PROGRESS REPORT FORM – 2014

1.	Principal Investigator's Family Name: Wist	Given Names: Tyler	Position: Research Scientist	Date: Aug 15 2024
	Institution: AAFC SRDC		Department: Entomology	
	Address: 107 Science Place		City: Saskatoon	
	Postal Code: S7N 0X2	Phone: 306-251-2592	Fax:	E-Mail: Tyler.Wist@agr.gc.ca
2.	Title of Research Project: *Continuing to watch the winds: the origin and arrival of migrant aster leafhoppers and diamondback moths.* AAFC Reference AGR-17913; WGRF # AGR2105; SaskCanola # CARP ADF2020.409			
3.	Start Date: March 1 2021	Completion Date: Aug 15 2024		

1. Name of the Principal Investigator and contact information.

Dr. Tyler Wist, Research Scientist

Saskatoon Research and Development Centre

Agriculture and Agri-Food Canada

107 Science Place

Saskatoon, SK, S7N 0X2

Telephone: (306) 251-2592

Email: Tyler.Wist@agr.gc.ca.ca

2. Name of the collaborators and contact information

Dr. Sean Prager

University of Saskatchewan Department of Plant Sciences

Saskatoon, SK, S7N 5A2

Telephone: (306) 966-8359

Email: sean.prager@usask.ca

Dr. Tim Dumonceaux

Saskatoon Research and Development Centre

Agriculture and Agri-Food Canada

107 Science Place

Saskatoon, SK, S7N 0X2

Telephone: (306) 385-9450

Email: Tim.Dumonceaux@agr.gc.ca

Dr. Chrystel Olivier

Saskatoon Research and Development Centre

Agriculture and Agri-Food Canada

107 Science Place

Saskatoon, SK, S7N 0X2

Telephone: (306) 385-93

Email: Chrystel.Olivier@agr.gc.ca

Dr. Keith Hobson

University of Western Ontario Department of Biology

Biological & Geological Sciences Building London, ON N6A 5B7

Telephone: (519) 661-2111

Email: khobson@uwo.ca

Dr. Jose A. Andres, Faculty Fellow
Cornell Atkinson Center for Sustainability
Cornell University
200 Rice Hall | 340 Tower Road

Cornell University
Ithaca, NY 14853
Email: jaa53@cornell.edu

Dr. Jack Gray
Department of Biology
University of Saskatchewan
Saskatoon Saskatchewan.
Email: Jack Gray@usask.ca.

Please complete the following sections and attach any relevant tables and/or charts.

(a)

Abstract/Summary – Maximum of one page in lay language. This must include project objectives, results, and conclusions for use in publications and in the **WGRF** database.

3. Abstract/Summary: Include activities during the progress period and status of the Research Project (is it on schedule) and any significant observations related to the progress of the Project.

Each year, aster leafhoppers (ALH), *Macrosteles quadrilineatus* (Hemiptera: Cicadellidae) migrate into Western Canada, bring in with them the aster yellows phytoplasma (AYp) that causes aster yellows (AY) disease in canola, *Brassica napus* (Brassicaceae), and really, in every other field crop in North America. Currently, the only crop that is monitored consistently for aster yellows disease by the three Prairie provinces is canola, and the data generated by the yearly aster yellow's survey is invaluable and was used in this project, with thanks to our provincial Ag Ministries. Some years however, aster leafhoppers migrate, and we do not have a subsequent outbreak of aster yellows in canola or other crops. We witnessed this phenomenon in 2020, where large numbers of ALH arrived in Western Canada, but aster yellows was not rampant across the canola growing regions. Two back to back projects on this phenomenon, with an extended year of sampling have given us much insight into the factors that have to align to create aster yellows outbreaks. In addition, we looked at another migratory insect that afflicts canola, the Diamondback moth (DBM). Diamondback moth haplotyping did not reveal any insights into their source locations, but did reveal a dominant haplotype that the majority of our tested moths could be classified into based on single nucleotide polymorphisms in their CO1 genetic barcoding region. This dominant haplotype likely indicates that each year there is a common source for migrating DBM into Saskatchewan.

In this project, we have created and applied a novel molecular tool, developed for aster leafhoppers (ALH), that gives unique insight into the ALH nuclear makeup and is the first attempt at revealing the unexplored ALH population structure in North America. The manuscript describing the genetics is ready for submission. It, and this report, detail a microsatellite approach consisting of 22 markers that we developed through this project to evaluate population structure to determine if source populations of migratory aster leafhoppers could be determined based on molecular markers. Through this, we hoped to pinpoint the origins of migratory aster leafhoppers arriving in Saskatchewan each year, but have instead revealed that the migratory ALH population has no discernable genetic structure, likely from their

migratory nature and mixing of genes. Two other techniques, to determine the migratory origins of these insects fared better in helping us determine where the leafhoppers that came into Western Canada each year, originated. Wind, and stable isotope results indicate a great plains migration event for the aster leafhoppers while winds indicate that diamondback moth came from the Pacific Northwest, and likely Oregon and Washington. The migrations of these two insect pests do not seem to be closely linked and therefore, ALH cannot be predicted by DBM pheromone trapping. DBM in 2022 migrated after the typical 6-weeks trapping period but with the Sask Ministry of Agriculture extending the trapping period that year and subsequent years, a later migration of DBM was captured on yellow sticky cards. In 2023, spring DBM migrants were noted on sticky cards and populations of larvae increased in our sentinel canola fields. Larval populations of DBM were evident in Saskatchewan canola in all project years, with 2023, having the highest population in all project years. DBM haplotyping revealed a dominant haplotype in each year and across all seasons, but Barcode of Life data base searches of it's source location revealed that the dominant haplotype is been found in multiple source locations in the US.

The winter wheat growing region of the Great Plains is likely the first stop on the Northward migration for the aster leafhoppers, and their heavy hydrogen isotopic profiles most closely match to that latitude. Southern winds that track back 24 to 48 hours and travel close to the ground (5 m Above Ground Level) also corroborate that region as the likely source of the aster leafhoppers that migrate each year into Western Canada. However, the migratory patterns and deuterium ratios in this and our last project, suggested that in one year, one long migration from the Gulf States occurred and that in other years, adult aster leafhoppers can spend two weeks in Montana and the Dakotas before moving into Canada on a short northward migration. So, the migratory path is not always the same each spring. In Western Canada, we should start looking for aster leafhopper migrations around the May long weekend each year with subsequent testing of migrant populations for the presence of phytoplasma. Alfalfa fields and roadside ditch grasses are the places that sustain aster leafhoppers until crops are large enough for them to move into. Sampling these sites of migrant leafhopper concentration in the spring gives the best chance to understand the risk of aster yellows that growing season.

Several perennial and biennial plants, including alfalfa, were identified as positive with aster yellows prior to any aster leafhopper activity in Saskatchewan indicating that these had been infected with AYp the previous year and carried over the infection from one growing season to the next as a potential "green bridge" between seasons. Population monitoring of the F1, or offspring generation of the migratory leafhoppers continues in each year and reveals a clear difference in isotopic values from the migratory generation. The 2021 and 2022 aster leafhopper migrations were not large ones but the 2023 migration, was a large migration with many infected individuals. We used the LAMP assay developed in the last project (WGRF project # AGR 14988) to determine the AYp infection status of individual ALH in all of these years, and this test is sensitive enough to use on single ALH. AY disease incidences in fields in 2021 and 2022 were not very significant, but they were 10x higher in 2023 than in most other years with 65% of all fields in Saskatchewan sampled showing evidence of AY disease with an incidence from 1 to 36% of plants. Sporadic outbreaks of AY in Western Canada are likely tied to drought in the Great Plains winter wheat growing regions that force the leafhoppers onto broadleaf weeds that are AYp reservoirs before they migrate into Canada.

Frequent Abbreviations used:

ALH=Aster leafhoppers

DBM=Diamondback moth

AY= aster yellows

AYp= aster yellows phytoplasma

Introduction

Aster leafhoppers, *Macrosteles quadrilineatus* Forbes, (Hemiptera: Cicadellidae) and diamondback moths (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae), migrate to the Canadian Prairies in spring and early summer on wind currents originating in the USA (Nichiporik 1965, Hopkinson and Soroka, 2009, Bertaccini et al. 2019). Neither of these insects overwinters well in cold-Canadian winters so infestations of these insects are tied to migration on the winds. Early detection and monitoring of both migratory insects is essential. Catching aster leafhopper populations early is crucial for aster yellows management, because aster leafhoppers vector the pathogen Aster Yellows phytoplasma (AYp) (16SrI subgroup) (Bertaccini et al. 2019) which causes Aster Yellows (AY) disease and affects crops across the Prairies including cereals (less susceptible) and oilseeds (most susceptible) such as canola and flax. Monitoring of the aster leafhopper migrations has been ongoing since 2018 in the previously funded WGRF project AGR 14988 at the AAFC Saskatoon Research and Development Centre while DBM monitoring has been ongoing since 1997 through the Provincial Monitoring Network. In that project, we aimed to determine the origin of the aster leafhoppers that migrate into Saskatchewan using a combination of reverse trajectory monitoring from the Prairie Pest Monitoring Network (and eventually HYSPLIT), genetic evaluation of the migrant leafhoppers and determination of the stable isotope ratios of the migrant and F1 (and potentially, F2), generations of aster leafhoppers, and to associate the arrival of aster leafhoppers with the arrival of diamondback moths using the pre-existing pheromone monitoring network administered by the province of Saskatchewan. More years of data will increase the precision of our predictions on the probable origins of both the migrating aster leafhoppers and diamondback moths, especially since each year basically represents one replication if there is only one discernable migration. Much of the work on the development of an early warning system for AY was focusing on the spring migrant generation of aster leafhoppers as the most important generation to monitor for disease transmission. However, the F1 generation of leafhoppers produced by the migrant generation has often had higher infection rates and greatly increased population numbers than the migrants. A newly discovered and previously undetected AY infection in alfalfa as well as some perennial weeds, indicates that these perennial plants, present in ditches across Saskatchewan, could be the plant-reservoirs that infects the migrant and F1 generation of leafhoppers before they move into Canola, if the aster leafhoppers do not arrive very infected with AYp. This alfalfa “green bridge” was hypothesised in the previous project through sampling of plants and Sean Prager et al.’s project (CARP ADF 2017-2023).

Diamondback moth (*Plutella xylostella*) is a major pest of Brassica crops worldwide. It can cause significant crop losses to canola and other Brassicaceae crops in the Canadian Prairie provinces. Insecticide application is the primary control method against this insect. However, diamondback moth populations have developed resistance to a number of important insecticides, which has made management of this insect increasingly difficult and expensive resulting in more than $4B annual cost to the world economy. In the Canadian Prairie provinces, diamondback moth infestations establish yearly by annual migrations. However, moths arriving in western Canada can have varying levels of resistance to insecticides and other biological characteristics. Therefore, understanding of genetic traits of populations arriving in annual migrations will be useful in understanding and managing diamondback

moth populations. Here we propose to use established molecular genetic techniques to assess genetic variations among populations. Migrant DBM were collected from pheromone traps designed to monitor the arrival of diamondback moths from different locations in western Canada. This trapping network has been in effect since 1997 following millions of dollars in losses to DBM in 1995 (Hopkinson and Soroka 2009). We used COI (mitochondrial cytochrome-c oxidase subunit 1, cytochrome P450 and SNP (single nucleotide polymorphism) markers to assess the genetic variations. This genetic analysis has been successfully used to identify migratory DBM into BC and Alberta and the genetic sequences are available for comparison on the Barcode of Life Database so there was no need to collect DBM extensively in the United States and Mexico for genetic comparisons. These molecular techniques provide powerful tools for population studies of various insects (Behura 2006, Hoy 2019). Mitochondrial COI gene is a maternally inherited and well conserved marker for determining genetic relationships and geographical studies (Hoy 2019). Various studies analysed COI in diamondback moth populations worldwide including Canada (Shijun 2017, Saw et al. 2006). Studies revealed lack of differentiations among some geographical locations (Saw et al. 2006) and considerable variations in some others especially among those separated by long distances (Niu et al. 2014). This project helps to fill in gaps on the Barcode of Life database about migratory diamondback moth populations in Western Canada.
Therefore, this study carried on the AY monitoring and AY risk estimation, along with the wind monitoring, analysis of the genetics and stable isotope ratios of the aster leafhoppers to determine their origins in each season and to differentiate diamondback moth populations among pheromone trap catches in western Canada using COI gene analysis and SNP markers. The hypothesis of an alfalfa green bridge was also evaluated.

This project continued two of the objectives of the WGRF project AGR 14988 (2018-2021), awarded to Tyler Wist through the 2017 ADF call and expands the genetic methods to include evaluating the genetic population structure of the diamondback moth captured in the Provincial pheromone monitoring network. The current project builds on two innovations that were created in the previous project; the rapid extraction and analysis LAMP test for determining AYp status of single leafhoppers (Pusz-Bochenska et al. 2020) and the microsatellite marker approach to identifying the genetic origin of aster leafhoppers. In that previous project, set to close out in March 2021, we set out with the ultimate goal to determine the origin of the aster leafhoppers that migrate into Saskatchewan with the potential to spread the phytoplasma that causes aster yellows disease. We also monitored Aster leafhoppers for the presence of AYp and estimation of the aster yellow risk index. As well, we aimed to associate the arrival of aster leafhoppers with the arrival of diamondback moths using the pre-existing pheromone monitoring network administered by the province of Saskatchewan. The arrival of aster leafhoppers and diamondback moths on the same winds however was not well correlated though in the previous project. We carried on the AY monitoring work along with the wind monitoring, analysis of the genetics and stable isotope ratios of the aster leafhoppers to determine origins in each season. Also, confirming the presence of an alfalfa AY green bridge between the aster leafhoppers and canola crops allowed a more accurate early warning system. Adding the DBM genetic work helped to further pinpoint the origins of the migrant insect infestations.

Literature Cited (Introduction)

Behura, S.K., 2006. Molecular marker system in insects: current trends and future avenues. Mol. Ecol. 15,3087-3113

Bertaccini, A., P. G. Weintraub, G. P. Rao, and N. Mori. 2019. Phytoplasmas : Plant Pathogenic Bacteria - II Transmission and Management of Phytoplasma - Associated Diseases. Springer Singapore.

Frost, K.E., Esker, P.D., Haren, R.V., Kotolski, L. and Groves, R.L. 2013a. Factors influencing aster leafhopper (Hemiptera: Cicadellidae) abundance and aster yellows phytoplasma infectivity in Wisconsin carrot fields. Environmental Entomology 42(3): 477-490. doi:10.1603/en12239.

Frost, K.E., Esker, P.D., Van Haren, R., Kotolski, L. and Groves, R.L. 2013b. Seasonal patterns of aster leafhopper (Hemiptera: Cicadellidae) abundance and aster yellows phytoplasma infectivity in Wisconsin carrot fields. Environmental Entomology 42(3): 491-502. doi:10.1603/en12240.

Hagler, J. R., S. E. Naranjo, S. A. Machtley & F. Blackmer 2014. Development of a standardized protein immunomarking protocol for insect mark–capture dispersal research. Appl. Entomol. 138 (2014) 772–782.

Hopkinson, L.F. and Soroka, J.J. 2009. Air trajectory model applied to an in-depth diagnosis of potential diamondback moth infestations on the Canadian Prairies. Ag. Forest Meterol. 150: 1-11.

Hoy, M.A., 2019. Insect molecular genetics. An introduction to principles and applications, 4th edition Academic Press, Elsevier Inc.

Nichiporick, W. 1965. The aerial migration of the Six-Spotted Leafhopper and the spread of the virus disease Aster Yellows. Int. J. Biometeorol. 9: 219–227.

Niu, Y. Q., Nansen, C., Li, X.W, Liu, T. X. 2014. Geographical variation of *Plutella xylostella* (Lepidoptera: Plutellidae) populations revealed by mitochondrial COI gene in China. Journal of Applied Entomology. 138-139.

Pusz-Bochenska K. Perez-Lopez , E. Dumonceaux T.J., Olivier, C., Wist, T.J. 2020. A Rapid, Simple, Laboratory and Field-Adaptable DNA Extraction and Diagnostic Method Suitable for Insect Transmitted Plant Pathogen and Insect Identification. Plant Health Progress 21(1): doi.org/10.1094/PHP-09-19-0063-FI

Saw, J., Endersby, N.M., Mckechnie, S.W. 2006. Low mtDNA diversity among widespread Australian diamondback moth *Plutella xylostella* (L.) suggests isolation and a founder effect. Insect Science. 13, 365-373.

Shijun, Y., 2017. Analysis of the evolutionary patterns of genetically varied populations of diamondback moth, *Plutella xylostella* (L.) Ph.D. thesis, University of British Columbia, Canada.

4. **Methods:** Include approaches, experimental design, methodology, materials, sites, and etc. Major deviations from original work plan must be indicated and the reason(s) for the change(s) should be specified. Significant changes from the original work plan will require written approval from the WGRF.

1.Diamondback moth (DBM) genetic structure

Objective description: Assess the genetic variations of the migrant and new generation of DBM to pinpoint their origin. CO1 (mitochondrial cytochrome-c oxidase subunit 1, cytochrome P450 and SNP (single nucleotide polymorphisms) markers to assess the genetic and geographic origins of DBM.

By using the Provincial network of pheromone traps supplemented by our own traps at sentinel sites and combining genetic markers such as DNA analysis we hoped to study geographical origin, population connectivity and migration patterns of diamondback moth in Saskatchewan. In order to understand variations in DBM genetic structure, we studied the genetic make-up of diamondback moth populations arriving in spring and early summer and evaluate the temporal and spatial changes in genetic composition using genetic CO1 and SNP markers.

Cytochrome c oxidase 1 (CO1):

One line of investigation of diamondback moth populations will be to examine the populations through the analysis of sequence variation in the mitochondrial cytochrome c oxidase 1 (CO1) gene in *P. xylostella*. CO1 region is used as a global bioidentification system for animals (Hebert et. Al., 2003). It uses a primer set to amplify 438-bp region of the CO1 gene in diamondback moth by the PCR followed by sequencing. The sequence data along with diamondback moth sequence already deposited in the database will be used to determine the extent and nature of the genetic variation in diamondback moth populations.

