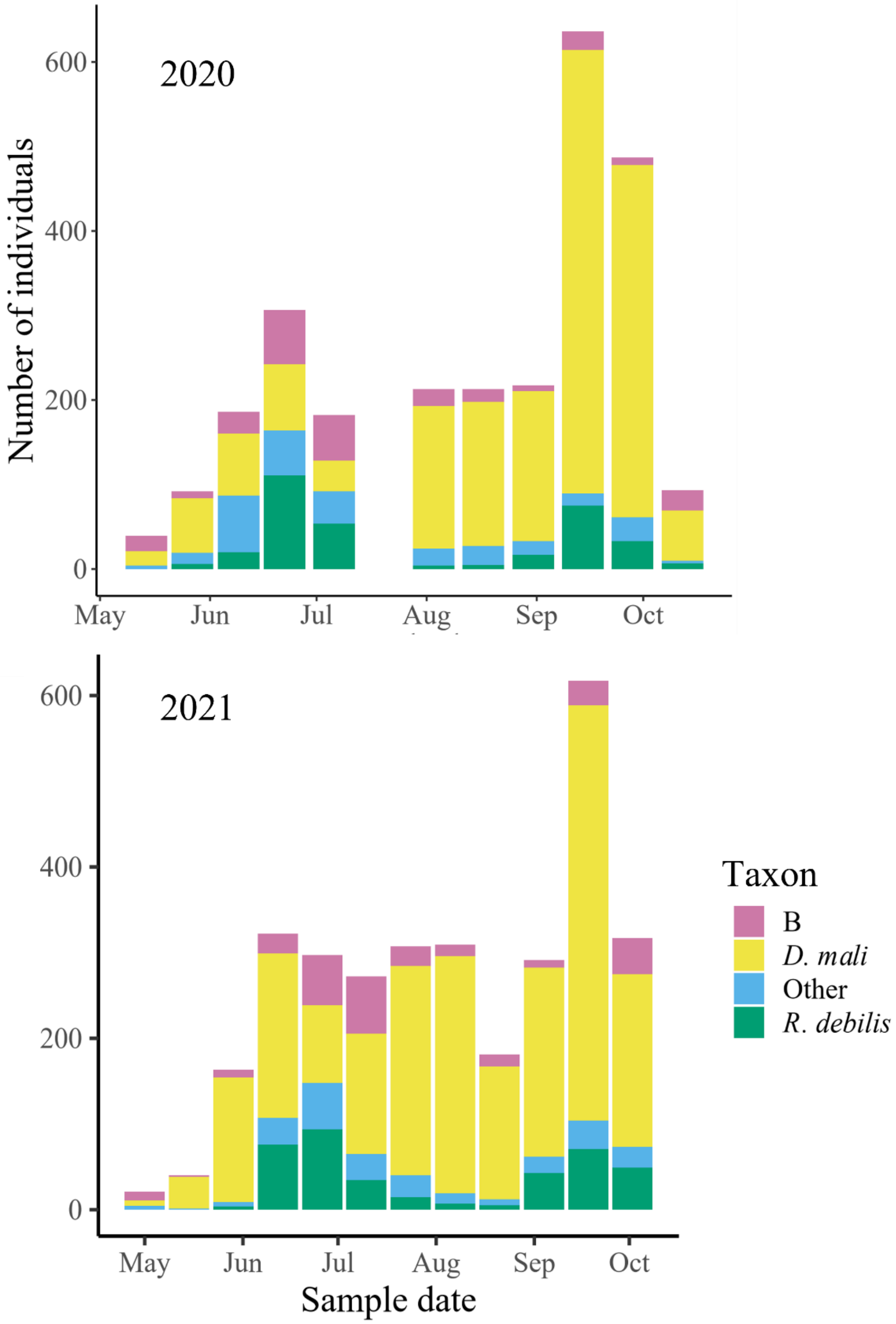

Figure 3. Total abundance of the three most abundant taxa, *Ribautiana debilis*, *Dikraneura mali*, and unidentified morphospecies B, collected in each two-week sample between May and October in 2020 and 2021 in Metchosin, British Columbia. The "Other" group represents 20 other leafhopper taxa that constitute the rest of the sample community.