CO1 sequence analysis will be used for haplotypes identification, phylogenetic analysis using maximum parsimony and maximum likelihood (ML) methods and genetic distance and migration estimate using population pairwise genetic distance (FST). Distance between DNA sequences will be calculated by Kimura's 2-parameters method (Kimura 1980)

Single nucleotide polymorphism (SNP)

Next generation sequencing (NGS) techniques provide powerful genotyping methods for population genetic studies in a wide range of species. Restriction-site-associated DNA sequencing (RAD-Seq) enables sequencing of targeted short regions across the genome (genome-wide) allowing identification of single nucleotide polymorphism (SNP) markers to study genetic diversity in diamondback populations. SNPs were analyzed for individual diamondback moths and compared.

In 2021, we employed bucket traps similar to the Bertha Armyworm trap to collect adult diamondback moth (DBM) because the sticky residue of the sticky cards typically used to catch DBM on pheromone traps might interfere with genetic analysis and will interfere with stable isotope analysis. In subsequent project years however, we did use DBM from sticky cards and there was no issue with the genetic tests.

2. Aster leafhopper population monitoring

Objective description: To assess the genetic variations of the migrant F1 generation of aster leafhoppers to pinpoint their origin using a microsatellite marker approach with Illumina sequencing.

Additional DBM and aster leafhoppers were sourced from several sites in the putative source locations through entomologist working for BASF as a trade for bertha armyworm moths. Unfortunately, no ALH were found in these source locations in May-June 2022. These insects were used to bolster the genetic analysis from source leafhoppers.

Our initial attempts to determine the origins of migratory aster leafhoppers using a two-region system, CO1 and NADH, revealed that all of the genetic sequences from all leafhoppers, from all locations, were identical with no different base-pairs (SNPs) at these two regions. Two hypotheses can be suggested based on those previous results. 1. The two-target approach is simply not sensitive enough to detect population differences, but population differences exist in other locations on the genome. And 2. There are no genetic differences among aster leafhopper populations due to constant genetic mixing due to migration and distinct populations cannot be determined using genetic methods. To test these two hypothesis we developed a more sensitive molecular test to evaluate if leafhoppers from different years and locations differed genetically. This analysis used leafhoppers caught in the last project as well as a few caught prior to that project to increase the potential number of migrations that we were able to evaluate. Development of the more sensitive technique is detailed in the below methods.

a) Specimen sampling

Sweep sampling and yellow sticky card traps were used to collect the aster leafhoppers (ALH). Collected specimens were removed from the cards and preserved in 70% ethanol. To understand the spatial population structure of ALH in SK we sampled 17 sites in SK as well as four reference populations in USA. 98 field-collected *Macrosteles quadrilineatus* from different locations in North America between years 2016 and 2021 were included in this study. Most of ALH were collected in SK (Fig. 2.1). Sampling sites in the USA constituted of Oregon, Wisconsin, Illinois and Kansas.

ALH populations in Saskatchewan go through a spring colonization and a winter extinction phase. While some ALH eggs may be able to survive the harsh Saskatchewan winters, no data exists on the percentage of eggs that overwinter every year. Therefore, the local recruitment is likely to be very low compared to the migration influx of LH. Since it was reported that the number of migratory ALH that arrive on winds in Saskatchewan every year differ, and we do not have data on the local ALH population from overwintering eggs, we further analyze data for each year separately.

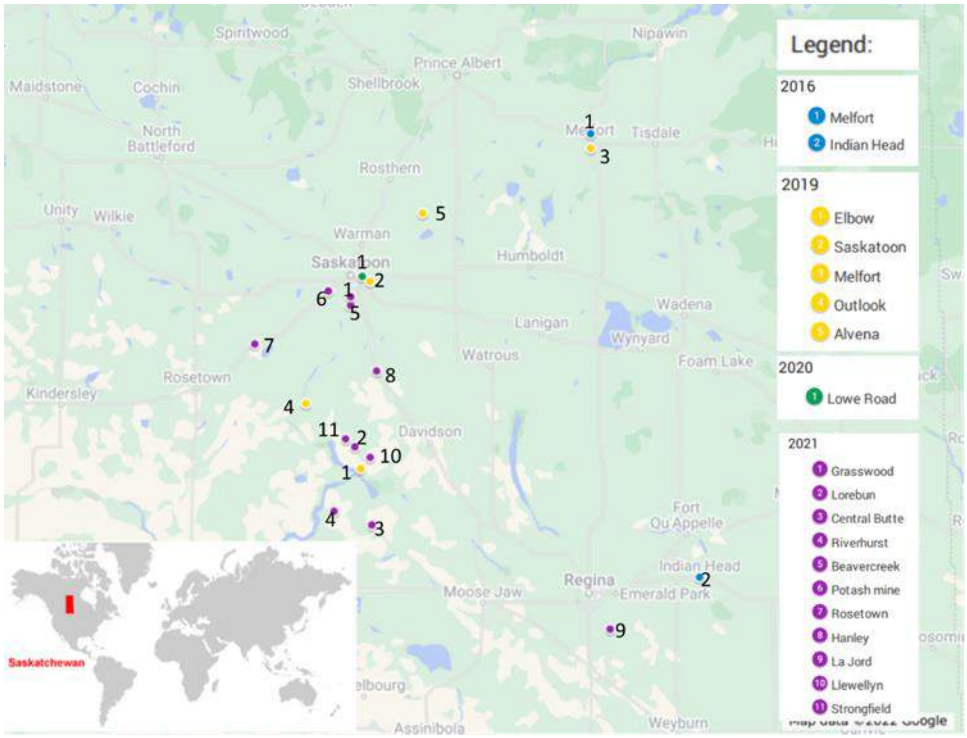


Figure 2. 1. Sample sites and number of individuals collected between years 2016 and 2021 in Saskatchewan used in the genetic analysis of population structure.

b) Microsatellite primers development

Construction and screening of a *Macrosteles quadrilineatus* genomic DNA library enriched for microsatellite loci was conducted at Cornell University in 2020, following procedures described in Rueger et al. 2021 with modifications. Briefly, genomic DNA (50-100 ng) from Agriculture Agri-Food Canada (AAFC) lab colony from five individuals was endonuclease-digested with AluI, RsaI, and Hpy166II, and pooled for subsequent adenylation with Klenow (exo-) and dATP. Restricted/adenylated DNA was then ligated to an Illumina Y-adaptor sequence using T4 DNA ligase in the presence of 1mM ATP. Genomic fragments with repeats were captured by hybridization to biotinylated repeats and streptavidin-coated magnetic beads. These genomic fragments were amplified/indexed with OneTaq polymerase and a pair of Illumina primers (one universal, one index primer). PCR products were quantified with a Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA), verified by electrophoresis on a 1.0% agarose gel, and size selected (300-600 bp) with AMPure beads (Beckman Coulter, Indianapolis, IN).

The resulting library was screened for 28 unique tetrameric motifs (pattern with four bases; e.g., ACTG, AATC, AAAC). Paired reads (2 × 250 bp) were collected on an Illumina Miseq platform at the BioResource Center at Cornell University. *De novo* contig assembly was performed with NGEN (version 11) software (DNASTAR), using Transcriptome project type. The software trimmed reads of adapter and low-quality regions with standard parameters of minimum Phred quality=20, k-mer length=99, and minimum match percentage=93. Gap penalty was set to 10 and maximum gap length was set to 50; these values were chosen because, during the assembly process, repetitive regions are inherently going to pair in length and may have alleles that vary in length. As gaps are expected for this type of data, the penalty of opening a gap should be small, and the penalty for standing the gap should be relatively low. Contigs shorter than 150 bp were not kept. The assembly generated 30692 contigs, with an N50 of 408 base-pairs and an average coverage of 18 reads.

Microsatellite repeats and associated genotyping primers were summarized with msatcommander software (Faircloth, 2008; Rozen and Skaletsky, 1999). The “design primers” function of msatcommander 1.0.3 software was used to produce repeat and primer output files. Next, contigs (microsatellites) with associated primers were selected in a semi-random manner to minimize ascertainment bias. Microsatellites with 25-30 repeats, though highly variable, were excluded because their length might exceed the sequencing capacity of MiSeq. Additionally, microsatellites with fewer than 6 repeats were rejected because they lacked variability and would not be informative. A total of 130 primer pairs were first tested on genomic DNA of 3 or 4 leafhoppers from each population across North America, resulting in 22 microsatellites that showed robust amplification across all tested populations (Table 1).

c) Multiplex genotyping

All ALH individuals were sequenced at 22 microsatellite loci using a multiplex PCR protocol for targeted amplicon sequencing (D’Aloia et al., 2017). Using newly developed primers from previous section (listed in Supplementary Table 3.), these loci were amplified with multiplex PCR reactions in 98 well plates using QIAGEN Multiplex PCR kits. There were several multiplex reactions that included 22 microsatellite loci. For each insect sample, these multiplex groups were pooled and barcoded using llumina’s S5 and N7 Nextera indexes set (D’Aloia et al., 2017). Then, to generate a single library for sequencing, all bar-coded individuals were pooled and cleaned with Ampure XP bead-based reagent? (Beckman Coulter, Inc.). The library was diluted to a 4 nM concentration and sequenced on an Illumina MiSeq with paired 300 bp reads at AAFC (see D’Aloia et al., 2017; Wu et al., 2020). The overall quality of the raw reads was first inspected using FastQC/MultiQC. Individual multilocus genotypes were called based on the primary nucleotide sequence using amplicon.py, with the parameters: -c 1, -a 0.005, -l 150 (Sun and Zou, 2023). These parameters were chosen as sequencing microsatellites leads to higher levels of polymorphism as compared to traditional size-based scoring methods, because of single-nucleotide polymorphisms and insertions/deletions in the flanking regions of the simple sequence repeats motif (Šarhanová et al., 2018). A minimum of two reads were

required for each allele; otherwise, the diploid genotype was recoded as missing data. Individuals with >20% missing loci and loci missing in >20% of individuals were filtered. The remaining 22 loci and 98 individuals were retained for subsequent analyses.

d) Population structure analysis

In northern latitudes, ALH populations are likely to show signs of recent admixture, driven by secondary dispersal and complex multiple colonizations. Complex multiple colonizations refers to the recolonization of SK by ALH sourced from different wind events and overwintered eggs, leading to multiple genetic footprints being present in one area. Each wind event has an unknown ALH density (i.e., there may be tens in one wind event and thousands in the next). There is a high level of stochasticity within this system; therefore, we did not apply population structure inference methods based on minimizing deviations from HWE but used a multivariate approach (Vander Wal et al., 2012). However, before multivariate analyses were applied, we conducted hierarchical clustering in the R programming language. The “complete linkage” method was used to calculate the distance between clusters based on the maximum dissimilarity between any pair of points in the two clusters being merged (details in supplementary material). Next, we used discriminant analysis of principal components (DAPC) (Jombart, Balloux et al., 2010) to determine the number of genetically distinct populations (*i.e.,* genetic clusters). DAPC maximizes between-cluster variance and minimizes within cluster variance using synthetic variables that represent linear combinations of alleles. Briefly, PCA-transformed data are used in a sequential k-means clustering algorithm to choose the number of clusters (k) that maximizes inter cluster variation. In addition, we applied PCA to compare DAPC results. Next, we used Bayesian information criterion (BIC) to choose the optimal k value and ran a discriminant analysis to assign individuals to clusters. To further characterize the partition of genetic variation between and within population, an Analysis of Molecular Variance (AMOVA) based on the genetic distance for exploring within and between population variation was performed. To test for a correlation between geographic and genetic distances, we performed a Mantel test (Mantel, 1967) using IBD (Bohonak, 2002), which compares the matrices of pairwise genetic and geographic distances. Because populations disappear every year, we analyzed each year independently.

Table 1. Sequences of microsatellite marker primers for ALH

Locus	primer sequences (5'-->3')
MQu9681	F: CTGACTCCATTCTGTACACAATTTGC
	R: GATACCTCCAAATGTACCTTCCAAG
MQu2996	F: AGAATGGAGAATGAAAGAATCCCG
	R:TACGAGTTGAAGCTGAGGACG
MQu3214	F: GCTGGGTATCATACATCAAGCC
	R:CATGCCAAATTTCAGCCCAATC
MQu438	F: TCAGTGTACCAAATTTCAGCCTC
	R:TCAAGGTTAAGTGCCAAATACCC
MQu572	F: CAGTGTACCAAAGTTCAGCCTC
	R:TCAAGGTTAAGTGCCAAATACCC
MQu2193	F: GATTGGGCTGAAATTTGGCATG
	R:TAATCCGTCCAGTGGTTTATGC
MQu4772	F: TTTCCCTCTCTTCCCATAGCAC
	R:ACGACATAGGAAGTGGCTTAGG
MQu4742	F: GGGAAGTGGGAGAATTAGTGATG
	R:AGCTGTAAACATTCAGCATTTGTG
MQu4147	F: TCAGTGTACCAAATTTCAGCCTC
	R:TGGTTCGGATTCCACTATAAACTG
MQu4288	F: TCCTAGGTAAATGGGAAGTGGG
	R:GCTTACGATCTAGTGTAAGGACG
MQu3839	F: TCCTAGGTAAATGGGAAGTGGG

This genetic approach to determining the origins of aster leafhoppers did not reveal genetic structure that was able to define populations of ALH. The genetic work being time consuming and expensive did not continue after the 2021 season, where we did not pursue this line of investigation with testing on any more migrant leafhoppers.

3. Aster yellows risk index and transmission risk in Saskatchewan.

Objective description: Rapid transmission of aster yellows data: continue testing migrant leafhoppers for AYp and transmitting the results to growers. Determination of what the aster yellows index means to canola production.

We have employed the rapid LAMP technique for AYp testing that we developed in the previous WGRF funded project (Pusz-Bochenska et al. 2020). In the laboratory, we used a modified version of the rapid test, with DNA extraction performed using a Qiagen Blood and Tissue DNA extraction kit to ensure that the most DNA was extracted per leafhopper sample.

Aster leafhoppers (ALH) were caught using an ALH Network that was established in 2018, and since that time approximately 20-30 sites across Saskatchewan were monitored for the first arrival of ALH, *Macrosteles quadrilineatus,* for eight weeks at the start of the growing season. The sites are run by cooperators who are also part of the PPMN Diamondback Moth pheromone trapping sites. At each site cooperators place a yellow sticky card adjacent to the diamond moth trap. Cards are changed weekly for 8 weeks from early May to the middle of June. The yellow sticky cards are returned along with the Diamondback moth trap inserts and are processed by Saskatoon Research and Development Center (SRDC) staff. The number of male and female *M. quadrilineatus* on the cards are recorded and the leafhoppers are removed from the sticky cards for AYp testing. In an attempt to pinpoint a closer date of first arrival an enhanced ALH monitoring grid was established in 2022 and 2023. Sites were selected close to the SRDC and monitored every 1 to 3 days depending on the site, weather and staff availability. Yellow sticky cards were placed at the sites the end of April prior to any aster leafhopper arrivals and checked every 2-3 days depending upon staff availability. The DBM/ALH network was used in 2022. In this season, the DBM traps stayed out for a longer period of time, which was a advantageous, because the largest DBM migration event happened later in the season (Week of June 20th 2022) and many fields had outbreaks of DBM larvae, including our fields at the SRDC farm.

An enhanced ALH monitoring grid was established in 2023 again (in a similar fashion as above) and we did not use the DBM trapping network again for yellow sticky card monitoring purposes for ALH. We found that rapid changing of yellow traps, coupled with sweep sampling at locations with ALH, as well as technicians trained in ALH identification and field survey methods were superior to relying on the DBM trapping network. As well, DBM arrival and aster leafhopper arrivals had not correlated for any other years of the projects.

5. Evaluation of aster leafhopper movement from alfalfa to canola and an evaluation of alfalfa as a "green bridge" reservoir.

Objective description: Determine if alfalfa is a green bridge between the population of migrant leafhoppers and the F1 population that move into canola crops. Proposed methods: through the sentinel monitoring sites (described above) and with a controlled field experiment, we hope to track the movement of aster leafhoppers of the F1 generation into canola crops and determine from where these leafhoppers originate locally and what their tendency to move from alfalfa into canola crops might be. Two techniques to track aster leafhopper movement across the landscape that have been successfully used in other project, fluorescent marking and ELISA determination.

Alfalfa was planted in summer 2021 at the AAFC Saskatoon Lowe Road experimental Farm. Untreated canola seed was sourced to use in movement experiment. Experiments indicated that fluorescent powder will stick to aster leafhoppers and be detectable at least one week later. A world-wild shortage of ELIZA plates and materials (due to the Covid pandemic) made the protein-marking of the leafhoppers too challenging.

Alfalfa and perennial weeds from areas of Saskatchewan where the Provincial survey detected the presence of aster yellows in fields were surveyed and ten plants of each species were brought back to be tested for the presence of AYp through our laboratory methods in 2021, 2022 and 2023.

Molecular testing for the presence of AYp in plant tissue

Small pieces of plant tissue (alfalfa and perennial weeds) were collected from a random three plants of each species (of the ten plants) that were brought back to the lab for AYp testing. The number of samples that we generated with ten plants per species per site as well as testing of the aster leafhoppers caught in ditches in the spring was overwhelming for AAFC lab staff and the budget attributed to this objective and we had to compromise where we could.

The three plant samples from each site were screened with fluorometric LAMP for the presence of AYp and any positive samples by fluorometric LAMP were then tested using R16S nested PCR. RFLP analysis was performed on insect and plant samples that tested positive for the presence of phytoplasma with the 16S nested PCR to determine which strain of AYp was infecting the plants. Detection of phytoplasma DNA was performed using PCR (Polymerase Chain Reaction) technology as the "gold standard" in the laboratory of Drs Wist and Olivier, AAFC-Saskatoon Research Centre. Leaf, stem and root tissue of collected plants were tested for the presence of phytoplasma DNA where possible until the number of samples were too many to accommodate multiple testing of single plants. These small pieces of plant tissue were then freeze-dried, lysed, and their DNA was extracted using the CTAB method (2018-2019) the DISC-method extraction (Pusz-Bochenska et al. 2020), or a QIAgen DNeasy® Blood and Tissue kit for 96 well plates. DNA titers were checked to ensure that sufficient amounts had been extracted for analysis. 16 S Nested PCR involved an initial round in the thermocycler using P1 and P7 primers on a 1:10 dilution of extracted DNA, followed by a second run in the thermocycler using primers R16F2-N and R16R2-N on a 1:30 dilution of P1/P7 amplicon. Conditions were 95ºC for 10 minutes as an initial denaturing step, followed by thirty-four cycles at 95ºC for 1 minute, 55ºC for 1 minute, and 72ºC for 1 minute and 45 seconds for annealing. Extension is at 72ºC for 10 minutes, and finally held at 12ºC indefinitely. The PCR product was run on 1.5% agarose gel to check for presence of R16S gene, indicating presence of AYp. Confirmation that the sample was the aster yellows phytoplasma and any identification of the phytoplasma strain was performed by RFLP analysis. The forward and backward segments (R16R2-N/R16F2-N) of the 16S DNA section sequenced and compared to the AYp 16S segment

using the VectorNTI software. In subsequent years of the project, the 16S strain was determined from these to understand if the infected plants carried the 16SrI-A, 16SrI-B or another AYp strain.

Potential reservoir weeds from RMs where the Saskatchewan Provincial survey the previous year (2021 to start) detected the presence of aster yellows in fields were sampled in spring 2022, and again in 2023 based on 2022 Saskatchewan Ministry results. Dandelions, and alfalfa were targeted at each site and if stinkweed, clover or sow thistle was found it was also sampled.

The aster leafhopper movement experiment was attempted in the field in summer 2022. The 30 foot wide alfalfa ditch planted the previous summer grew well in spring 2022 and these plants were interspersed with brome grass like a typical Western Canadian ditch. Rows of canola with only a fungicide seed treatment were sown in the early summer. Canola plants emerged at the same time as the summer generation of flea beetles and between the flea beetle damage and the hot and dry conditions, the canola was destroyed. With the failure of the ditch to canola experiment in the field in 2022 we ran this experiment in lab bioassays in cages using aster leafhoppers from our AYp-infected and AYp-uninfected colonies. Leafhoppers were reared in growth chambers set to 21℃ during the day and 17℃ at night on an 16h photoperiod and sustained on barley, with new plants added weekly. The AY negative colony was reared without exposure to aster yellows phytoplasma (AYp) (ALH-). Acquisition cages of ALH infected with AYp (ALH+) were created when required for experiments. ALH+ leafhoppers were created by placing ALH- leafhoppers into cages with AYp-infected periwinkle plants, *Catharanthus roseus* (Gentianales: Apocynaceae), which are good hosts for the phytoplasma. Periwinkle is vegetatively propagated by cutting and healthy new plants are grown in the greenhouse until they are infected with the AYp. Over 48 hours, the ALH are confined in cages with a previously infected periwinkle plant showing symptoms of AY infection such as yellowing, phyllody and abnormal propagation of leaves. These leafhoppers are forced to feed on the infected periwinkle plants and after 48 hrs a pot of young barley is added into the cages. To sustain the AYp infection in periwinkle, ALH+ from cages with infected periwinkle are moved into cages with uninfected periwinkle, where the leafhoppers will transmit the phytoplasma among the periwinkles as they feed. Both the periwinkle plants and the aster leafhoppers are screened periodically via PCR or the newly developed colourimetric LAMP to insure that AY infection is sustained in the colony.

Plant Varieties and Growing Conditions

All plants that were used in this experiment were grown from seed in a greenhouse at 21℃ on a 16h photoperiod, were watered 3-times a week, and were potted in PRO-MIX BX potting soil. Canola, C.V. AC Excel was used for the canola “field” and *Bromus inermis* was used as the brome grass for the “ditch”. For the initial control trial, canola was planted 21 days before being placed into the experiment where alfalfa and brome were planted 28 days in advance. For the first experimental trial, brome, alfalfa, and canola plants were grown for 19 days, until canola plants were between the 3rd to 5th leaf stage, before use in trials. During the second trial, alfalfa and brome were planted 24 days before experimentation began while canola was grown for 13 days, until it was in the 2nd and early 3rd leaf stage before use in trials. Younger canola plants were used to match the approximate growth stages in the field where we theorize that the ALHs move into the canola fields. Younger plants are highly susceptible to AY infection as well (Olivier *et al*., 2014). For the 3rd and final trial of this experiment, brome and alfalfa were planted 19 days before use and canola was grown for 14 days and plants were between the 2nd and 4th leaf stage. For the bioassay, plants were moved into a temperature controlled room set to 21℃ on the same 16h photoperiod and were watered on Monday, Wednesday and Friday. During the control trial and the first experimental trial, temperature controls were not functioning properly, so the temperature in the bioassay room averaged slightly warmer, typically between 21℃ and 24℃.

Experimental Trials

The experimental design was five mesh cages measuring 47.5cm in width x 47.5cm in height x 93.0cm in length. Each cage had an experimental "ditch" containing one alfalfa plant and one pot of young brome in circular pots measuring 12.5cm in diameter x 12cm in height and a "field" containing four rows of five potted canola plants in square pots measuring 6cm in width x 8.5cm in height (Figure 4.1.). Canola plants were spaced with a similar density to that suggested by the Canola Council of Canada (2022) of five to eight plants/ linear foot. Since the dimensions of the field portion of the cage was 65cm in length x 47cm in width, the 20 canola plants placed in the field portion of the cage had a lower density of 6.08 plants/linear foot. During the control trial, where no leafhoppers carrying AYp were used, was aimed at seeing if ALH would move into the canola field. Numbers of leafhoppers in each replicate of the experiment had to changed depending upon the availability of leafhoppers from the colonies. In the initial trial, 20 ALH were placed into each cage, which made it easier to check if insects could be seen on the plants in the cages. Before adding leafhoppers to cages, they were dusted with a fluorescent powder that glows under UV light. This powder was detectable on leafhoppers under UV light after the 2-weeks of experimentation had ended. For the first experimental trial with AYp infected leafhoppers, each bioassay cage contained 50 ALH of different ratios of ALH+/ALH-, creating five cages with 1/49, 3/47, 5/45, 7/43, and 9/41 ALH+/ALH- which made 2%, 6%, 10%, 14%, and 18% infection proportions (Table 4.1.). These ratios were chosen as they spanned infection rates of normal and outbreak years of AY (Frost *et al*., 2013a). During the second trial, the number of leafhoppers per cage was doubled to 100 insects, and the ratios of infected/uninfected ALH were increased to 5%, 10%, 15%, 20% and 25% to increase the chances of AY infection. For this trial, 10 ALHs from the ALH+ colony were screened per cage via

Table 4.1. Experimental design of each cage across all three experimental trials. For the last two columns, the five percentages and ratios in each row represent each cage within the trial, listed from lowest to highest aster yellows pressure. Changes in infection rate of aster yellows acquisition cages resulted from different success rates when attempting to produce aster yellows infected aster leafhoppers (ALH+). Three different acquisition cages were set up over the three trials, with 0/10*, 4/10, and 1/10 ALH+ testing positive for AY after PCR and this ratio was accounted for when creating the attempted infection ratios. In the third trial cages 1 and 2 contained ALH+ from an acquisition cage with a 1/10 infection success, cage 3 contained ALH+ from both the 1/10 and 4/10 acquisition cage, and cages 4-5 used ALH+ from the 4/10 acquisition cage. This information is represented in the final column with the acquisition cage displayed in brackets.

* This ratio was obtained after the trial had concluded and the attempted infection ratio had assumed 100% infection.

Trial	Number of ALHs per Cage	Infection Rate of ALH+ from Acquisition Cage used in Trial	Attempted Infection Ratio (5 Cages)	Number of ALH from ALH+/ALH- Cages (5 Cages)
1	50	0/10*	2%, 6%, 10%, 14%, 18%	1/49, 3/43, 5/45, 7/43, 9/41
2	100	4/10	5%, 10%, 15%, 20%, 25%	12/88, 25/75, 37/63, 50/50, 63/37

3	50	1/10 (Cages 1-2) 1/10 + 4/10 (Cage 3) 4/10 (Cages 4-5)	4%, 10%, 14%, 20%, 24%	20(1/10)/30, 50(1/10), 14(1/10) + 14(4/10) /28, 25(4/10) /25, 30(4/10) /20

our newly developed colourimetric LAMP test for AY infection (Pusz-Bochenska et al. 2020). Four insects tested positive for AYp which suggested that the acquisition cage only created an infection rate of 40%, so an increased number of ALH+ were added to each cage to try and obtain the attempted infection ratio, making the exact numbers of ALH+/ALH- added to each cage 12/88, 25/75, 37/63, 50/50, and 63/37 (Table 4.1.). For the third trial, the colonies of ALH+ were depleted so only 50 insects were possible. Insects from the ALH+ acquisition cage used in the second trial were added in along with insects from a newer acquisition cage which was found to have only 1/10 ALH+ infected with AY using the cLAMP. The attempted infection ratios were 4%, 10%, 14%, 20% and 24% which were created in the same way as the second trial by accounting for the infection rate of the initial cage, and the exact number of ALHs added to each cage are represented in Table 4.1.

For all trials, the first 48hrs had the introduced leafhopper settle into the "ditch" area of the cage by placing the alfalfa and brome grass into the experimental cages and adding the appropriate ratio of ALH+ to ALH- leafhoppers (Table 4.1.). In this 24 hour period, the leafhoppers were monitored to see if they chose to feed on alfalfa or brome in the ditches. Canola was added 48 hours after to see if the leafhoppers would move from their settled position in the ditch without any mechanical interference like shaking or mowing. The ditch simulates the dominant vegetation of the roadside margins, or "ditches" in Western Canada and these are often the only green plants in the spring when the initial migration of aster leafhoppers into Western Canada occurs. Following this 48 hour period, the 20 potted canola plants, (Figure 4.1), were added to the cage and left undisturbed for 12 additional days. Observations were made twice daily on weekdays and the settling location of each observable ALH was recorded (brome, alfalfa, or on the numbered canola plants, numbered by their distance from the ditch to see how far the leafhoppers would travel into the "field". The number of number of dead ALHs were also tallied in order to estimate the number still alive in the cage. After the 14 days, live ALHs were carefully extracted from the cage using an aspirator and frozen for AYp testing. Most of these have not been tested yet. The cages were then searched for additional leafhoppers that had died during the experiment which were also frozen. The canola plants were returned to the greenhouse where they could mature and develop AY symptoms if they had become infected. Symptoms such as "purpling" of leaves, stunting of plants, failure to bolt, and phyllody and virescence of floral parts and development of bladder-like pods were all used as visual symptoms of AYp infection Canola plants were grown to maturity in the greenhouse, then harvested, and their fresh and dry weight was taken, as well as yield measures consisting of seed counts, weights and thousand kernel weights.

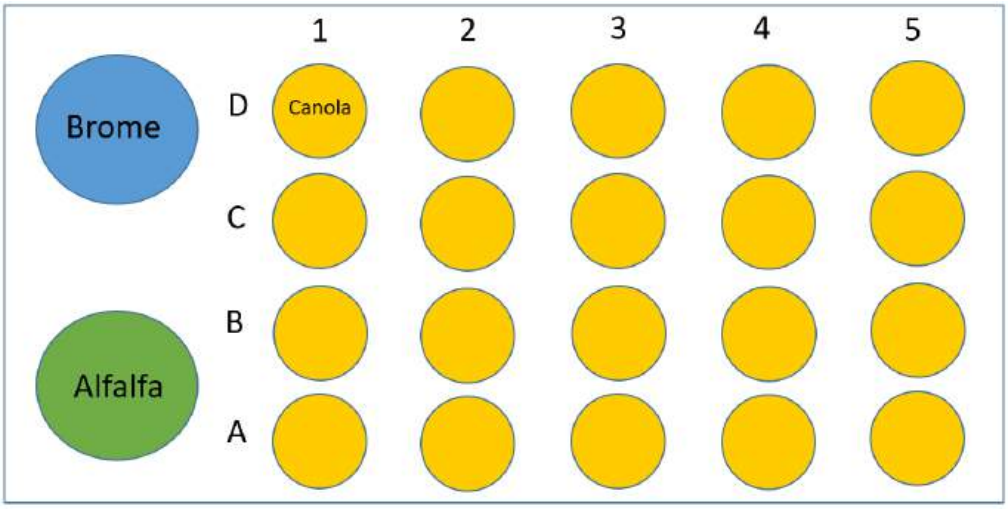


Figure 4.1. Orientation of plants inside cages. The letters and numbers adjacent to canola represent the labeling system for individual canola plants.

Aster Yellows Testing and Visual Assessment

During the control trial where no infected leafhoppers were used and in the first trial, no plants were tested for AY. After the first trial, plants were visually monitored for AY in accordance with a guide to identifying AY in canola, by Olivier *et al*. (2014), for several weeks but none appeared to have become infected. After testing 10 ALH+ with cLAMP that were in the same AYp acquisition cage used in the first trial, no leafhoppers were found to have acquired the AYp infection in this sample group. This check was performed after the trial had finished, so it is likely that none, or only a few, ALH+ assumed leafhoppers actually were infected during that trial, out of the 25 total ALH+ that were used across all cages. After the second and third experimental trial, where ALH+ leafhoppers from the AYp acquisition cages were known to be infected, canola leaf-midrib tissue was sampled to test for AYp using cLAMP. Samples were taken 7 weeks and 3 days after they were initially exposed to ALH+ leafhoppers, in concordance with other studies testing for AY (Olivier *et al*., 2014), in order to confirm infection of visually symptomatic plants and test for cryptic AYp infection in asymptomatic plants. Half of the canola from each cage were sampled in the second trial and all the canola in the third trial were sampled.

Reproductive host potential of Alfalfa and Brome

After the second and third trial, when all ALHs were removed from cages, brome and alfalfa that were present in cages during experiments were placed into separate cages in order to test their viability as reproductive hosts for ALH. These plants were still watered Monday, Wednesday and Friday, and were monitored over the following weeks to see if any ALH nymphs would emerge. Counts were not performed on any emerging nymphs, but each plant pot from each cage that produced nymphs was recorded.

Statistical Analyses and Calculations

All graphs and statistical tests were performed in R Studio version 4.2.2. The ggplot2 package was used to create graphs and the patchwork package was used to arrange plots within a figure. To compare settling behavior and choices between cages and experiments, and because not all insects were able to be counted at one time, proportions for each plant were calculated for graphs and statistics. Relative proportions were calculated by dividing the counted insects on each plant by the total number of counted insects in each cage.

This was used to compare between cages and trials which had fluctuating amount of visible insects since trials had different numbers of ALHs added to each cage. These proportions were plotted over time for each trial to visualize the choices of ALHs when settling and feeding. This data was also analyzed with ANOVAs to see if settling choices differed between cages where the gradient of increasing ALH+ existed.

To analyze the spread of observed leafhoppers on canola plants, Statistical Analysis of Distance Indices (SADIE) was used with SADIEShell v1.2 software. This method has become a popular and respected way to analyze spatial count data in ecological studies (Winder *et al*., 2019). The index of aggregation (I_a) shows if spatial count data is clustered ($I_a > 1$), randomly distributed ($I_a = 1$), or regular ($I_a < 1$) and the P_a represents the probability that the observed distribution of counts are random. Each time a leafhopper was observed on a plant it was recorded and the sum of these observations over time across all cages was inputted into this analysis. Shapiro-Wilk tests were used to test for normality in these datasets. Spatial count data was also plotted to help visualize which canola plants were preferred by ALHs to settle on.

Objective 4 references

Frost, K. E., P. D. Esker, R. V. Haren, L. Kotolski, and R. L. Groves. 2013a. Factors influencing aster leafhopper (Hemiptera: Cicadellidae) abundance and aster yellows phytoplasma infectivity in Wisconsin carrot fields. Environmental Entomology 42:477-490.

Frost, K. E., P. D. Esker, R. Van Haren, L. Kotolski, and R. L. Groves. 2013b. Seasonal patterns of aster leafhopper (Hemiptera: Cicadellidae) abundance and aster yellows phytoplasma infectivity in Wisconsin carrot fields. Environmental Entomology 42:491-502.

Pusz-Bochenska, K., et al. (2020). "A rapid, simple, laboratory and field-adaptable DNA extraction and diagnostic method suitable for insect-transmitted plant pathogen and insect identification." Plant Health Progress **21**(1): 63-68.

Winder, L., C. Alexander, G. Griffiths, J. Holland, C. Woolley, and J. Perry. 2019. Twenty years and counting with SADIE: Spatial Analysis by Distance Indices software and review of its adoption and use. Rethinking Ecology 4:1-16.

5. Use of wind trajectories and stable isotopes to continue gathering data on DBM and aster leafhopper migration flights and origins.

Monitoring for Diamondback moth (BM) and aster leafhoppers (ALH) was achieved using backwards and forwards air trajectory models from the Hybrid Single Particle Lagrangian Integrated Trajectory Model (HYSPLIT) system whose data is publicly available from the US government and compared with insect surveyed using sticky cards and sweep nets and from monitoring of sentinel canola fields and nearby ditches and cereal crops. Stable isotope ratios based on the deuterium content of migrant leafhoppers aiming at determining the origin of the migrant leafhoppers, will continue under this objective.