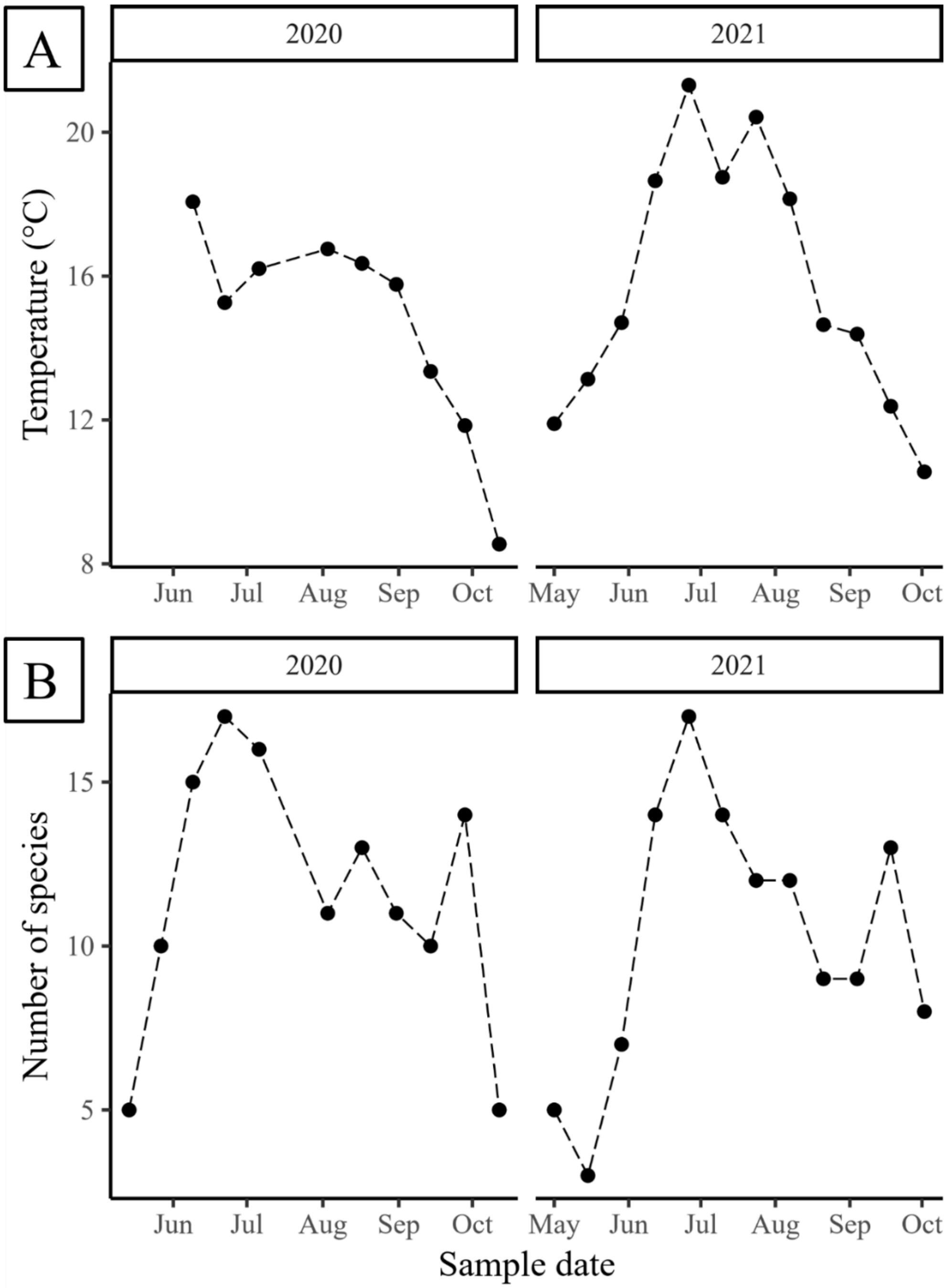

Figure 4. A) Average daytime temperature at the sample site in each sample period over the collection interval. Temperature data for May in 2020 was unavailable. B) Leafhopper species richness between May and October, 2020 and 2021, in Metchosin, British Columbia. Each sample date represents species richness over a two-week collection period, with collection being continuous over time.

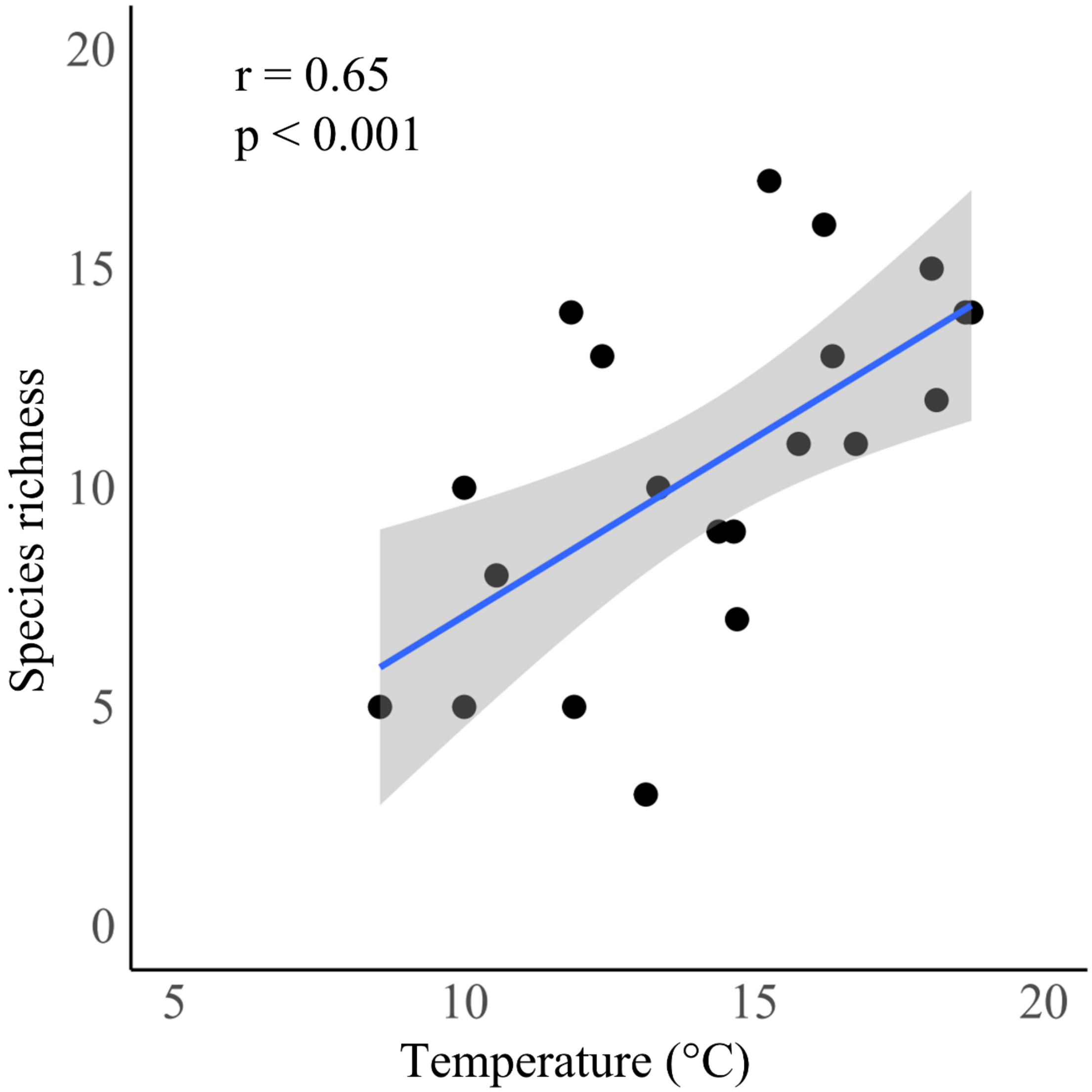

Figure 5. The positive linear relationship between temperature and species richness of leafhoppers collected in Metchosin, British Columbia, between 2020 and 2021. Pearson's correlation (r) was determined significant ($p < 0.001$). Grey area represents 95% confidence interval.

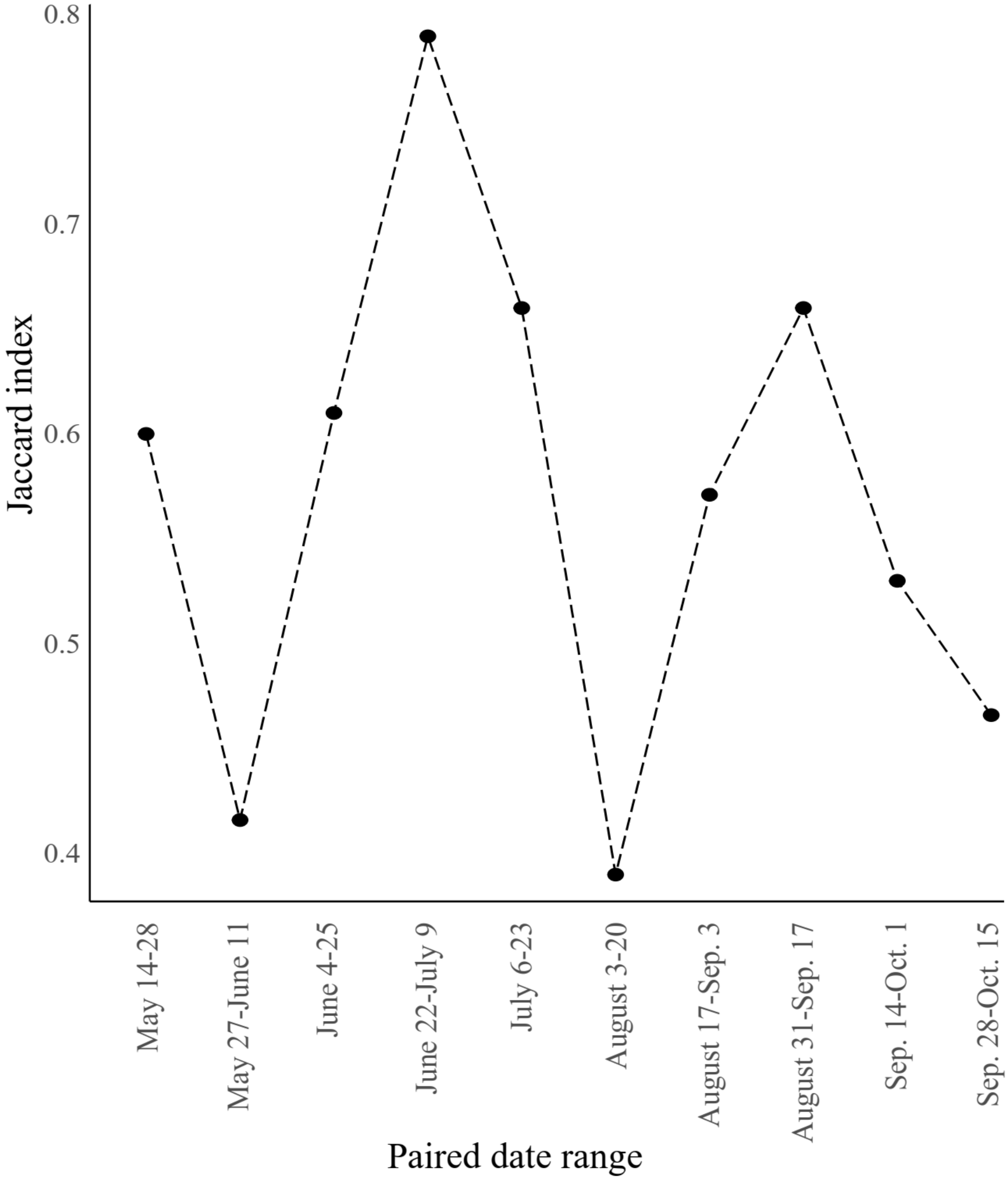

Figure 6. Jaccard similarity of the species composition in a leafhopper community in Metchosin, British Columbia, sampled in 2020 and 2021. Paired between-year samples were compared to estimate the interannual similarity of leafhopper species richness over the active season.

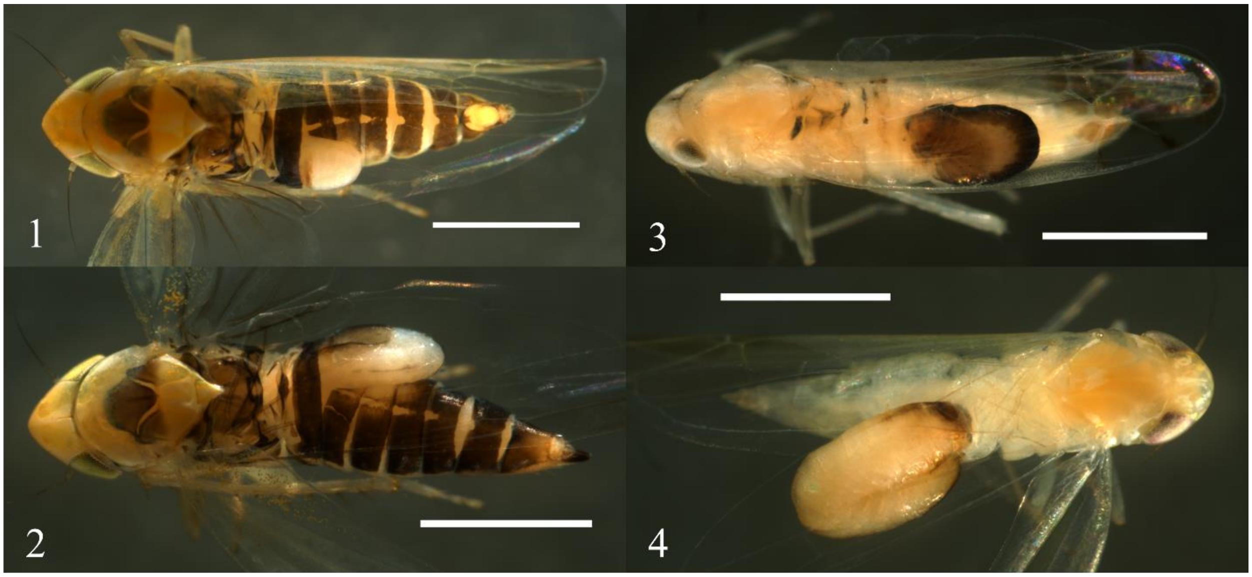

Figure 7. Parasitoid larvae (Hymenoptera: Dryinidae) attached to the abdomen of leafhopper hosts. Four morphologically distinguished types of larvae were observed on leafhoppers sampled in Metchosin, British Columbia. 1) Type 1 on *Dikraneura mali*; 2) Type 2 on *Dikraneura mali*; 3) Type 3 on *Ribautiana debilis*; 4) Type 4 on unidentified morphospecies B. Scale bar represents one millimetre.