In spring 2021 and 2022, Yellow sticky cards were placed at 33 sites in Saskatchewan to monitor arrival of aster leafhoppers, *Macrosteles quadrilineatus* along with 33 DBM pheromone traps. In 2022, this technique was employed again with the addition of an enhanced monitoring network (2022) using sticky cards and sweep samples. This network is a follow-up to our previous project where an ALH Network was established in 2018, and since that spring approximately 20-30 sites across Saskatchewan have been monitored for the first arrival of *Macrosteles quadrilineatus* for eight weeks at the start of the growing

season. The sites are run by cooperators who are also part of the PPMN Diamondback Moth pheromone trapping sites. Thanks to our Provincial cooperators and the Saskatchewan Ministry of Agriculture. At each site, provincial cooperators placed a yellow sticky card adjacent to the diamond moth trap. Cards were changed weekly for 8 weeks from early May to the middle of June. The yellow sticky cards were returned along with the Diamondback moth trap inserts and were processed by Saskatoon Research and Development Center staff in the Wist Lab. The number of male and female *M. quadrilineatus* on the cards are recorded and the leafhoppers are removed from the sticky cards for molecular analysis of the presence of aster yellows phytoplasma (AYp). First captures of aster leafhoppers and Diamondback moths were used to determine the possible sources of origin. Migration routes were examined using Hysplit to calculate Ensemble RTs. Unlike single trajectory modelling which calculates a single trajectory based on a single meteorological grid cell, Ensemble trajectories calculate 27 trajectories with each trajectory calculated by offsetting the grid cells. Trajectories were run at height of 1500m AGL and for 48 hrs. The run time was selected because, although the estimated time leafhoppers can remain in flight for 24-30 hrs, they are not passive while in flight so they may cover a distance greater than 24 hrs when assisted by the wind. The start time for the model runs was set at 3UTC or 9:00pm local time which corresponds to sunset in May. Based on the arrival date, the Ensemble reverse trajectory model is run using NOAA Ready Hysplit 4 (Stein *et al.* 2016, Rolph *et al.* 2017). Hysplit allows the user to run a Normal trajectory model which generates a single trajectory or an Ensemble trajectory model which generates multiple trajectories. The drawback to using a single trajectory model is that it represents a single release point in space and time (Hopkinson and Soroka 2010). The Ensemble trajectory model calculates 27 trajectories from a single starting point. This is done by offsetting the meteorological data set by 1 grid point in the X, Y, Z directions (Draxler and Hess 1998). In essence this is akin to a “standard error” giving a geographic range of possible origin sites. The parameters used in the ALH model runs assumed nocturnal migration with a flight initiation at dusk (Reynolds *et al.* 2017) and a maximum flight period of 30 hrs (Nichiporick 1965). In addition leafhoppers require a temperature of 15C for flight and it is also known that rainfall can result in termination of migration (Huff 1963, Nichiporick 1965) so ambient air temp (K) and rainfall (mm/hr) were plotted along the trajectory. The 30 hour trajectory runs showed origin points in the Dakotas. One limitation of the ALH Network is that cards are changed weekly this means that the arrival window was considered to be 10 days (3 days prior to the date card was placed in the field and the 7 day trapping period). Ensemble Rts were run daily for this 10 day period to look for RTs originating south of the 49th parallel. However, if you have multiple days and a broad geographic range of origin points occurring in the United States this can lead to confirmation bias by selecting the locations that fit your underlying assumptions.

Objective 5 References

Draxler, R. R. and G. Hess (1998). "An overview of the HYSPLIT_4 modelling system for trajectories." J Australian meteorological magazine 47(4): 295-308.

Hopkinson, R. F. and J. J. Soroka (2010). "Air trajectory model applied to an in-depth diagnosis of potential diamondback moth infestations on the Canadian Prairies." Agricultural and Forest Meteorology 150(1): 1-11.

Huff, F. (1963). "Relation between leafhopper influxes and synoptic weather conditions." Journal of Applied Meteorology 2(1): 39-43.

Nichiporick, W. (1965). "The aerial migration of the Six-Spotted Leafhopper and the spread of the virus disease Aster Yellows." International Journal of Biometeorology 9(3): 219-227.

Reynolds, R. D., et al. (2017). "Windborne migration of Auchenorrhyncha (Hemiptera) over Britain." 114(1): 554-564.

Rolph, G., et al. (2017). "Real-time Environmental Applications and Display sYstem: READY." Environmental Modelling & Software 95: 210-228.

Stein, A. F., et al. (2016). "NOAA's HYSPLIT Atmospheric Transport and Dispersion Modeling System." Bulletin of the American Meteorological Society 96(12): 2059-2077.

Wallis, R. L. (1962). "Spring Migration of the Six-Spotted Leafhopper in the Western Great Plains." Journal of Economic Entomology 55(6): 871-874.

Westdal, P. H., et al. (1961). "The six-spotted leafhopper, *Macrosteles fascifrons* (Stål) and aster yellows in Manitoba" Canadian Journal of Plant Science 41(2): 320-331.

Winder L, Alexander C, Griffiths G, Holland J, Wooley C, Perry J. 2019. Twenty years and counting with SADIE: Spatial Analysis by Distance Indices software and review of its adoption and use. Rethinking Ecology 4: 1-16.

6. Results: Report laboratory, growth chamber, greenhouse, and field experiments; chemical analysis; data analysis; and model development.

1. Diamondback moth (DBM) genetic structure

Description of objective. Assess the genetic variations of the migrant and new generation of DBM to pinpoint their origin. CO1 (mitochondrial cytochrome-c oxidase subunit 1, cytochrome P450 and SNP (single nucleotide polymorphisms) markers to assess the genetic and geographic origins of DBM.

In 2021, we evaluated sticky cards from the Provincial DBM traps There were only 43 DBM males and these were used for the molecular determination of origins using PCR techniques on the CO1 region. Our bucket trapping at sentinel AAFC research farms did not yield any DBM moths.

We had some input with the Provincial DBM monitoring network and the DBM trapping period has been extended until the end of July to catch any late migratory flights. In 2021, the 43 DBM caught in the initial 6 week trapping period failed to predict the DBM larvae that eventually were found in fields and were started from a migratory flight that came after the monitoring network had stopped monitoring. This extension of the monitoring network helped us source more moths for genetic analysis in the next years of the project as well as gave us more insight in regards to the later migratory winds that we can back-track using HYSPLIT.

Our contact in the Southern US, and entomologist who worked with BASF, Dr. Rammohan Balusu put out the 15 DBM pheromone traps that we shipped to him, with his ento-contacts across the Southern US. Traps were run in May of 2022 but no male DBM moths were caught. He then made a road trip to sample canola fields for larvae at the end of May and beginning of June in 2022. Canola and other cole crop fields in Kentucky, Mississippi, Tennessee, Florida Georgia, Alabama and North and South Carolina, were sampled for DBM larvae. In addition, forage grasses were sampled with a sweep net for aster leafhoppers. DBM larvae were caught and some were preserved for genetic analysis and others were reared to adulthood (5/field) for our stable isotopic study on diamondback moths.

In 2022, DBM migrated later in the season than usual but the decision to run the pheromone traps into July paid off in catching the later migration, which then established larval populations in fields that we were monitoring. The week of June 20th was indicated as the largest migration of DBM into Saskatchewan. Another peak of population monitoring on the pheromone traps indicated DBM adults in the week of the 20th of July, which were likely the F1 generation offspring of the migrants from one month before. The DBM traps ran until the end of July and caught a total of 1283 male DBM over 52 traps. The wind currents that brought the DBM were discussed in the last project but were not the same winds that the migrant aster leafhoppers arrived on. The winds that brought DBM came out of the Pacific Northwest. In 2023, we had stopped comparing DBM pheromone trap catch to ALH sticky card catch and we did not take the provincial data except for DBM males off of the pheromone traps for haplotyping.

In 2024, DBM adults that looked like they had newly eclosed were caught on pheromone traps early in the season in Saskatchewan, and we received these to determine if there was a common haplotype present that might be more prone to overwintering. This was an interesting situation, that we felt the isotopes, but perhaps, also the genetics was able to answer. The first samples were caught on traps in the last week of April 2024, as far North as Meadow Lake, with subsequent catches in the first week of May 2024.. The CO1 regions were compared and matched against any DBM entries in the Barcode of Life Data Base to determine if common haplotypes came from similar migratory origins in North America. Single Nucleotide Polymorphisms (SNPs) within the approximately 681 base-pair barcode region, were identified by sequence alignments revealing a total of 21 haplotypes within these samples (Table 1.1). The 'Main Haplotype' had 30 individuals and contained 54% of the total samples sequenced and it was a common haplotype in all 4 years. Most of the other haplotypes had only a single individual. There seems to be no correlation between location caught in North America and haplotype except that the 3 samples tested from Manitoba were all Haplotype 1. (Table 1.1). Two of the Haplotypes, 3 and 9, mapped to entries outside of North America (Appendix Figure 1 A-D), but have also been detected within North America.

Table 1.1 Haplotypes based on Single Nucleotide Polymorphisms within the CO1 mitochondrial genome region of diamondback moths caught between 2021 and 2024.

Haplotype	**Info**	**# of individuals**
1	Main Haplotype	30
2	2024 sample 87, 123 and 162, 2021 sample 10	4
3	2021 sample 5	1
4	2024 sample 118	1
5	2024 sample 119	1
6	2024 sample 129	1
7	2024 sample 56 and 2022 sample 4	2
8	2024 sample 159	1
9	2024 sample 160 and 2022 sample 7	2
10	2024 sample 163	1
11	2024 sample 124	1
12	2024 sample 166	1

13	2024 sample 41	1
14	2024 sample 42	1
15	2021 sample 7	1
16	2021 sample 8	1
17	2022 sample 2 and 13	2
18	2022 sample 1	1
19	2022 sample 11	1
20	2023 sample 7	1
21	2023 sample 6	1

2. Aster leafhopper population monitoring

Description of objective: Assess the genetic variations of the migrant F1 generation of aster leafhoppers to pinpoint their origin using a microsatellite marker approach with Illumina sequencing.

The genus *Macrosteles* includes over 100 holartic leafhopper species, several of which cause severe agricultural damage, primarily through the transmission of aster yellows phytoplasmas (AYp). AYp is transmitted primarily by the migratory aster leafhopper (ALH), *Macrosteles quadrilineatus* (Cicadellidae). Understanding the dispersal patterns of ALH is key to developing effective control strategies, and so our work addresses the genetic diversity of ALH populations and provides a snapshot of the genetic makeup of populations in Saskatchewan. Our graduate student, in collaboration with Dr. Jose Andres (Cornell University) developed a multiplex assay for 22 polymorphic microsatellite markers (Figure 2.1). Across North America, surveyed loci were highly variable (average of 33 allele/locus) (Figure 2.2). Consistent lack of heterozygosity across loci suggests that the sampled populations were of admixed origins, and that migration–drift equilibrium had not been reached at the time of sampling. Multivariate population structure and AMOVA analyses indicate that most genetic variation is found within populations, and that geographically closed populations have very different allele frequencies. Accordingly, there was no correlation between genetic and geographic distances (Figure 2.3). The inferences made here are supported via multiple analyses of 22 polymorphic loci (Figure 2.1) with powerful resolution and suggest that the ALH population is too genetically mixed to define individual populations. Overall, these results are consistent with complex multiple colonizations (and secondary dispersal) of ALH in Saskatchewan that vary year to year. The summary of this section is that this microsatellite study revealed a lack of structure and high within-population genetic variance in populations of aster leafhopper, *Macrosteles quadrilineatus*. Results support the hypothesis that the migratory lifecycle of *M. quadrilineatus* mixes the genetics of the population each year and that distinct source populations cannot de distinguished through genetic techniques. Results showed that each LH is genetically unique and individuals carry a genetic footprint from everywhere suggesting much mixing of populations. There is no detectible population structure, and the genetic distance between individuals is quite large. If there were any kinship relationships among the migratory leafhoppers it would be detected with the panel of these microsatellite markers because they are highly polymorphic, high-resolution molecular markers.

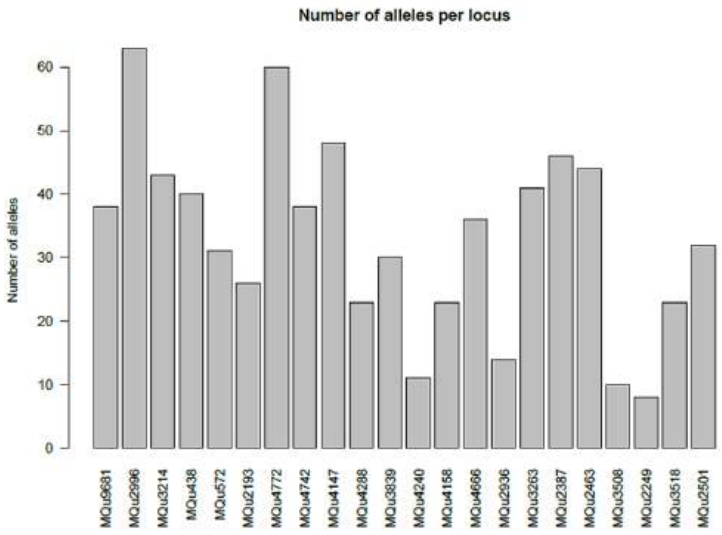


Figure 2.1. Number of alleles per locus in the panel of 22 microsatellite loci in the study.

- Proportion of shared alleles between and within populations.

A.

B.

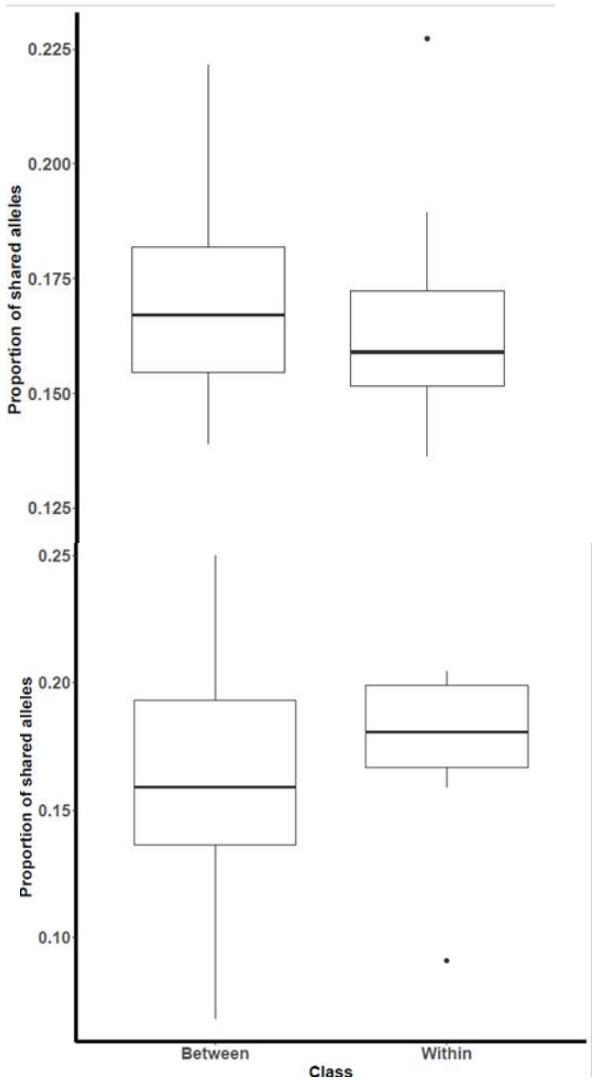
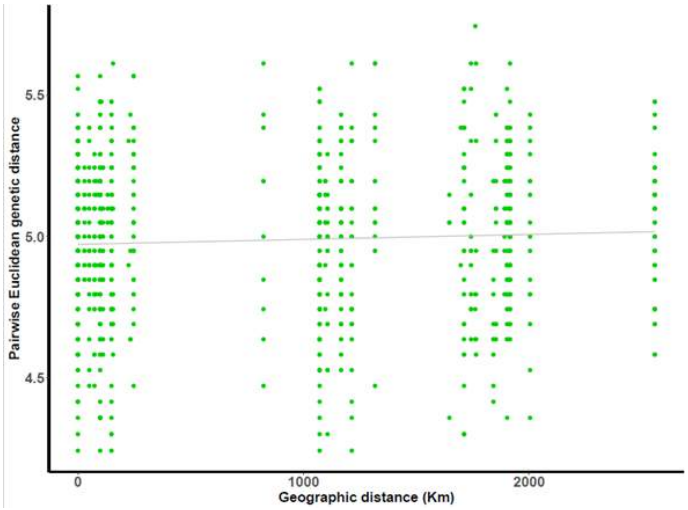


Figure 2.2. Proportion of shared alleles between and within populations in A) ALH collected in 2019 in Saskatchewan and US locations , B) ALH collected in 2021 in Saskatchewan.

- Correlation between geographic and genetic distances.

A.

ALH



B.

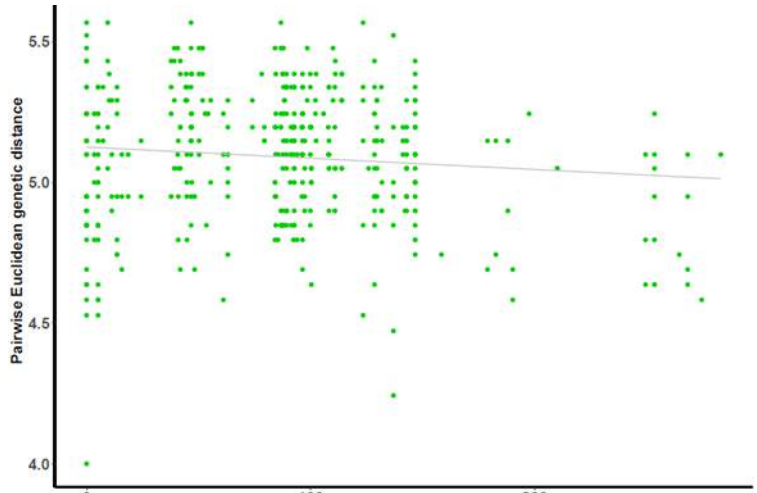
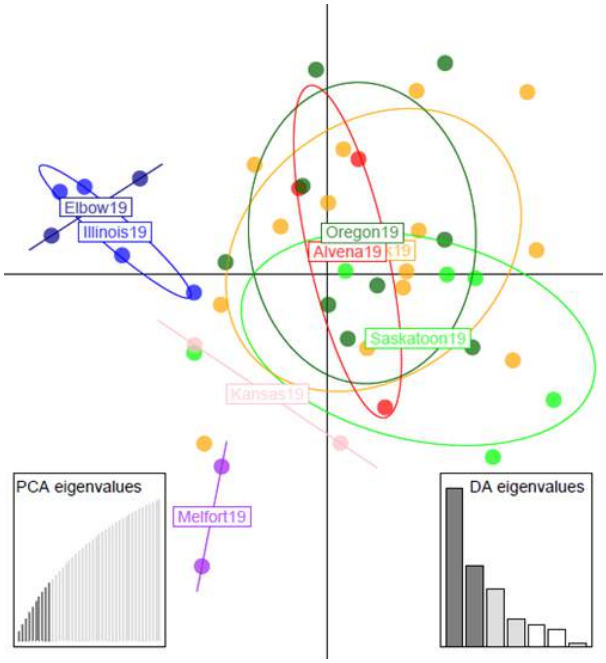


Figure 2.3. Correlation between geographic and genetic distances in A) ALH collected in 2019 in Saskatchewan and US locations, B) ALH collected in 2021 in Saskatchewan

- Genetic clusters based on discriminant analysis of principal components (DAPC)

A.