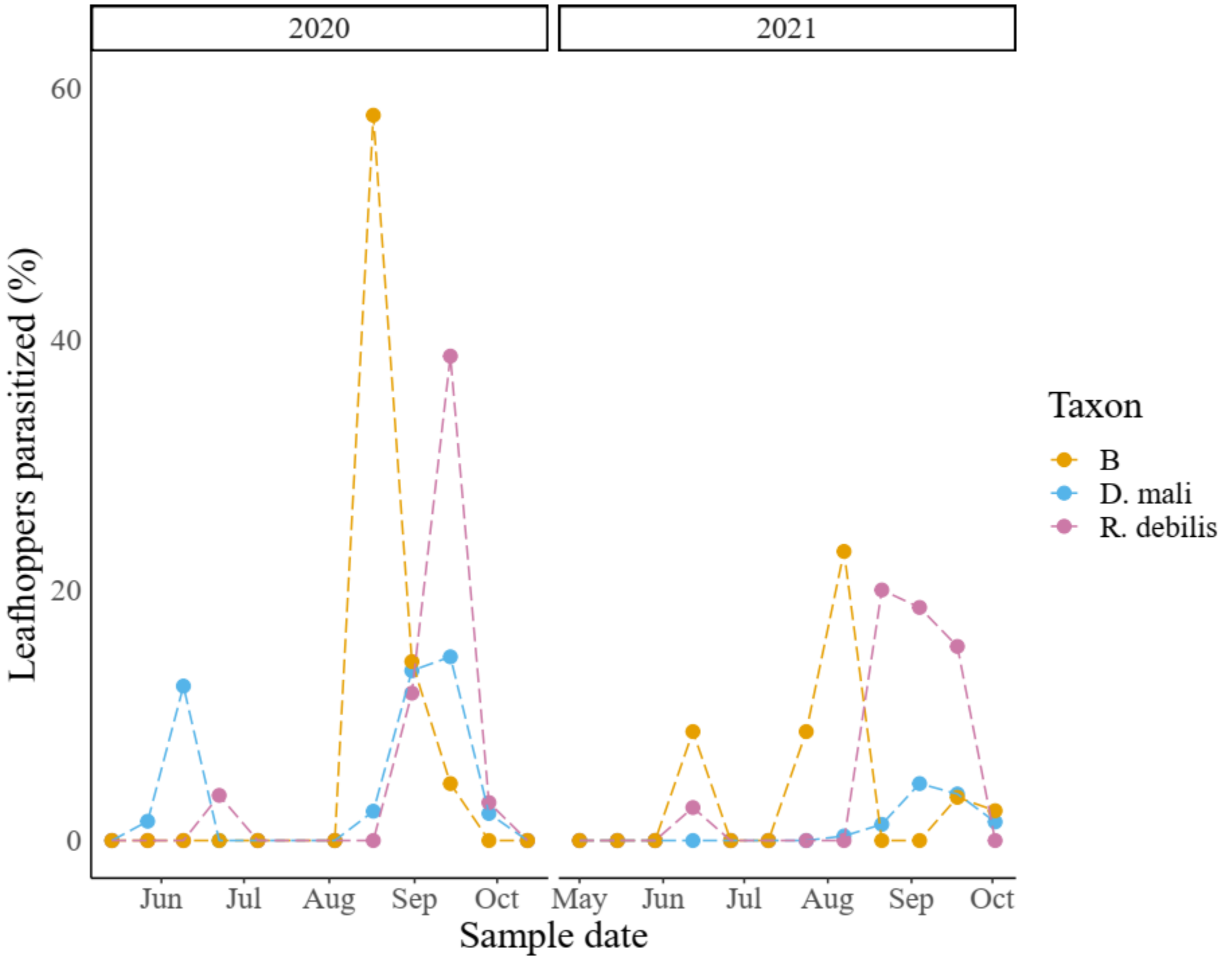

Figure 8. Parasitism rates of two parasitoid wasps (Hymenoptera: Dryinidae) from three leafhopper hosts, *Dikraneura mali*, *Ribautiana debilis*, and unidentified morphospecies B, collected over two years in Metchosin, British Columbia. *Dikraneura mali* and *R. debilis* were host to the same unidentified dryinid, while another unidentified dryinid parasitized morphospecies B.

Discussion

Community composition

Measurements of overall and seasonal leafhopper abundance indicate that this community is dominated by *Dikraneura mali* and *Ribautiana debilis*. Many arthropod communities appear to be assembled in this way, where a relatively small number of species dominate a community composed mostly of rare species (Nickel and Hildebrant, 2003). While both dominant leafhoppers in this community are in the subfamily Typhlocybinae, they differ in host plant association and origin. *Ribautiana debilis* is a European leafhopper and a generalist of Betulaceae and Rosaceae, while *Dikraneura mali* is native to North America and appears to be a generalist on grasses (Ball and Delong, 1925; Dmitriev, 2022). *Dikraneura mali* is more common east of the Rocky Mountains, but it has been reported in Greater Victoria since at least 1918 (Downes, 1918). Although *R. debilis* has never been formally described here, a leafhopper with an identical COI barcode was collected nearby. The congeneric *R. tenerrima* is prevalent on Southern Vancouver Island and *R. debilis* has been misreported in British Columbia before due to its similarity to the more common *R. ulmi* (Hamilton, 2014; Raine, 1960). However, partial COI sequence similarity of the *R. debilis* from the present study to *R. ulmi* was low. No COI sequences for *R. tenerrima* were available for comparison.

Of leafhoppers identified to species in the sample community, there are several examples of European introduced species that have been previously recorded around Metchosin. *Allygus mixtus* is a European leafhopper that has become prominent on Southern Vancouver Island, especially in Garry Oak meadow remnants like that of the sample site, as it feeds and lays its eggs on various oaks and grasses (Dmitriev, 2022; Hamilton, 1983). *Anoscopus serratulae* is another introduced European leafhopper of local abundance and a generalist on grasses. *Empoasca luda*, whose hosts are specific to *Betula* (birches), is a more recently arrived European leafhopper first found in Canada in 1984 (Dmitriev, 2022; Hamilton, 1983).

The genus *Balclutha* has a holarctic distribution and is the subject of taxonomic disagreement about the synonymy of individuals found across continents. *Balclutha rhenana* is a European species that has been synonymized in the past with *B. punctata*; the latter has a native range within North America (Hamilton, 1983). This synonymy is not always maintained in the literature, however, and likely the best descriptor for leafhoppers in this genus is by their distribution rather than their historical identity as debate continues about synonymy (Knight, 1987). Both *B. rhenana* and *B. punctata* are generalists on grasses (Dmitriev, 2022).

Two leafhoppers were found that have important economic roles as major pests in agriculture. *Graphocephala atropunctata* has a native range encompassing the Pacific Northwest, where it causes heavy agricultural losses due to its role as vector of *Xylella fastidiosa* (Redak *et al.*, 2004). *Macrosteles quadrilineatus* is an economically important agricultural pest in North America as it has a wide host range that includes many crops and is the main vector of aster yellows, a mycoplasma-like organism-induced disease (Hagel and Landis, 1967). It was recently revealed that *M. quadrilineatus* houses an obligate nutritional symbiont with the smallest genome of any known bacteria (Bennett and Moran, 2013). Despite its economic and scientific importance, little is known about *M. quadrilineatus* populations over its native range, making geographically broad molecular data of potential importance to future research of its ecology (Hagel and Landis, 1967).

Unlike many studies on leafhoppers in other anthropogenic (e.g., agriculture-associated) environments, which found even nearby native remnant areas inundated with generalist migratory pest species (Saguez *et al.*, 2014; Keene *et al.*, 2020), I did not find an overwhelming presence of agriculture-associated leafhoppers in this community. Although farms are common in the Metchosin area, they are often small and with diverse crops; Metchosin does not have a history of large-scale monoculture (District of Metchosin, 2012). Agriculture-associated species are found here but their presence is not significant compared to other members of the sample community.

Anthropogenic influence has introduced generalist European leafhoppers to Metchosin, which appear to be taking hold of the community (as in *R. debilis*). Many of the leafhoppers identified, including the dominant species *D. mali*, were generalists on broad plant groups, potentially indicating an advantage to this life habit in the area. There is evidence suggests that generalists are more likely to proliferate in urban areas (Nickel and Hildebrant, 2003; Keene *et al.*, 2020). Leafhopper diversity can be correlated to plant diversity and since urban areas are defined by lowered plant diversity, more host plants remain available to generalists than specialists in urban areas (Keene *et al.*, 2020). Although this site likely represents only a portion of the leafhopper community in the vaster area and not all members of this community were identified, I did not identify any endemic leafhoppers at the urban sample site. More sampling is needed to address endemism, as Hamilton (2014) suggested that Greater Victoria is an important remnant area for leafhopper endemism.

Phenological trends and life histories

Previously unreported life history traits of the dominant leafhoppers *Dikraneura mali* and *Ribautiana debilis* can be gleaned from abundance data. The bimodal abundance distributions of *D. mali* and *R. debilis* suggest that these are multivoltine species that exhibited at least two generations over the sample seasons. In this case, leafhoppers laid early in the season would complete a full life cycle, while leafhoppers laid late would enter diapause and emerge in spring (Oman 1949). The breeding habits of *D. mali* and *R. debilis* have not been previously recorded, though as voltinism is a temperature-linked trait, it has the potential to differ between populations (Kong *et al.*, 2019). Although species-level phenology can only be identified in two species, it appears that a multivoltine habit, specifically in June and September, is the common trend in this community.

Insect seasonality is largely influenced by temperature and host plant availability (Wolda, 1988). However, because leafhoppers have such close associations with host plants, phenology may be more reliant on the availability of food and brood resources. Some leafhoppers oviposit on host plants at specific times of the year. For example, species that oviposit on woody plants, such as *R. debilis*, are predicted to have their major reproductive period in the beginning of the season when they can access growing tissues that have not yet developed protective wood (Oman, 1949). *Dikraneura mali* was the only taxon with an observable phenology that appeared most prominently in the second half of the season. As this taxon is a generalist on grasses, host plant availability may not be as great of an influence on phenology. It is possible that this seasonal niche differentiation allows for the abundance of *D. mali*, however there is little evidence that interspecific competition is a significant determinant of seasonality in leafhoppers (Wolda, 1988).

A smaller third abundance peak of *D. mali* in late July/early August of 2021 but not in 2020 could be an example of phenotypic plasticity in voltinism. Altered voltinism may be of benefit during periods of high temperature, where high developmental rates allow another brood cycle within the season (Macgregor *et al.*, 2019). Macgregor *et al.*, (2019) demonstrated that the phenotypic plasticity associated with multivoltine species of Lepidoptera can increase abundance over the season. Overall, abundance was higher in 2021 and it is noteworthy that this year also experienced higher temperatures than 2020. With only two years of data on interannual variation, it is not possible to discern whether this trend is an effect of natural variation or environmental conditions.

Species richness was the only significant temperature-related diversity variable within years. Most leafhopper species in the community, despite their varying life histories, favour an occurrence that peaks in June, contemporaneous with high temperatures. Although a causal relationship cannot be determined, it may be that most leafhoppers favour a phenology associated with high temperatures that favour nymphal development times and survival (Kong *et al.*, 2019). In a study of the spatial biodiversity of urban heat islands, McGlynn *et al.* (2019) found higher fly species diversity correlated positively only with temperature over other biotic or abiotic factors. As the period of highest species diversity was the most consistent period of community composition between years, it is likely representative of a consistent pattern in the seasonality of this community.

Leafhopper species richness can be negatively correlated with the management intensity of an area and used as an indication of a healthy grassland habitat (Nickel and Hildebrant, 2003). As species richness estimates on a small spatial scale in this climate are rare, it is difficult to evaluate the relative biodiversity of our sample site. Saguez *et al.* (2014), in their survey of leafhoppers present in vineyards of British Columbia, collected 91 species from 22 sites across the province over three years. Vineyards may hold more abundance of leafhoppers than surrounding non-agricultural areas and are often rich in generalist species (Moya-Raygoza, 2020). While some species benefit from abundant but narrow food resources in agricultural areas, leafhopper diversity is often lower in monoculture than natural systems (Moya Raygoza, 2020). Our single urban sample site holds around ¼ of the richness of provincewide estimates of vineyard leafhoppers.

Leafhoppers have nearly worldwide ecological importance and as such are used as indicator species in the study of ecosystem health across other parts of the temperate hemisphere. Nickel and Hildebrant (2003) used leafhopper species richness as an indicator of the intensity or sustainability of land use in agricultural grassland areas of Germany. They concluded that some agricultural land uses, such as inconsistent cattle grazing, can boost leafhopper diversity by making the landscape more heterogenous. Some agricultural and urban activity is not necessarily detrimental to ecosystem health and the diversity of species found at this urban site may corroborate this (Nickel and Hildebrant, 2003; Ardanuy Gabarra, 2017).

This sampling effort likely encompasses the full range of the leafhopper season, between early May and late October. Because leafhoppers often rely on day length as signal for exiting diapause, this is expected to be consistent and future efforts at determining leafhopper diversity in Metchosin should concentrate their sampling in this range (Wolda, 1988).

Dryinid parasitism on Dikraneura mali and Ribautiana debilis

An important component shaping community dynamics of leafhoppers are their parasitoids. In the sample community, two dryinids, one that parasitizes both *R. debilis* and *D. mali* and one that parasitizes morphospecies B, show distinct seasonal trends. In addition to molecular data, the distinct seasonality of parasitism rates in these host species supports the existence of two dryinid parasitoids. It appears that both species of dryinids collected in Metchosin samples are multivoltine, with a bimodal distribution of parasitism rates. The second generation of dryinids are those performing higher parasitism rates than the first. Jervis (1980) observed this pattern in bivoltine *Aphelopus* species, where the second generation in a season appears more active in parasitoid habits, though this pattern has not been explained.

The dryinid parasitoid of *D. mali* and *R. debilis* is of unknown identity. The apparent developmental stages of this dryinid are distinct in size and color, representing larval growth and development of the thylecium, a hardened sac which protects the larva in its final stages (Buntin, 1989). Both *R. debilis* and *D. mali* are of the family Typhlocybinae but are of different tribes (Dmitriev, 2022). This parasitoid may be a generalist to the level of subfamily, concurring with evidence of generalist parasitoid habits in many dryinids (Guglielmino *et al.*, 2013). Dryinids of the genera *Aphelopus* and *Neodryinus* are known to parasitize the Typhlocybinae (Guglielmino *et al.*, 2013). The most recent catalogues describing host associations identifies only one species of dryinid, *Aphelopus albopictus*, known to parasitize the genus *Dikraneura* (Guglielmino *et al.*, 2013). *Ribautiana debilis* has no previously known dryinid parasitoid; however, *R. tenerrima* and *R. ulmi* are known to be parasitized by two other *Aphelopus* species. Alignment of the unknown dryinid COI partial sequence did not return high similarity to any of these three

Aphelopus parasitoids. Therefore, the dryinid found here likely represents a new host record for its association with *D. mali* and *R. debilis*. It cannot be said with certainty that it represents an unknown dryinid species, as COI databases remain incomplete (Tribull, 2015; Guglielmino *et al.,* 2013).

Parasitoids are often important regulators of leafhopper abundance in agricultural systems where host abundance is high (Moya-Raygoza, 2020). In wild systems, parasitism rates are often much lower, with many determined as less than 10% and at this level dryinids may not considered to be a major natural enemy in a community (Buntin, 1989). High dryinid parasitism rates can be considered to exceed 20%; rates in *M. quadrilineatus* have been recorded as high as 37% in fall sampling of a Manitoban population (Hagel and Landis, 1967). In the present study, high parasitism rates are observed in *R. debilis* and morphospecies B, peaking at 39% and 58%, respectively. Average rates of parasitism are low in the sample population of *D. mali*, peaking at ~14% in August and September of 2020.