B.

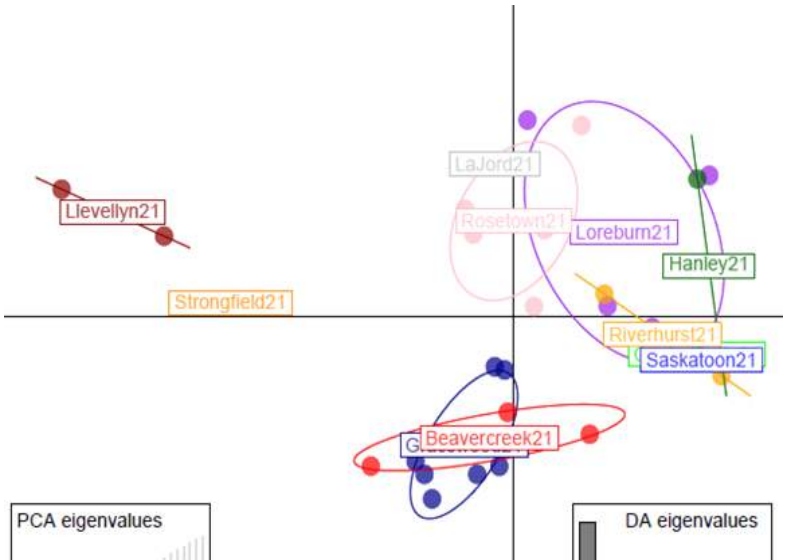


Figure 2.4. Genetic clusters based on discriminant analysis of principal components (DAPC) in A) ALH collected in 2019 in Saskatchewan and US locations, B) ALH collected in 2021 in Saskatchewan.

The genetic analysis of the aster leafhoppers showed no genetic structure in the overall population so no distinct populations of leafhoppers could be determined. This analysis was time-consuming and expensive and not worth continuing. The stable isotope analysis is working to help determine origins of leafhoppers and so we focus on that technique and the wind migrations.

3. Aster yellows risk index and transmission risk in Saskatchewan.

Objective description: Rapid transmission of aster yellows data: continue testing migrant leafhoppers for AYp and transmitting the results to growers. Determination of what the aster yellows index means to canola production.

2021 aster leafhoppers and AYp in sweep samples

From three early season sample dates (May 22, June 1, 3 2021), we tested aster leafhoppers caught using sweep nets from 14 sites in Saskatchewan. Of these 32 leafhoppers caught in Saskatchewan, only one tested positive for the presence of AYp in 2021. We also tested leafhoppers that appeared to be of the same Northward migration into Canada but landed in Carmen Manitoba and of these 94 aster leafhoppers from 3 sites around Carmen, no AYp positives were found. Another 4 aster leafhoppers were caught on June 6 2021 at Fork River MB and none were positive for AYp. Forty-four aster leafhoppers caught on the 2nd of June from Morris MB had one AYp positive leafhopper. On yellow sticky cards, we pooled all the leafhoppers from individual sticky cards into pools of maximum five leafhoppers for testing. For example, if there were 21 leafhoppers on one cards, we could have four pools of five leafhoppers and one pool of one leafhopper. 32 of 192 pools (17%) tested positive for the presence of AYp. However, twenty positive pools were from near the end of June and these leafhoppers could have arrived earlier and picked up infection in the province before being trapped. Of the first arrivals (May-June 9 2021), 12 pools were positive which indicates that some ALH likely did arrive to Saskatchewan with active AYp infections. On the whole, the migratory aster leafhoppers in 2021 did not come into Western Canada with much aster yellow infection and their numbers were low and with one AYp positive leafhopper from the entire catch we did not use the AY index to assess risk.

2022 aster leafhoppers and AYp in sweep samples

The 2022 yellow sticky cards from the DBM network were received at the end of the growing season and leafhoppers were enumerated and removed. However, the enhanced monitoring protocol that we enacted worked well to detect and narrow the window of data resolution from one week (with weekly changes of sticky cards) to two days, with frequent checks and sweep net sampling (sample = 50 to 100 standard sweeps of a 15 inch net) of ditches along our driving network. When leafhoppers were detected however, a base of 1000 sweep samples was used to enhance the number of individuals caught. The first arrival of leafhoppers to Saskatchewan was likely the 25th of May (see 2022 wind results above) and 8 ALH total were caught on two yellow sticky cards set on the 24th of May in Prudhomme Saskatchewan. Sweep sampling in ditches at

Vonda captured 11 ALH and one of these tested positive for the aster yellows phytoplasma (AYp). At Aberdeen on the 28th of May 2022, 6 ALH were captured by sweep netting and one of the six was positive for AYp. On the 29th of May Overall, 4 of 36, (11%) of these migrant leafhoppers were positive for AYp (Table 3.1) which is higher than most years when testing for AYp has occurred. In carrot production, the severity of AY outbreaks is related to the level of infectivity and the abundance of ALH in a susceptible crop (Chapman 1971). The Aster Yellows Index (AYI) combines the abundance and infectivity of aster leafhoppers to give an assessment of the AY risk at that location. s(Table 3.1). The AY Risk index was therefore very low at this site (Table 3.1). The highest AYI (0.4) was at Tuxford on June 7 2022 where 4 of the 72 ALH caught were positive and at Keeler on the 7th of June (AYI=0.4) where 4 of 41 ALF caught in 100 sweeps were positive for AYp (Table 3.1).

Table 3.1. Catches of early season migratory aster leafhoppers (1000 sweeps or 1 yellow sticky card), the percentage infected by aster yellows phytoplasma and the aster yellows risk index (2022).

Sample Location	Sample Date	Collection Method	#leafhoppers	Number of AY(+)	% of ALH infected	AY Index
Vonda	27-May-22	Sweep	11	1	9.090909091	0.10
Aberdeen	28-May-22	Sweep	6	1	16.66666667	0.10
Letkeman Rd (Vonda and Abeerdeen)	29-May-22	Sweep	8	1	12.5	0.10
Esperance Rd (Prudhomme)	30-May-22	Sweep	1	0	0	0.00
Vonda	02-Jun-22	Sweep	2	0	0	0.00
Prudhomme	May 24-27	StickyCard	5	0	0	0.00
Prudhomme	May 24-27	StickyCard	3	1	33.33333333	0.10
Chamberlain	07-Jun-22	Sweep	5	0	0	0.00
Tuxford	07-Jun-22	Sweep	72	4	5.555555556	0.40
Pasqua	07-Jun-22	Sweep	4	0	0	0.00
Rouleau	07-Jun-22	Sweep	19	0	0	0.00
Tuxford	07-Jun-22	Sweep	1	0	0	0.00
Keeler	07-Jun-22	Sweep	41	4	9.756097561	0.40
Eyebrow	07-Jun-22	Sweep	1	1	100	0.10

2023 aster leafhoppers and AYp in sweep samples

With a large ALH migration in 2023, we were able to study the within crop-dynamics of leafhopper movement over the course of the growing season. In 2023, the first migrant aster leafhoppers were caught on May 23rd 2023 in roadside ditches, weedy patches and in several alfalfa fields (Fig. 3.1). Females dominated the initial population of migrants (Fig. 3.1), which has been found previously with ALH migrants following a long-distance migration North (R. Wallis, 1962). When following their population over the course

of the season in three crops at several sites, alfalfa was the first host crop (Fig. 3.1) followed by a slow movement and population buildup into wheat (Fig. 3.2) starting on the 13 of June 2023 (Fig. 3.2) (when the wheat was tall enough to sweep sample), with a corresponding drop in the alfalfa fields (Fig. 3.1). No reproduction occurred in the alfalfa, with no nymphal production, but a generation of nymphs was produced in the wheat through July with a peak at the end of July 2023. Leafhoppers trickled into canola on a relatively steady pace (Fig. 3.3) (note the small sums even in the peaks) whose peaks correlated with the population peaks in wheat (Fig. 3.2) but no nymphs were produced in the canola, which is typical. At the end of July, the population peak in wheat dwindled (Fig. 3.2) as the wheat matured and the ALH population moved back to alfalfa in August (Fig. 3.1) with nearly equal numbers of males and females, which indicates short population movements (R. Wallis, 1962). This return movement to alfalfa is likely because it is not only the first green crop in the spring, but also the last remaining green crop in the fall which likely plays into the movement of the aster yellows phytoplasma among crop types.

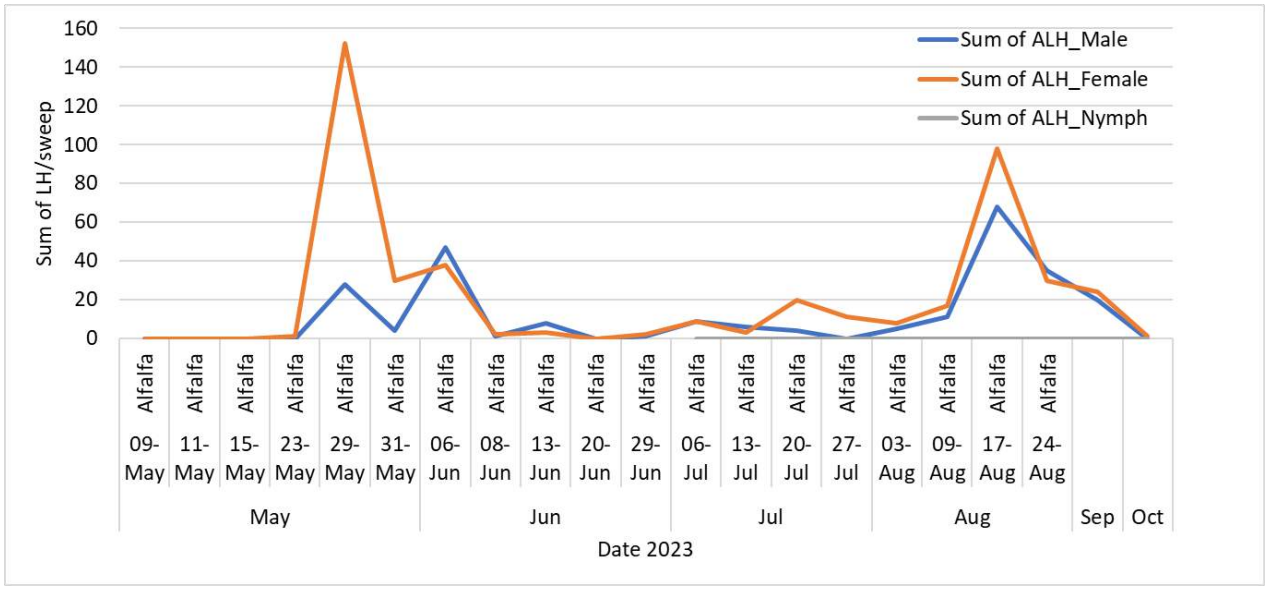


Figure 3.1 Aster leafhopper populations in alfalfa (n=2 sites) over the course of the 2023 growing season

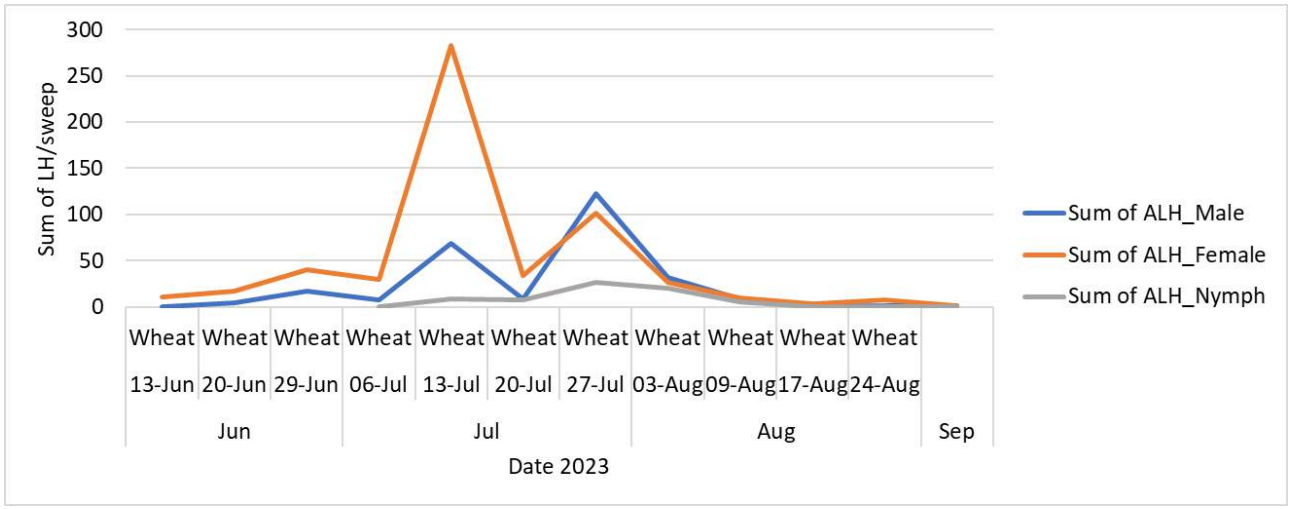


Figure 3.2. Aster leafhopper populations in wheat (n=2 sites) over the course of the 2023 growing season

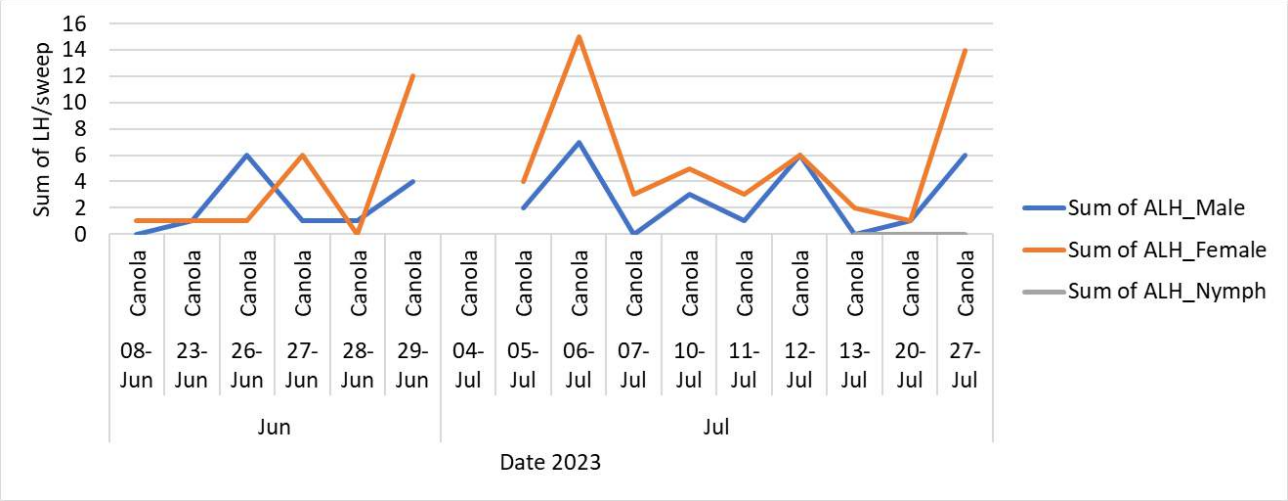


Figure 3.2. Aster leafhopper populations in canola (n=2 sites) over the course of the 2023 growing season

In 2023, between our roadside surveys, sentinel fields, and agronomist collaborators, we collected 162 independent samples of leafhopper sweeps that we standardized to 100 sweeps for the purposes of comparisons. From many of these samples, we tested every leafhopper for the presence of AYp. In the migrant generation, 44 of 56 sites surveyed in the roadside driving survey had leafhoppers with arrival date as the 23rd of May with 15 of the 56 (27%) sites having migrant leafhoppers that tested positive for AYp (Fig.3.3). From all of the surveyed fields throughout the year, 36% of the 162 samples had infected leafhoppers, with percentages where positive leafhoppers were found with a mean infection rate of 30.6 ±4.1 % ranging from 2.2% to 100%. Some sites where 1 leafhopper was caught and was AYp positive, skew the results by coming in as 100% infection of the sample. When calculating the Aster Yellows Index from these samples, the results where one positive leafhopper per 100 sweeps seems high at an AY Risk of 100 (1 ALH/100 sweeps x 100% infection = AY Risk of 100), which is double the AY Risk index that triggers a spray in carrots (Frost et el. 2013), it was eclipsed by some of the AY Risk numbers of the migrant generation, where there was a mean of AY Risk of 433.5 ± 200.8 (range 33-6700, n-=33). Low samples numbers such as 1ALH/100 sweeps, when trying to determine the outcome of having one infected leafhopper in a ditch on the subsequent AY infections in a field, likely would be taken out of any data set. The next step with these AY Risk numbers, with the blessing of the Provincial ministries for using their 2023 AY sample data, would be to determine if we have enough data points from our sweeps and their Rural Municipality survey to determine a relationship between spring migrant AY Risk and incidence of aster yellows in a field (Fig. 3.3). We have overlaid the data and there are regions with AY where our sweep sampling didn't find AY infected leafhoppers and regions where AY infected leafhoppers migrated but no AY incidence was recorded in the RM (Fig. 3.3) so the resolution of the two data sets are likely not going to be precise enough to draw anything more that overall conclusions for the province (Fig. 3.3). The figure does however, highlight future areas of

migrant leafhopper surveying that should be explored to better match with the Rural Municipalities surveyed by the Provincial Ministry.

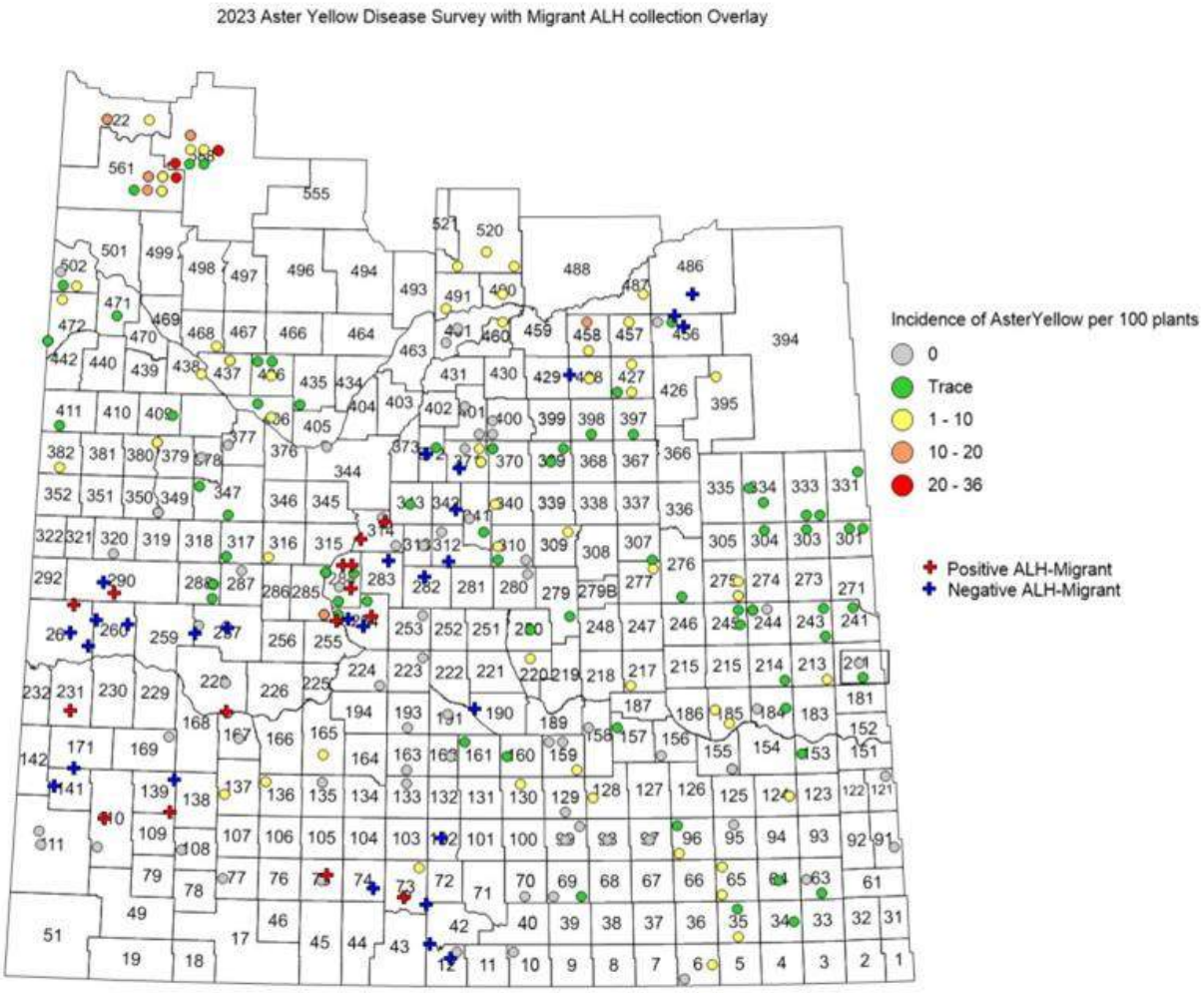


Fig. 3.3 2023 Saskatchewan Ministry of Agriculture Aster yellows survey across Saskatchewan with Aster Yellows phytoplasma positive and negative pools of migrant aster leafhoppers overlaid.

4. Evaluation of aster leafhopper movement from alfalfa to canola and an evaluation of alfalfa as a "green bridge" reservoir.

A 31x 185 ft strip of alfalfa was seeded at the AAFC Lowe Road experimental farm in summer 2021. This strip was to be used as the source to model aster leafhopper movement into canola from alfalfa. The extreme drought however, prevented any germination of the alfalfa. The seed sat dormant until spring 2022 when rain prompted its germination and growth. Canola seed without an added insecticide was sown in plots perpendicular to and touching the alfalfa edge in 2022, but were wiped out by summer-generation flea beetles as soon as they germinated. The alfalfa plot though, served as an excellent "sink" for newly arriving

migrant leafhoppers and provided a second site at the AAFC research farm, to follow the movement of aster leafhoppers among different crops.

Molecular analysis of plants for AYp as "green bridges"

In total, 123 plants from 26 locations were tested for the presence of AY from 2021. Of these plants, 27 were positive by fluorometric LAMP and are in the process of confirming the phytoplasma strain through 16S PCR followed by sequencing. In the late summer, plants with AY symptoms that were tested were fleabane, *Erigeron canadensis* (Asteraceae) (positive for AYp), cleavers, *Galium aparine* (Rubiaceae) x 2 (both positive for AYp), and flax, *Linum usitatissimum* (Linaceae) (five of eight positive for AYp), and buckwheat, *Fagopyrum esculentum* (Polygonaceae) (two of four positive for AYp).

From 32 sites in the spring 2021 we collected potential perennial and biennial reservoir plants based on the criteria that they had been confirmed as AYp hosts before and that the plant had the potential to bridge one growing season to the next (hence, perennial and biennial species) and were common in the prairie landscape. We settled on alfalfa, common dandelion, *Taraxum officinale* (Asteraceae), perennial sow thistle (first to emerge in the spring), *Sonchus arvensis* (Asteraceae) and stinkweed (field pennycress), *Thlaspi arvense* (Brassicacea). These plants were collected as we detected arrival of the 2021 aster leafhopper spring migrants (just prior to the May long weekend), May 18-19 2021, to ensure that any AYp infections resulted from overwintering of the AYp in the plant itself and not from spring infection by migrating leafhoppers. These samples were mostly taken from fields margins (ditches) and we qualified the dominant vegetation in all of these ditches as Alfalfa/Brome grass (*Bromus* spp.), with Stinkweed, Sow Thistle and Dandelion often present where soil was disturbed (Stinkweed, Sow Thistle) or everywhere (dandelion). It was here that our resources (personnel, budget and ability to test) fell short so we screened 3 of each of the ten plants per species per site. We had one full time technician and an FSWEP student helper working in winter 2021, and recruited the help of another technician in 2022. We had several molecular biological setbacks which is not uncommon. Initial tests on the plants failed to extract DNA (suspected expiration issue with the DNAEasy extraction kit over the Covid shutdown) but subsequent extraction worked well and we achieved results with the fluorometric LAMP (Jan 13 2021). Subsequent 16S PCR to confirm results and sequence the AYp strain were unsuccessful. Results from the LAMP test are very interesting though. From the first sample date (May 18-19), plants in all of the four species collected from sites within a two hour drive from Saskatoon tested positive for aster yellows. Seven alfalfa plants from five of the 14 sites (n=42 plants total) tested positive (17%) for AYp. Five of the 30 (16%) dandelions from four out of ten sites tested positive for AYp. One sow thistle plant (7%) from one of five sites (n=15 plants) tested positive for AYp. Two stinkweed plants from two sites (n=12 plants, 17%) tested positive for AYp.

The Saskatchewan Ministry of Agriculture survey in canola fields in 2022 found only low levels of aster yellows infection in canola fields across the province. Seven fields of canola of 205 surveyed (3%) had AY incidence scores, where aster yellows symptomatic plants were recorded on the 100 plant transect (Mode was 1% infection with 5 times occurrence, and 7%,4%,3% in the other fields). Aster yellows plants (called trace) were evident in 25% of the surveyed canola fields.

The Saskatchewan Ministry of Agriculture survey in canola fields in 2023 found high levels of aster yellows infections (Incidence range, 1-36%) which indicates that the high infection rate and large numbers of the migrating aster leafhoppers that we detected in the spring of 2023 resulted in widespread Aster Yellows infection (Fig. 3.3). 66% (143 of 218) of surveyed fields had plants with detectable aster yellows symptoms,

scored as “trace” if plants with AY infections were not detected in the 100 plant random survey. Alberta and Manitoba’s surveys also indicated much higher levels of aster yellows than in the previous few years.

In 2022, and 2023, weedy, potential reservoir plants were sampled from RMs where aster yellows was found on the Saskatchewan Ministry of Agriculture Provincial aster yellows survey as well as from sites on our enhanced grid (18 sites, 10 plants from each species/site). Alfalfa and dandelions were found at all sites, with stink weed (4 sites), clover (5 sites) and perennial sow thistle (1 site) sampled where they occurred. These plants were all collected before the 2022 aster leafhopper migration so any positive plants would be attributed to infection of rosette stage plants the year before. None of the 400 plants tested positive for the aster yellows phytoplasma (AYp) in 2022. Our weedy reservoir survey in 2023 focused on common plants that could be found at all sites, that we know can host AYp such as alfalfa and dandelion, and we sourced plants prior to the 2023 leafhopper migrants arrived from the areas in 2022 (n=19) where aster yellows was noted in the Provincial surveys and on our own research farms where we had aster yellows infected plants in 2022 (Fig. 3.4). None of the weeds collected in spring showed any aster yellows symptoms and when we tested alfalfa and dandelions (n=10 each) from our research farms, none of the samples were positive for AYp. We sourced the same two weeds again in June at the AAFC Research farms (n=10 each) and none of these tested positive for AYp either. We decided to not test any more weedy plants that we had collected to save money and time and to focus on the large numbers of leafhoppers that started to arrive in May 2023.

The reservoir results support our theory that AYp is moved into these perennial plants in the fall when they are the last green plants in the environment and then AYp becomes active from the roots again in the spring as reservoir plants. The “green bridge” then might only function for one season as a transitory reservoir, and not form a yearly reservoir for aster yellows. The aster yellows recorded in Saskatchewan in 2021 and 2022 was low, with subsequent potential weedy reservoirs also low. In 2024, we noted many weedy biennial plants such as stinkweed *Thlaspi arvense*, and prickly lettuce, *Lactuca serriola*, in the spring and late summer when the plants had fully grown, at the AAFC research farm with symptoms of aster yellows.

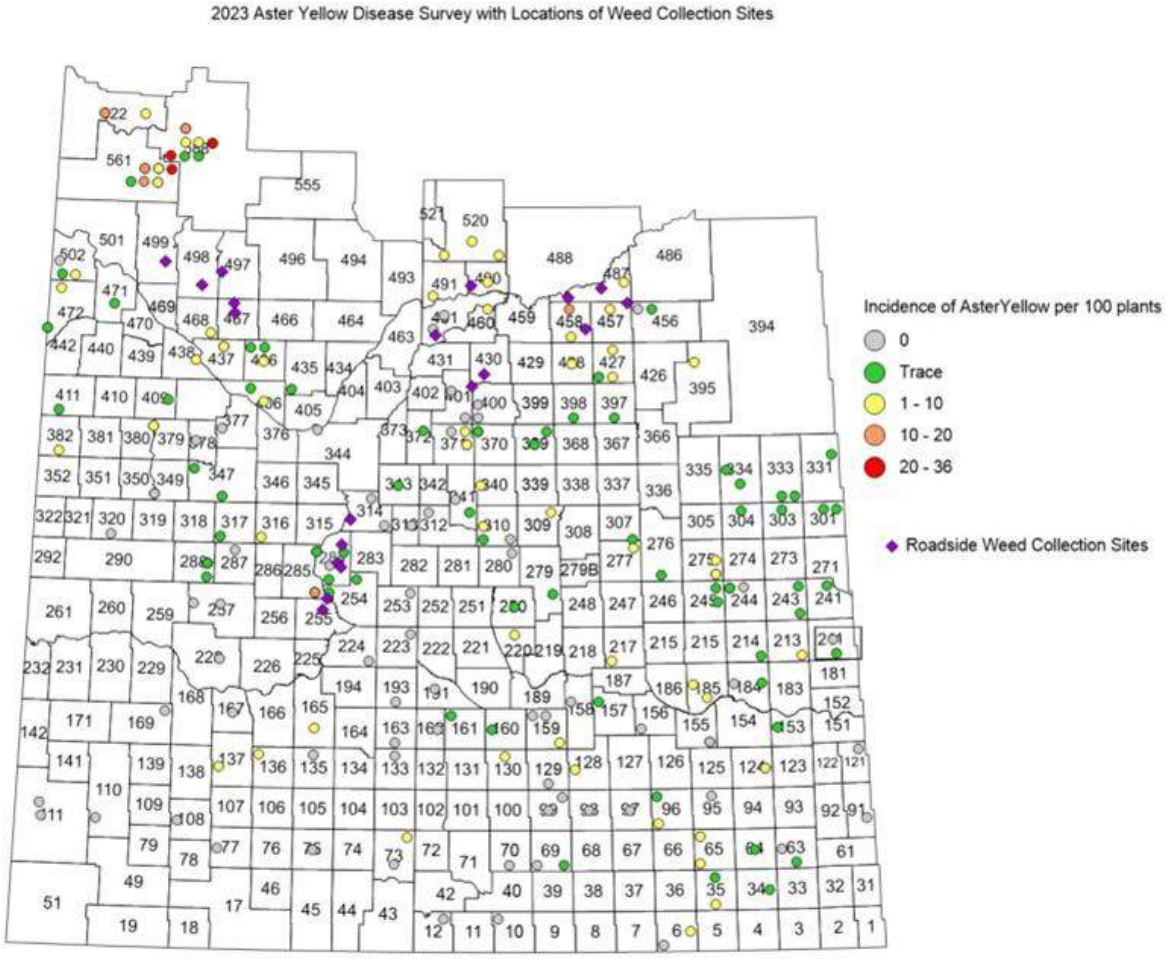


Figure 3.4. 2023 Saskatchewan Ministry of Agriculture Aster yellows survey across Saskatchewan with potential spring weedy reservoirs that were sampled in mid-May 2023, overlaid.

Evaluation of aster leafhopper movement from alfalfa to canola and an evaluation of alfalfa as a "green bridge" reservoir.

The 31x 185 ft strip of alfalfa that was seeded at the AAFC Lowe Road experimental farm in summer 2021 came up nicely in 2022. Canola seed without an added insecticide was be sown in plots perpendicular to and touching the alfalfa edge in early summer but the summer generation of flea beetles wiped out these young, unprotected plants even with Matador sprays having been applied. So, we took the experiment inside where conditions were controlled (ie. No rampaging flea beetles) and studied aster leafhopper (ALH) host choice and movement, leafhopper reproductive potential on these three key plants, ratios of AYp un-infected to AYp infected leafhoppers and development of aster yellows symptoms on plants.

Settling Behavior of Aster Leafhoppers

Over each trial of the experiment, brome was by far the most popular choice for ALH to settle and feed on, and this was quite similar across all three trials (Figure 4. 2., Table 4.2.). Alfalfa was the least popular plant for

settling while canola was consistently the second most popular choice once it was added to cages (Figure 4.2., Table 4.2.). The settling preferences of ALH between the five cages in each trial had varying degrees of similarity (Table 4.2.). For canola, ANOVA indicated that there were significant differences among cages in each trial, with cages having less AYp pressure (lower ratios of AYp infected leafhoppers) with more ALHs on canola (Table 4.2.). For the first trial, the average percentage of ALHs with standard error was 33.3% ± 3.3% and 0.399 ± 0.047 for the 2% and 6% trial respectively, and 0.269 ± 0.033, 0.071 ± 0.014, and 0.268 ± 0.038 for the 10%, 14%, and 18% respectively (Table 4.2.). During the second trial, the average proportion of ALH was 0.156 ± 0.014 and 0.165 ± 0.013 for the 5% and 10% trial and 0.102 ± 0.009, 0.114 ± 0.018, and 0.100 ± 0.009 for the 15%, 20%, and 25% respectively. During the third trial, the average proportion of ALH was 0.103 ± 0.013 and 0.145 ± 0.016 for the 4% and 10% trial respectively and 0.060 ± 0.009, 0.102 ± 0.019, and 0.058 ± 0.014 for the 14%, 20%, and 24% cage respectively. The proportions of ALH in each experiment changed over both experiments, more notably in the first and second trial (Figure 4.2.). In the first and second trial, the proportion on canola increased over time while the proportion on brome decreased. The proportions stayed fairly consistent during the third experiment. The other statistically significant result was in alfalfa in the second trial, where the highest proportion on alfalfa was in the 5% AY cage, and the second highest on alfalfa in the 10% AY cage. There were no statistically different settling proportions among cages for settling on brome grass in any experiment. Interestingly, in the first and second trial especially, the proportion of ALH on brome decreased throughout the experiment while the proportion increased on canola. This demonstrates the movement of ALH from brome onto canola over time. This trend is not quite visible in the third trial however, with proportions remaining relatively steady throughout the trial but still demonstrating that around 25% of the leafhoppers chose to leave brome and move into canola when canola was presented.