Because parasitoids often have a broad range of available hosts, it is thought that host choice may simply be a function of the ease of finding a host, i.e. dependent on the host's abundance in the population (Hassell, 2001). This may be especially true for dryinids as the females are usually flightless (Mita *et al.*, 2013). Neither the dryinid that parasitizes *R. debilis* and *D. mali* nor that of morphospecies B showed significant correlation between larval parasitoid abundance and the abundance of their hosts. Some dryinids parasitize other abundant hemipterans, such as planthoppers, alongside leafhoppers; it could be that the abundance of other hosts determines their density (Guglielmino *et al.*, 2013). Moya Raygoza (2020) observed parasitoid wasps to be density-dependent in their selection of hosts in a maize monoculture but less so in an adjacent wild habitat. Biotic factors other than abundance, such as the plant structure of communities, may also influence the host selection and finding ability of dryinids.

Parasitoids may affect competition between sympatric species by favouring parasitism on dominant species (Hertäg and Vorburger, 2018). Here, parasitoids may be increasing the diversity of this leafhopper community by mediating coexistence between apparent competitors. Leafhoppers in the community other than *R. debilis* and *D. mali* are known to host dryinids. For example, *M. quadrilineatus* is known to be parasitized by three species over its range (Hagel and Landis, 1967). Buntin (1989) found that leafhoppers with less than 20 individuals in a sample were not found to be parasitized, despite relationships with dryinids being known. Therefore, sampling bias may have affected the dryinid species found in this community.

Parasitism can affect annual cycles in insect abundance by producing low survival rates in hosts and reducing abundance in the following year (Hassell, 2001). If this were the case in our community, we would expect to see high parasitism correlated with high abundance, and vice versa. Instead, parasitism rates and raw numbers decreased in 2021 despite the high numbers and apparent reproductive success at the end of the previous season. As well, numbers of leafhopper hosts increased in 2021, ruling out a 'boom-and-bust'-type response of host and parasitoid (Hassell, 2001). In 2020, a year with 15% less leafhoppers, numbers of larval parasitoids were ~3x higher, resulting in much higher overall rates of parasitism than found in 2021. These trends could be accounted for if there was an unseen mortality event of dryinids post-sampling in fall or winter 2020 that reduced the number of parasitoids in the following year. Dryinids overwinter as prepupae in the substrate, with a hardened larval cocoon to protect them from their external environment but are likely still vulnerable to abiotic stress (Jervis, 1980). Environmental factors can have extreme effects on the survival rates of insects during diapause, especially precipitation and very cold temperatures (Bale and Hayward, 2010). Longer temporal surveys may elucidate factors contributing to interannual parasitism cycles in this community.

Wolbachia and Rickettsia of Dikraneura mali and Ribautiana debilis

I found symbionts in each of *D. mali* and *R. debilis* that are often facultative in insects and have known defensive functions. Obligate symbionts known to occur in other leafhopper subfamilies were not found in *D. mali* or *R. debilis*. This concurs with the lack of known occurrence of obligate symbionts in the parenchyma-feeding leafhopper family, Typhlocybinae (Bennett and Moran, 2013).

Ribautiana debilis contains a *Wolbachia* that shares strong sequence identity with various *Wolbachia pipientis* strains in arthropods. *Wolbachia* are widespread symbionts in insects, though not always due to the provision of beneficial service; they are well known for securing their maintenance in a population by manipulating the reproductive success of their hosts (Weeks *et al.*, 2007). Defensive functions of *Wolbachia* symbionts have been reported in *Drosophila* *melanogaster*, where a single strain of *Wolbachia* protects against multiple viruses, significantly increasing host survival after viral infection (Hedges *et al.*, 2008). *Wolbachia* has been reported in leafhoppers as well as dryinids previously and their influence on host-parasitoid relationships deserves further investigation (Wangkeeree *et al.*, 2020; Duplouy *et al.*, 2015; Espinosa *et al.*, 2017). However, with the data gathered no conclusions can be drawn about the relationship of *Wolbachia* to *R. debilis*.

All *Rickettsia* found in phytophagous insects are facultative symbionts (Perlman *et al.*, 2006). Significant matches to *Rickettsia* in *D. mali*, including those from *Bemisia tabaci* and various ticks, represent *Rickettsia bellii*-like insect endosymbionts (Gottlieb *et al.*, 2006, Binetruy *et al.*, 2020). *Rickettsia* have been shown to provide defensive functions against pathogens in insects. In *B. tabaci*, endosymbiotic *Rickettsia* protect against pathogenic bacteria (Hendry *et al.*, 2014). Besides the known defensive function of *Hamiltonella defensa* (Oliver *et al.*, 2003), some aphids contain *Rickettsia* sp. symbionts that reduce infection frequencies of pathogenic fungi (Łukasik *et al*., 2013). *Rickettsia* endosymbionts have been previously reported in deltocephaline leafhoppers, but not in the subfamily Typhlocybinae.

Infection frequencies of defensive symbionts can vary between and within host species; a planthopper symbiont that had a positive correlation with rates of parasitism occurred with up to 75% frequency in different populations (Hansen *et al*., 2007). With 100% infection frequency in a putatively uninfected subset of the host population, the data I gathered indicate the possibility of a strong symbiotic association of this *Rickettsia* with *D. mali*. Ishii *et al.* (2014) also reported 100% frequency of *Rickettsia* endosymbionts in laboratory populations of *M. quadrilineatus*. However, the identity of my *Rickettsia* sequences were not confirmed by sequencing, therefore it remains unclear the exact identity of the putative *Rickettsia* surveyed in the unparasitized *D. mali* population. While leafhoppers chosen for this experiment were free of ectoparasitoids, it also remains possible that individuals collected for these surveys contained the early internal stages of dryinid larvae.

Symbiont status is often inferred from identifying a microbe's presence in the eggs of its host, indicating probable vertical transmission and therefore its maintenance in the population (Binetruy *et al.*, 2020). When a symbiont's infection frequency is related significantly and positively to the occurrence of a natural enemy, a defensive function can be inferred. No such relationship was tested for in this study, making the relationship of this *Rickettsia bellii*-like microbe to *D. mali* uncertain. With such high infection frequencies, a beneficial symbiotic relationship of this *Rickettsia* to its host deserves further investigation.

References

Ardanuy Gabarra, A. (2017). *A trophic chain as indicator of agricultural practice chain in maize*. [Unpublished doctoral dissertation]. Universitat de Lleida.

Bale, J. S. & Hayward, S. A. L. (2010). Insect overwintering in a changing climate. *Journal of Experimental Biology, 213*(6), 980–994. doi.org/10.1242/jeb.037911

Ball, E. D. & Delong, D. M. (1925). The genus *Dikraneura* and its allies in North America. *Annals of the Entomological Society of America, 18*, 324–340.

Beecher Bay First Nation. Sc'ianew First Nation. (2022). https://beecherbay.ca/

Bennett, G. M. & Moran, N. A. (2013). Small, smaller, smallest: The origins and evolution of ancient dual symbioses in a phloem-feeding insect. *Genome Biology and Evolution*, *5*(9), 1675–1688. doi.org/10.1093/gbe/evt118

Binetruy, F., Buysse, M., Barosi, R. & Duron, O. (2020). Novel *Rickettsia* genotypes in ticks in French Guiana, South America. *Scientific Reports, 10,* 2537. doi.org/10.1038/s41598-020-59488-0

Buntin, G. D. (1989). Dryinid (Hymenoptera: Dryinidae) parasites of leafhoppers and planthoppers (Homoptera) in forage-type bermudagrass. *Journal of the Kansas Entomological Society*, *62*(4), 602–606.

Chisté, M. N., Mody, K., Kunz, G., Gunczy, J, & Blüthgen, N. (2018). Intensive land use drives small-scale homogenization of plant- and leafhopper communities and promotes generalists. *Oecologia, 186,* 529–540. doi.org/10.1007/s00442-017-4031-0

Chua, T. H., & Dyck, V. A. (1982). *Assessment of* Pseudogonatopus lavifemur *E. & H. (Dryinidae: Hymenoptera) as a biocontrol agent of the rice brown planthopper*. Proceedings of the International Congress in Tropics 1–4: 253–265.

District of Metchosin. (2012). *Agriculture*. District of Metchosin. Retrieved March 18, 2022, from http://www.metchosin.ca/content/agriculture

Dmitriev, D. A. (2022). 3I interactive keys and taxonomic databases. dmitriev.speciesfile.org/

Douglas, A. E. (2010). *The symbiotic habit*. Princeton University Press. (pp. 5-17)

Downes, W. (1918). Notes on a collection of Hemiptera. *Proceedings of the Entomological Society of British Columbia, 12*.

Duplouy, A., Couchoux, C., Hanski, I., & van Nouhuys, S. (2015) *Wolbachia* infection in a natural parasitoid wasp population. *PLOS ONE, 10*(8), e0134843. doi.org/10.1371/journal.pone.0134843

Espinosa, M. S., Virla, E. G., & Cuozzo, S. (2017). *Wolbachia* infections responsible for thelytoky in dryinid wasps. The case of *Gonatopus bonaerensis virla* (Hymenoptera: Dryinidae). *Neotropical Entomology, 46*(4), 409-413. doi:10.1007/s13744-016-0475-x

Forero, D. (2008). The systematics of the Hemiptera. *Revista Colombiana De Entomología, 34,* 1–21.

Gottlieb, Y., Ghanim, M., Chiel, E., Gerling, D., Portnoy, V., Steinberg, S., *et al.* (2006). Identification and localization of a Rickettsia sp. in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Applied and Environmental Microbiology*, *72*(5), 3646–3652. doi.org/10.1128/AEM.72.5.3646-3652.2006

Guglielmino, A., Olmi, M., & Bückle, C. (2013). An updated host-parasite catalogue of world Dryinidae (Hymenoptera: Chrysidoidea). *Zootaxa* 3740, 1.

Hagel, G. T. & Landis, B. J. (1967). Biology of the aster leafhopper, *Macrosteles fascifrons* (Homoptera: Cicadellidae), in eastern Washington, and some overwintering sources of aster yellows. *Annals of the Entomological Society of America, 60,* 591–595.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., *et al.* (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLOS ONE, 12*, e0185809. doi:10.1371/journal.pone.0185809

Hamilton, K. G. A. (1983). Introduced and native leafhoppers common to the old and new worlds (Rhynchota: Homoptera: Cicadellidae). *The Canadian Entomologist, 115*: 473-511.

Hamilton, K. G. A. (2002). Homoptera (Insecta) in Pacific Northwest grasslands. Part 1 - New and revised taxa of leafhoppers and planthoppers (Cicadellidae and Delphacidae). *Journal of the Entomological Society of British Columbia,ha 99*, 3-31.

Hamilton, K. G. A. (2014). Canadian grasslands and their endemic leafhoppers (Hemiptera: Auchenorrhyncha: Cicadellidae). In H. A. Cárcamo & D. J. Giberson (Eds.), *Arthropods of Canadian grasslands (volume 3): biodiversity and systematics part 1*. Biological Survey of Canada. (pp. 311–345). doi.org/10.3752/9780968932162.ch11

Hansen, A. K., Jeong, G., Paine, T. D. & Stouthamer, R. (2007). Frequency of secondary symbiont infection in an invasive psyllid relates to parasitism pressure on a geographic scale in California. *Applied and Environmental Microbiology, 73*, 7531–7535.

Hassell, M. P. (2000). Host-parasitoid population dynamics. *Journal of Animal Ecology, 69,* 543–566.

He, J., He, Y., Lai, F., Chen, X., & Fu, Q. (2020). Biological traits of the pincer wasp *Gonatopus flavimeur* (Esaki & Hashimoto) associated with different stages of its host, the brown planthopper, *Nilaparvata lugens* (Stål). *Insects 2020, 11*, 279. doi:10.3390/insects11050279

Hedges, L. M., Brownlie, J. C., O'Neill, S. L., Johnson, K. N. (2008). *Wolbachia* and virus protection in insects. *Science, 322*(5902), 702. doi:10.1126/science.1162418

Hendry, T. A., Hunter, M. S., Baltrus, D. A., & Schloss, P. D. (2014). The facultative symbiont *Rickettsia* protects an invasive whitefly against entomopathogenic *Pseudomonas syringae* strains. *Applied and Environmental Microbiology, 80*(23), 7161-7168.

Hertäg, C. & Vorburger, C. (2018). Defensive symbionts mediate species coexistence in phytophagous insects. *Functional Ecology, 32*, 1057– 1064. doi.org/10.1111/1365-2435.13040

Ishii, Y., Matsuura, Y., Kakizawa, S., Nikoh, N., & Fukatsu, T. (2013). Diversity of Bacterial Endosymbionts Associated with Macrosteles Leafhoppers Vectoring Phytopathogenic Phytoplasmas. *Applied and Environmental Microbiology, 79*(16), 5013-5022. doi.org/10.1128/AEM.01527-13

Jervis, M. A. (1980). Life history studies on *Aphelopus* species (Hymenoptera, Dryinidae) and *Chalarus* species (Diptera, Pipunculidae), primary parasites of typhlocybine leafhoppers (Homoptera, Cicadellidae). *Journal of Natural History 14***,** 769–780.

Keene, K., Malmstrom, C. M., Alexander, H. M., Wayadande, A., & Denning, K. R. (2020). Low conservatism of leafhopper communities in remnant and reconstructed prairie sites in a working agroecological landscape. *Journal of Insect Conservation 24*, 35–48.

Knight, W. J. (1987) Leafhoppers of the grass-feeding genus *Balclutha* (Homoptera, Cicadellidae) in the Pacific region. *Journal of Natural History, 21*(5), 1173-1224. doi: 10.1080/00222938700770731

Larsen, K. J., Madden, L. V., & Nault, L. R. (1990). Effect of temperature and host plant on the development of the blackfaced leafhopper. *Entomologica Experimentalis et Applicata 55*(3), 285–294. doi:10.1111/j.1570-7458.1990.tb01373.x

Łukasik, P., Guo, H., Van Asch, M., Ferrari, J., & Godfray, H. C. J. (2013). Protection against a fungal pathogen conferred by the aphid facultative endosymbionts *Rickettsia* and *Spiroplasma* is expressed in multiple host genotypes and species and is not influenced by co-infection with another symbiont. *Journal of Evolutionary Biology 26*, 2654–2661. doi: 10.1111/jeb.12260

Kong, J. D., Hoffmann, A. A., & Kearney, M. R. (2019). Linking thermal adaptation and life-history theory explains latitudinal patterns of voltinism. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 374, 20180547.