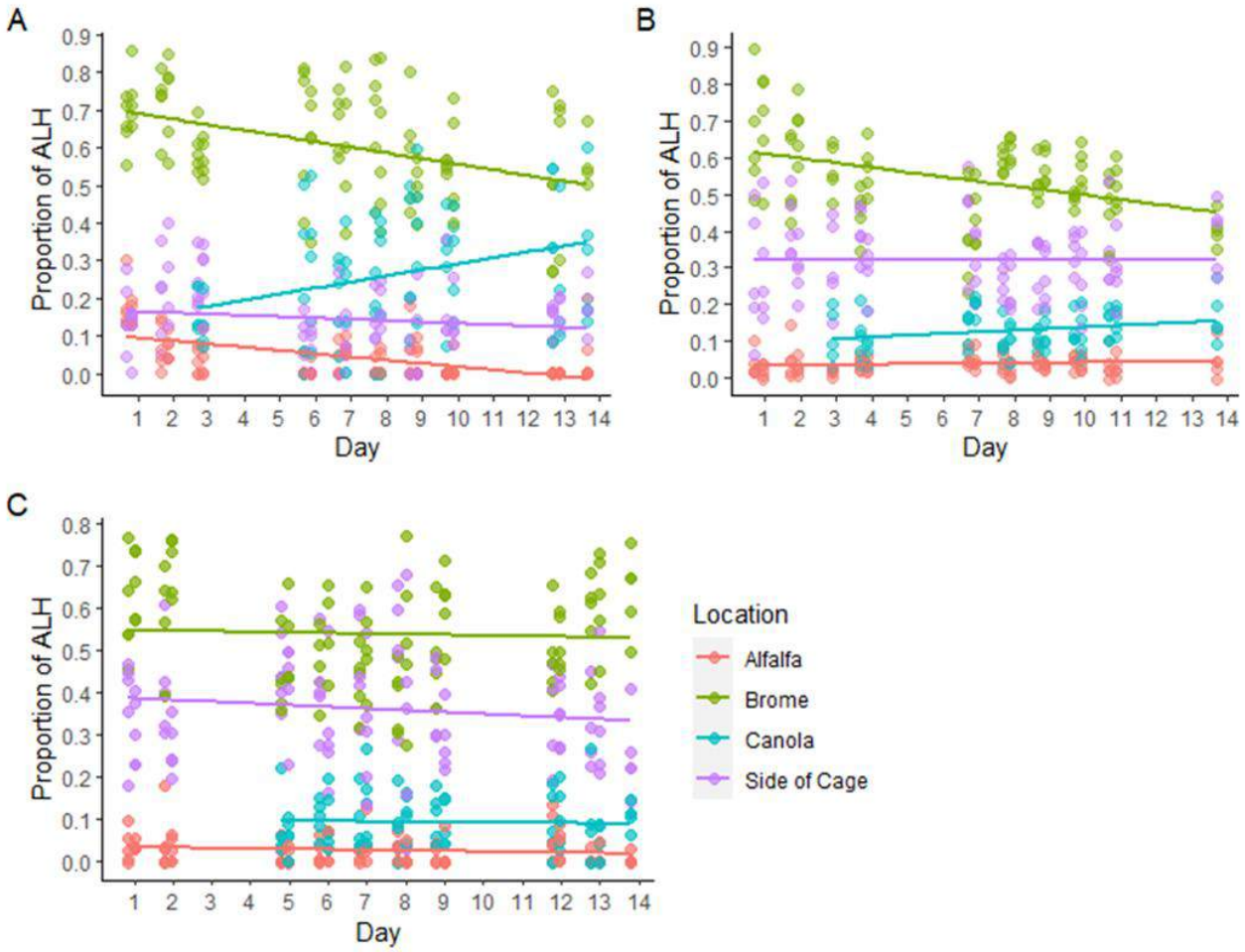


Figure 4.2. Settling locations of aster leafhoppers over time across all five cages and separated by trial. Observations were taken twice daily on weekdays, therefore gaps in data represent weekends. Each point represents the proportion of aster leafhoppers counted on each plant species, or on the side of the cage, during observations, divided by the total amount counted in each cage.

Table 4.2. Settling behavior of aster leafhoppers over the three experimental trials for each cage as well as for the average of the five cages. Proportions were calculated by dividing the number of counted individuals on each plant by the total number of counted insects per each cage for a specific observation period. ANOVA was used to test for differences in these proportions among experimental cages, with the different treatments of each cage represented in Figure 4.1 (materials and methods). N represents number of counts. Counts differ between brome and alfalfa and canola because canola was added 48 hours after brome and alfalfa.

Cage Number	Brome Trial 1	Alfalfa Trial 1	Canola Trial 1	Brome Trial 2	Alfalfa Trial 2	Canola Trial 2	Brome Trial 3	Alfalfa Trial 3	Canola Trial 3
1	**59.1%** ± 3.2% N = 19	**4.7%** ± 1.2% N = 19	**33.3%** ± 3.3% N = 15	**54.6%** ± 2.8% N = 18	**6.6%** ± 0.9% N = 18	**15.6%** ± 1.4% N = 14	**60.2%** ± 3.0% N = 19	**4.1%** ± 0.8% N = 19	**10.3%** ± 1.3% N = 15

2	**52.3%** ± 3.3% N = 18	**6.3%** ± 1.8% N = 19	**39.9%** ± 4.7% N = 15	**58.0%** ± 3.4% N = 18	**4.2%** ± 0.6% N = 18	**16.5%** ± 1.3% N = 14	**54.3%** ± 2.1% N = 19	**2.5%** ± 0.6% N = 19	**14.5%** ± 1.6% N = 15
3	**62.2%** ± 3.5% N = 19	**2.8%** ± 1.3% N = 19	**26.9%** ± 3.3% N = 15	**56.4%** ± 2.6% N = 18	**2.7%** ± 0.4% N = 18	**10.2%** ± 0.9% N = 14	**47.3%** ± 2.5% N = 19	**1.4%** ± 0.4% N = 19	**6.0%** ± 0.9% N = 15
4	**67.9%** ± 2.2% N = 19	**5.6%** ± 1.3% N = 19	**7.1%** ± 1.4% N = 15	**51.0%** ± 2.4% N = 18	**2.1%** ± 0.4% N = 18	**11.4%** ± 1.8% N = 14	**48.4%** ± 3.0% N = 19	**3.0%** ± 1.0% N = 19	**10.2%** ± 1.9% N = 15
5	**61.1%** ± 3.6% N = 19	**3.3%** ± 1.2% N = 19	**26.8%** ± 3.8% N = 15	**50.6%** ± 2.3% N = 18	**2.9%** ± 0.3% N = 18	**10.0%** ± 0.9% N = 14	**59.1%** ± 2.2% N = 190	**2.5%** ± 0.9% N = 19	**5.8%** ± 1.4% N = 15
Trial Average	**60.6%** ± 1.5% N = 94	**4.6%** ± 0.6% N = 95	**26.8%** ± 2.0% N = 75	**54.1%** ± 1.2% N = 90	**3.7%** ± 0.3% N = 90	**12.7%** ± 0.7% N = 70	**53.9%** ± 1.3% N = 95	**2.7%** ± 0.4% N = 95	**9.3%** ± 0.7% N = 75
ANOVA (P-value, F-Value)	**0.068** **3.405** df = 92	**0.429** **0.631** df = 93	**<0.001** **12.16** df = 73	**0.084** **3.064** df = 88	**<0.001** **27.57** df = 88	**<0.001** **15.04** df = 68	**0.363** **0.836** df = 93	**0.309** **1.048** df = 93	**0.010** **7.026** df = 73

Aster Yellows Infection in Canola

The first trial of the experiment did not result in any visually infected plants. Once the experiment was finished, 10 individuals from the ALH+ acquisition cage which were used during experimentation were tested via colourimetric LAMP (cLAMP) and none were found to be infected. Due to this lack of infection, none of the plants were tested for AY infection either to save time and molecular reagents. Plants were still monitored after the experiment in a greenhouse and none produced any symptoms over the following 6 weeks and bolted and flowered normally. These plants were saved to be harvested at maturity but we didn't have the resources (human and reagent) to test them all.

The second trial of the experiment involved LAMP testing of ALH+'s before experimentation and an adjustment to the number of ALH+ added to counteract the lower infection rates. In addition to this adjustment, twice the number of individuals were added to each cage. Following experimentation, one plant became heavily symptomatic, scoring a 5 on the 5-point scale developed by Olivier *et al.* (2014). This plant

came from the 20% infection cage at position B4 (Figure 4.3.), and there were six recorded instances of a leafhopper on this plant between day 8 to day 14 of the experiment, but also several days where the insect was not observed. After half of the plants in each cage were sampled, the B4 plant tested positive for AYp as well as another canola plant that had no visual symptoms, C1. This plant had three recorded instances of leafhopper feeding, and came from the same 20% infection cage. An infection score of 0 was assigned to this plant since it had no visual symptoms.

The third trial of the experiment produced eight infected canola plants which were all identified visually (Figure 4.3.). Five of these plants came from the 10% infection cage, (A3, A4, B1, B3 and D1), which had 3, 5, 5, 2, and 10 leafhopper observations respectively. All these plants had infection scores of 5 with the exception of B3 and A4 which had scores of 2 and 4 respectively. The 14% cage had the other three infected canola, which were plants A2, C3, and B5, which had 0, 5, and 1 ALH observations respectively and each plant had an AY score of 5.

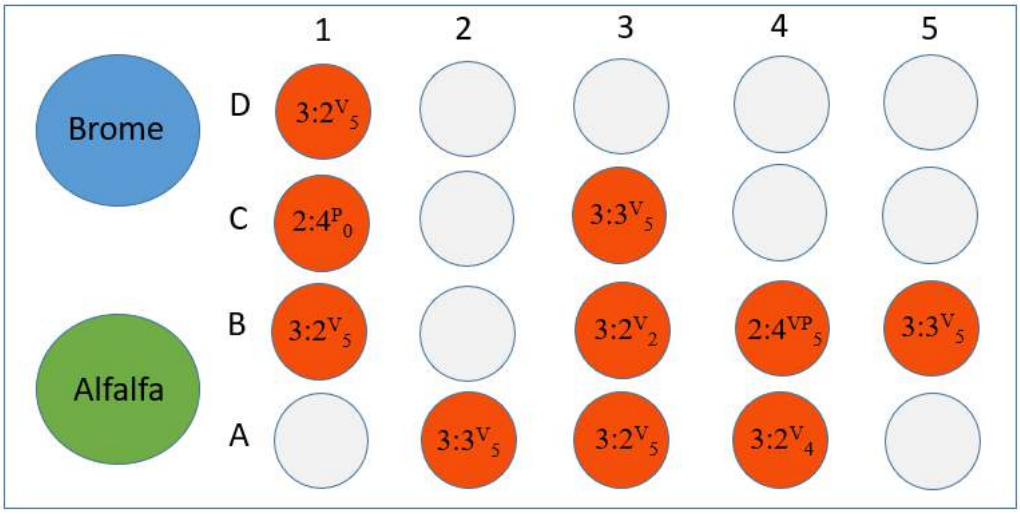


Figure 4.3. Aster yellows infection location, represented by orange circles, in canola across all cages across all three trials. Numbers and letters adjacent to canola locations represent the labeling system for pot location. The two numbers within circles represent trial number:cage. The "V" superscript represents infection was identified visually and the "P" superscript represents infection was identified with cLAMP. The subscript number represents the infection severity rating, as described by Olivier *et al*. (2014)., from least severe, 1, to most severe, 5. A rating of 0 was given when plants weren't visually symptomatic but PCR identified the plant as infected.

Leafhopper Distribution in Cages

In the first trial there were 272 observations of ALH on canola, this increased to 485 in the second trial when the number of ALH in each cage was doubled, and 190 in the third trial. Results from SADIE over the three trials showed that each trial showed evidence of aggregation, with all trials producing an $I_a > 1$, but none produced significant evidence that the distribution deviated from a random aggregation ($P_a > 0.05$; Figure 4.). The first trial had the strongest trend towards aggregation ($I_a = 1.281$) but no significant difference between the results and random aggregation ($P_a = 0.0688$). This P value is close to the alpha significance value of 0.05 however, and likely indicates aggregation due to males searching plants for calling females. We unfortunately could not determine male from female leafhoppers with these observations. The second trial produced

weaker but still a substantial trend towards aggregation (I_a = 1.038) and still no significant difference from random aggregation (P_a = 0.3475). The third trial produced data that was not normally distributed so the non-parametric version of this test was used. The data showed aggregation in the observations (I_a = 1.116) but no significant deviation from random aggregation (P_a = 0.2058).

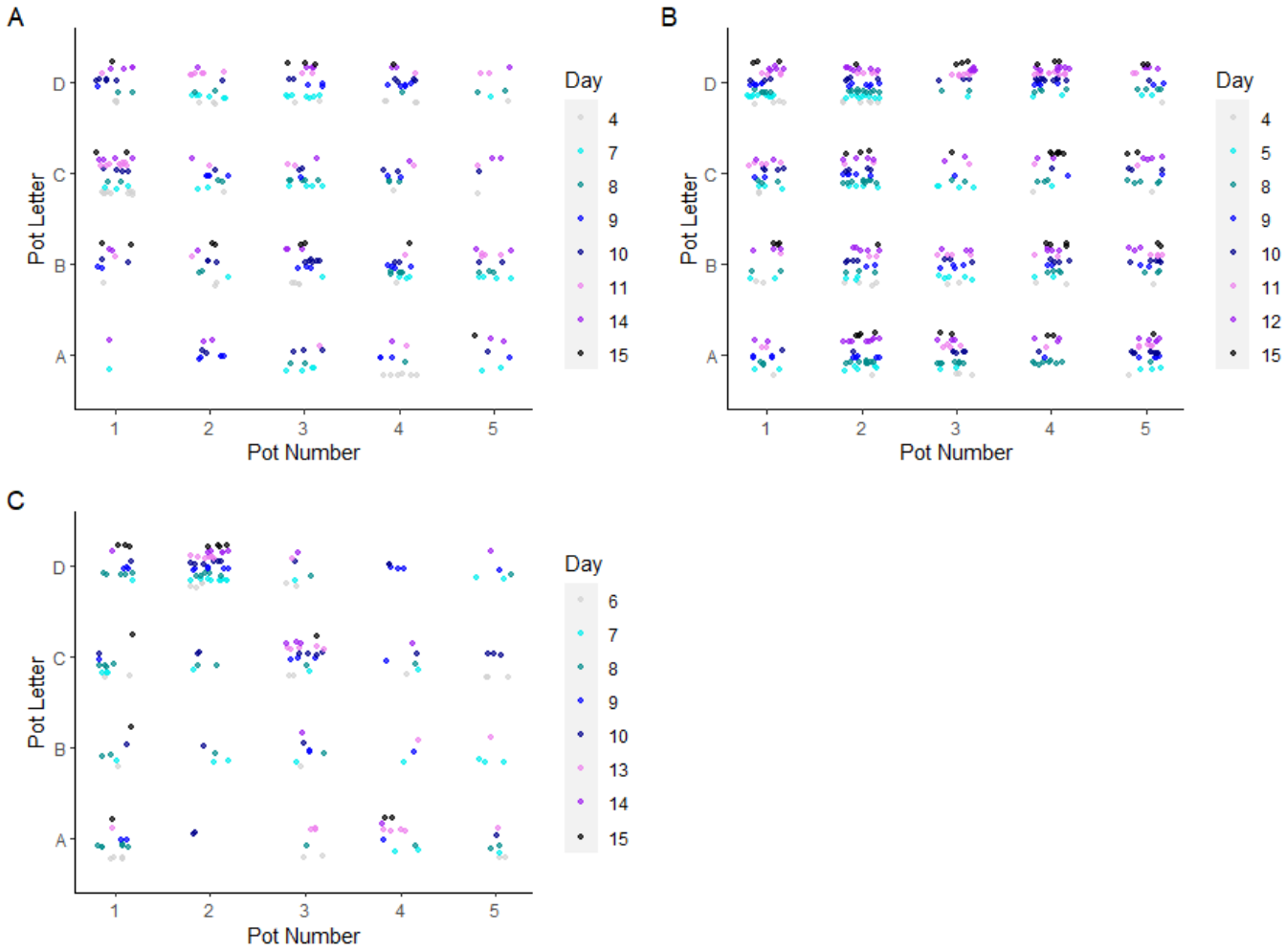


Figure 4.4. Location of counted aster leafhoppers within the canola field across all five cages for the first (A), second (B), and third (C) experimental trials. Observations were taken twice daily on weekdays and each point represents an observation, meaning one individual can be represented multiple times in the data.

Reproductive Viability of Brome and Alfalfa

After the brome and alfalfa that were used in the second and third trial were placed into separate cages, nymphs began emerging after a few weeks. For all 10 brome plants, nymphs emerged and some cages contained well over 100 insects. However, no nymphs emerged on any of the alfalfa plants, suggesting it is not a good host for ALH reproduction. No nymphs emerged on any of the canola plants, and we already knew that canola was a poor reproductive host for ALH.

Discussion

In this experiment, the main objective was to discover if ALHs in an alfalfa and brome-dominated ditch would move into a canola field. The spread of leafhoppers onto the canola was also investigated by analyzing where leafhoppers decided to settle, to understand if they prefer to stay near the ditch. By using varying ratios of ALHs infected with AYp, we investigated if higher AYp pressure in enclosures would increase disease transmission. Finally, to test the reproductive viability of alfalfa and brome, we followed nymphal production by ALH to see if nymphs would emerge.

Settling Behavior and Preferences Between Species

Over all three trials, brome was chosen for settling over the other two species, which is not a surprise because grasses and cereals are preferred by ALH for feeding and reproduction. (Figure 4.2., Table 4.2.). Alfalfa was the least preferred plant with sometimes no ALHs observed on the plants. This is interesting as it complicates the theory that alfalfa might act as a reservoir for AYp, as it appears to be an unpopular feeding choice. This experiment was not designed to test if ALH feed on brome or alfalfa, so it is difficult to say if the few ALH observed on alfalfa were feeding on the plant, or were just settling. Brome is also known as a host for AYp (Urbanavičienė 2005, Olivier et al. 2009) and is perennial so it could also be an overwintering reservoir for AYp. Since it appears to be the preferred host plant of the two ditch plants, perhaps it should be sampled adjacent to agricultural fields to test for AYp. Sweep nets could also be used to identify if ALH or other leafhoppers which transmit AYp, such as *A. argentarius*, are located in field margins. *A. argentarius* has also been shown to make up a significant portion of leafhopper populations in field edges within the last decade (Olivier *et al*., 2017). The theory that ALHs pick up AY infection from alfalfa, brome, or other weeds could use more investigation, as cereals such as barley, don't show much ability to propagate the phytoplasma or spread to aster leafhoppers (Bahar et al. 2018).

The observation that the proportion of ALH settling on canola varied between cages is an interesting result of this experiment (Table 4.2.). In general, in each trial, the two cages with the lower AYp infection rates in ALHs had a higher proportion of ALH settling on canola than those with higher treatments (Table 4.2.). While a similar study investigating ALH settling preference never found a pattern between AY-infection and settling choice, oviposition, or egg counts, they did notice some statistically significant differences (Bahar *et al*., 2018). Most interestingly, they found that when ALH- were given a choice between barley and canola, there was no difference in settling preference but when ALH+ were placed in the same position, more ALH+ settled on barley. This is similar to what was found here, with increasing proportions of ALH+ seeming to prefer to stay on brome, another grass. As indicated by these researchers, there are many examples of pathogens which can alter hosts behavior and feeding preferences, however in the case of AYp and the ALH, there is no strong evidence to suggest that this is the case, but the findings from this study and those by Bahar *et al*. (2018) prompts future exploration into this question, especially given the similarity of the findings. In the second trial, it was found that on alfalfa, the 5% ALH+ cage had higher settling proportion (6.6% ± 0.9%), followed by the 10% AY cage with (4.2% ± 0.6%), with the 15%, 20%, and 25% being between 2.7% and 2.1% (Table 4.2.). These averages were found to be significantly different when tested with ANOVA ($p = <0.001$, $F=27.57$). The study by Bahar *et al*. 2018 did not include alfalfa, or any legumes, but based on the findings that there are discrepancies between ALH settling when infected with AY and when not infected, this finding seems reasonable.