Macgregor, C. J., Thomas, C. D., Roy, D. B., Beaumont, M. A., Bell, J. R., Brereton, T., *et al.* (2019). Climate-induced phenology shifts linked to range expansions in species with multiple reproductive cycles per year. *Nature Communications 10,* 4455.

Madeira, F., Pearce, M., Tivey, A.R.N., Basutkar, P., Lee, J., Edbali, O., *et al.* (2022). Search and sequence analysis tools services from EMBL-EBI in 2022. Nucleic Acids Research. doi: 10.1093/nar/gkac240

Maw, H. E. L., Foottit, R. G., Hamilton, K. G. A., & Scudder, G. G. E. (2000). *Checklist of the Hemiptera of Canada and Alaska*. NRC/CNRC Research Press

McGlynn, T. P., Meineke, E. K., Bahlai, C. A., Li, E., Hartop, E. A., Adams, B. J., & Brown, B. V. (2019). Temperature accounts for the biodiversity of a hyperdiverse group of insects in urban Los Angeles. *Proceedings of the Royal Society B: Biological Sciences, 286*(1912), 20191818. doi:10.1098/rspb.2019.1818

Mita, T., Sanada-Morimura, S., Matsumura, M., & Matsumoto, Y. (2013). Genetic variation of two apterous wasps *Haplogonatopus apicalis* and *H. oratorius* (Hymenoptera: Dryinidae) in East Asia. *Applied Entomology and Zoology, 48,* 119–124.

Moya-Raygoza, G., Palomera-Avalos, V., Chacon-Torres, N. M. & Becerra-Chiron, I. M. (2006). The parasitoid *Gonatopus bartletti* reduces presence of plant-pathogenic *Spiroplasma kunkelii* within the leafhopper vector *Dalbulus maidis*. *Entomologia Experimentalis et Applicata, 119,* 189–196. doi.org/10.1111/j.1570-7458.2006.00409.x

Moya-Raygoza, G. (2020). Diversity and density-dependence relationship between hymenopteran egg parasitoids and the corn leafhopper (Hemiptera: Cicadellidae) in maize agroecosystem vs. teosinte wild habitat. *Florida Entomologist, 103*(1), 48-53. doi.org/10.1653/024.103.0408

Nikel, K. (2019). *Estimating flying insect biomass and its spatiotemporal distribution in Metchosin: Biodiversity on Southern Vancouver Island*. [Unpublished honour's thesis]. University of Victoria.

Nielson, M. & Knight, W. (2000). Distributional patterns and possible origin of leafhoppers (Homoptera, Cicadellidae). *Revista Brasileira de Zoologia, 17*(1), 81-156.

Oliver, K. M., Russell, J. A., Moran, N. A., & Hunter, M. S. (2003). Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences, 100*, 1803–1807.

Oliver, K. M., Smith, A. H., & Russell, J. A. (2014). Defensive symbiosis in the real world - advancing ecological studies of heritable, protective bacteria in aphids and beyond. *Functional Ecology, 28,* 341–355.

Oman, P. W. (1949). The Nearctic leafhoppers (Homoptera: Cicadellidae): A generic classification and check list. *Annals of the Entomological Society of America, 43(*3), pp. 7-18.

Perlman, S. J., Hunter, M. S., & Zchori-Fein, E. (2006). The emerging diversity of *Rickettsia. Proceedings of the Royal Society B: Biological Sciences, 273,* 2097–2106. doi.org/10.1098/rspb.2006.3541

R Core Team. (2018). *R: a language and environment for statistical computing*. R. Foundation for Statistical Computing. https//www.Rproject.org/

Raine, J. (1960). Life history and behavior of the bramble leafhopper, *Ribautiana tenerrima* (H.-S.) (Homoptera: Cicadellidae). *The Canadian Entomologist, 92*(1), 10-20. doi:10.4039/Ent9210-1

Redak, R. A., Purcell, A. H., Lopes, J. R., Blua, M. J., Mizell, R. F., 3rd, & Andersen, P. C. (2004). The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. *Annual Review of Entomology, 49,* 243–270. doi.org/10.1146

Saguez, J., Olivier, C., Hamilton, A., Lowery, T., Stobbs, L., Lasnier, J., Galka, B., Chen, X., Mauffette, Y., & Vincent, C. (2014). Diversity and abundance of leafhoppers in Canadian vineyards. *Journal of Insect Science* 14(73), 1-20. doi.org/10.1673/031.014.73

Schmitt, L. & Burghardt, K. T. (2021). Urbanization as a disrupter and facilitator of insect herbivore behaviors and life cycles. *Current opinion in insect science, 45,* 97–105. doi.org/10.1016/j.cois.2021.02.016

Shi, S. S., Zang, L. S., Liu, T. X., Ruan, C. C., & Sun, G. (2009). Host-feeding behaviors of parasitoids on hosts and implications for biological control. *Acta Entomologica Sinica, 52,* 424–433

Statistics Canada. (2022). *Population and dwelling counts: Canada and census subdivisions (municipalities)*. Statistics Canada. www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=9810000201

Sudakaran, S., Kost, C., & Kaltenpoth, M. (2017). Symbiont acquisition and replacement as a source of ecological innovation. *Trends in Microbiology, 25*(5), P375-390. doi.org/10.1016/j.tim.2017.02.014

Taylor, P. S. & Shields, E. J. (1995). Development of migrant source populations of the potato leafhopper (Homoptera: Cicadellidae). *Environmental Entomology, 24,* 1115–1121. doi:10.1093/ee/24.5.1115

Tribull, C. M. (2015). Phylogenetic relationships among the subfamilies of Dryinidae (Hymenoptera, Chrysidoidea) as reconstructed by molecular sequencing. *Journal of Hymenoptera Research, 45,* 15–29. doi:10.3897/ JHR.45.5010

Vulinec, K., Swift, K., Balke, V., Sweitzer, L., Sturgis, B., & Powell, D. (2017). Buckyballs and bats: trace evidence points to trophic interactions. *Frontiers in Ecology and the Environment, 15,* 108–109.

Wangkeeree, J., Tewaruxsa, P., Roddee, J., & Hanboonsong, Y. (2020). *Wolbachia* (Rickettsiales: Alphaproteobacteria) infection in the leafhopper vector of sugarcane white leaf disease. *Journal of Insect Science, 20*(3), 20. doi.org/10.1093/jisesa/ieaa053

Weeks, A. R., Turelli, M., Harcombe, W. R., Reynolds, K. T., & Hoffmann, A. A. (2007). From parasite to mutualist: Rapid evolution of *Wolbachia* in natural populations of drosophila. *PLOS Biology*, *5*(5), e114. doi.org/10.1371/journal.pbio.0050114

Wolda, H. (1988). Insect seasonality: Why? *Annual Review of Ecology and Systematics*, *19*(1), 1-18. doi.org/10.1146/annurev.es.19.110188.000245