Leafhopper Distribution in Canola Field

One of the main goals of this experiment was to determine if ALH would move into a canola field once it had settled in an alfalfa and brome ditch, even though canola has been shown as a unpreferred species compared to many grasses and cereals (Bahar *et al*., 2018, Romero et al *et al*., 2020, 2022). This experiment certainly succeeded in this aspect, as ALH seemed readily settled on canola (Figure 4.2.; Figure 4.4.) and feed on it for long enough to vector AYp, as demonstrated by the canola which contracted AY disease (Figure 4.3.), without any disturbance needed in the ditch to prompt movement. According to analysis by SADIE, each of the three experimental trials produced some degree of clustering, but none showed significant difference than from a random aggregation. Most notably in the third trial, clustering closer to the location of the brome in the ditch occurred which was to the left of C1 and D1 (Figure 4.1.), while alfalfa was next to B1 and A1. This trend is also visible in the first experiment, where canola closer to brome had more ALHs observations, and this is the trial which produced the data which was closest to being statistically significant in terms of clustering ($p = 0.0688$). Because brome is the preferred plant for settling and feeding, it seems logical that more ALHs would settle close to brome, as they likely would have moved from brome onto the closest canola after it was added to cages. This would mean that canola adjacent to ditches with brome and other preferred food plants which are able to harbour AYp can be a significant risk to introducing AYp into canola fields, where it could be spread by ALH or other leafhoppers. In low AY years, the few aster yellows symptomatic plants are usually found on field edges which is supported by the clustering of leafhoppers on plants near the “ditch” in this experiment. The canola “fields” we not that long but did demonstrate that ALH would penetrate into a field beyond the first available plants.

Aster Yellows Infection in Canola

Over all the trials completed in this experiment, 10 canola plants with were symptomatic for AY disease (Figure 4.4.). While only two canola plants showed symptoms in the second trial, one being highly symptomatic and the other being visually asymptomatic, eight plants became infected in the third trial. Most interestingly, five plants became symptomatic in the 10% cage of the third trial. This was a cage that received 50 ALH+s from a AY acquisition cage with only 1/10 leafhoppers testing positive before they were placed into the cage. In retrospect, there was probably a higher proportion of infected leafhoppers than was accounted for in the initial test; this could be verified later by testing the leafhoppers collected from this cage which are preserved in the freezer. Even with a higher infection ratio, this cage alone demonstrates the potential of infected ALH+ to vector AY to canola, in a recreated field setting. With enough AY in an ALH population, just a few individuals feeding on canola can spread the disease quite effectively over a long enough time frame. In the wild, abundances of ALH and other AY-transmitting leafhopper species can sometimes be much higher, especially towards field edges (Olivier *et al*., 2017). Out of the five canola plants in this cage, three plants scored a 5/5 on the AY symptom rating scale, while one scored a 4/5, and one a 2/5 (Figure 4.). All of the plants at this level of symptomology have no yield, which will be revealed once the full harvest results are ready. Olivier *et al*. (2014) investigated how many ALH+ were required over a 10-hour period to infect canola plants and found that even with 12 ALH+ on a single plant, only 17%, 40%, and 40% of canola became infected and scored 5/5, over the three replicates. Here, we showed that symptoms scoring 5/5 can be obtained by potentially only one ALH+ feeding on a single plant over a long enough time scale, while simultaneously providing leafhoppers with a preferred food source, brome. This is very interesting as a plant scoring 5/5 and 4/5 on the symptom rating scale produce no seed, meaning in this cage 20% of the plants produced no seed, which is a substantial yield loss in a real canola field. While we can’t conclusively say that only one individual can transmit AY, there were two plants from the 14% AY cage in the third trial which had 0 and 1 ALH observed over the whole trial which both developed 5/5 symptoms. While this does provide evidence that one, or at most only a few, ALH+ can cause heavily symptomatic canola, it also highlights one

of the drawback to this study which is the difficulty of observing leafhoppers in their cages. This difficulty could be tackled in future experiments by using other methods to visualize ALH, such as coating insects in a fluorescent powder and locating them with a UV light in the dark, a technique that appeared promising in our initial lab tests.

Reproductive Viability of Brome and Alfalfa

The lack of reproductive potential of ALH on alfalfa is very interesting. While it certainly doesn't diminish the suspected role of alfalfa in the spreading of AYp, it definitely suggests there may be some other weedy plants growing next to agricultural fields which are more appealing as food and reproductive hosts to ALHs. The fact that more ALHs were observed on canola compared to alfalfa also points to this, although it is always possible ALHs were simply not able to be seen as well on alfalfa due to its more "bushy" nature. The preference of ALHs for brome in this experiment and it's ability to act as a good host for ALH reproduction demonstrates a need for more testing of this plant and other common grasses for AYp and their potential as reservoirs. If there were to be a similar level of AY in these grasses as there has been found in alfalfa, it could be a dominant reservoir for the disease, and any nymphs that matured on brome would almost certainly pick up the phytoplasma if it was present.

Objective 4 References

Bahar, M.H., Wist, T.J., Bekkaoui, D.R., Hegedus, D.D. and Olivier, C.Y., 2018. Aster leafhopper survival and reproduction, and Aster yellows transmission under static and fluctuating temperatures, using ddPCR for phytoplasma quantification. *Scientific Reports. 8*(1): 227.

Frost, K. E., P. D. Esker, R. V. Haren, L. Kotolski, and R. L. Groves. 2013a. Factors influencing aster leafhopper (Hemiptera: Cicadellidae) abundance and aster yellows phytoplasma infectivity in Wisconsin carrot fields. *Environmental Entomology* **42**:477-490.

Frost, K. E., P. D. Esker, R. Van Haren, L. Kotolski, and R. L. Groves. 2013b. Seasonal patterns of aster leafhopper (Hemiptera: Cicadellidae) abundance and aster yellows phytoplasma infectivity in Wisconsin carrot fields. *Environmental Entomology* **42**:491-502.

Olivier, C., T. Dumonceaux, E. Pérez-López, T. J. Wist, R. B. H. Elliott, and S. Vail. 2017. Detection, symptomatology and management of aster yellows disease in canola. Pp. 233-246 in *Integrated Management of Insect Pests on Canola and Other Brassica Oilseed Crops* CABI International.

Olivier, C. Y., B. Galka, and G. Séguin-Swartz. 2010. Detection of aster yellows phytoplasma DNA in seed and seedlings of canola (Brassica napus and B. rapa) and AY strain identification. *Canadian Journal of Plant Pathology* **32**:298-305.

Olivier CY, Lowery DT, Stobbs LW (2009) Phytoplasma diseases and their relationships with insect and plant hosts in Canadian horticultural and field crops. The Canadian Entomologist 141, 425-462.

Romero, B., et al. (2020). "Oviposition Behavior and Development of Aster Leafhoppers (Hemiptera: Cicadellidae) on Selected Host Plants from the Canadian Prairies." Journal of Economic Entomology **113**(6): 2695-2704.

Romero, B., et al. (2022). "Do Options Matter? Settling Behavior, Stylet Sheath Counts, and Oviposition of Aster Leafhoppers (Hemiptera: Cicadellidae) in Two-Choice Bioassays." Environmental Entomology **51**(2): 460-470.

Urbanavičienė, L., 2005. Molecular detection and identification of phytoplasma affecting smooth bromegrass (Bromopsis inermis). *Botanica Lithuanica. 11*(3)*:1392-1665*.

5. Use of wind trajectories and stable isotopes to continue gathering data on diamondback moth (DBM) and aster leafhopper migration flights and origins.

Wind trajectories

2021 Wind trajectories and Yellow Sticky Card Sites

Yellow sticky cards were placed at 33 sites in Saskatchewan to monitor arrival of *Macrosteles quadrilineatus*. The trapping season in 2021 ran April 27 to June 23. The total capture of *M. quadrilineatus* across the season was 778, which was almost the same number as the ALH catch in 2020. The catch of these two years was substantially higher than the ALH caught in both 2018 and 2019, (2019 (n= 97) and 2018 (n=496). The two sites with the highest number of individuals captured in 2021 were Regina1 (n=90) and Pelly (n=96) the 3rd highest total was Ituna (n=72). There were three sites: Bruno, Lemburg and Prince Albert, where no *M. quadrilineatus* were captured.

Possible sources of origin and migration route were examined using Hysplit to calculate Ensemble reverse trajectories (RTs). Unlike single trajectory analysis which calculates a single trajectory based on a single meteorological grid cell, Ensemble RTs calculate 27 trajectories with each trajectory calculated by offsetting the grid cells. Trajectories were run at height of 1500m AGL and for 48 hrs. The run time was selected because, although the estimated time leafhoppers can remain in flight is 24-30 hrs, they are not passive while in flight. This means that they may cover a distance of greater than 24 hrs when assisted by the wind. Start time of the models was set to 3UTC or 9:00pm local time which is corresponds to sunset in May.

RTs were generated for the locations with the first capture of *Macrosteles*. In 2021 the first capture of *Macrosteles* occurred at 5 sites between May 10-19. Those sites were Drake (n= 1), Lumsden (n=1), Pelly (n= 2), Regina1 (n= 27), and Tisdale (n=2). There were 6 sites with the first capture between May 17-June 3: Craik (n=6), Dundurn (n=11), Outlook (n=2), Rouleau (n=1), Southey (n=9), and Regina 2(n=1). Based on the location of these 11 first-catch sites, Ensemble RTs were calculated for Regina, Pelly, Tisdale and Imperial (May 8-23). The Imperial location was used as it was deemed to be central to Dundurn, Drake, Southey, Craik and Outlook and would be indicative of the wind trajectories for all 5 locations. The time period was selected to cover a period of 4 days prior to the card placement but was extended 5 days after the card was removed to cover overlap between the first and second capture dates. Maps were created for the nine days with RTs south of 49th parallel (Fig 5.1-9). Points along the trajectory route where the trajectory was at ground level (0-5 m AGL) were plotted on the map as dots. At this level, aster leafhoppers would have had no trouble catching the prevailing winds and riding them Northward.

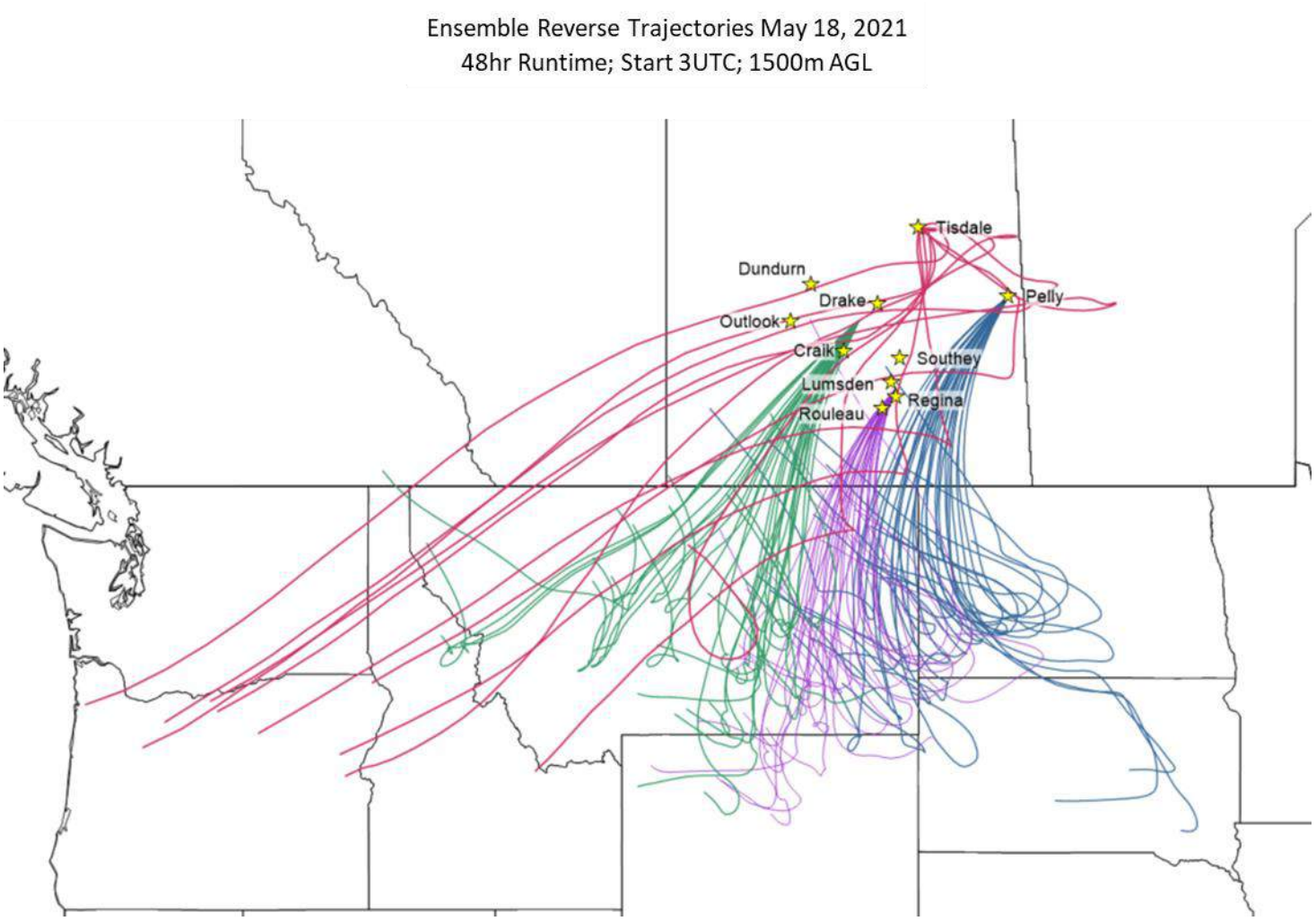
There were eight consecutive days (May18-25) with RTs originating in the USA. May 20 2021 was the only day out of the seven that did not have RTs originating in the Great Plain corridor. The origin on that day was the Pacific North West (PNW). The ensemble RTs on May 18, May 19 and May 21 2021 had points of origin

both in the PNW and the Great Plain corridor (GPC); May 20 the point of origin was PNW; May 22-25 origin was GPC. May 24th is of interest because all the RTs for all sites follow the same pattern.

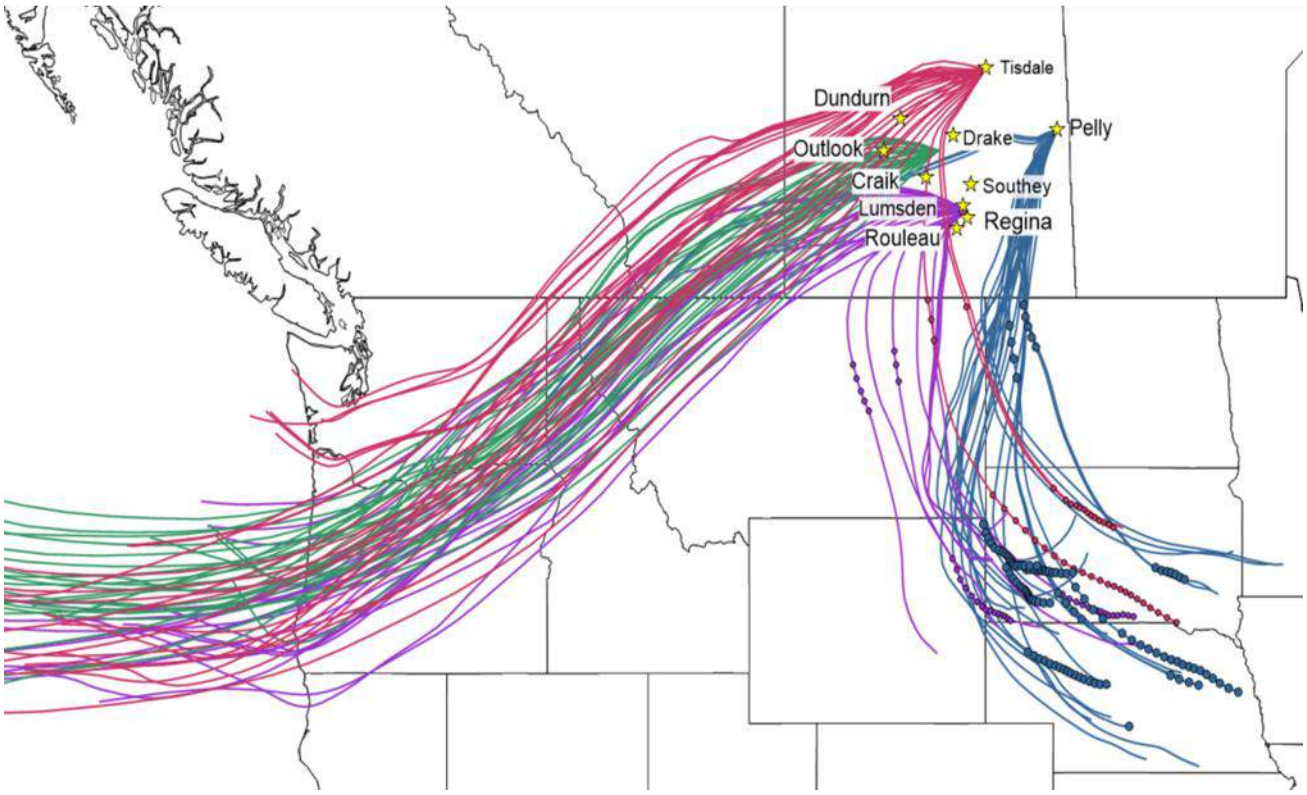
Table 5.1 2021 Yellow Sticky Card Trap Locations

Site	Lat	Long	Trapping Period	Date First Capture	Total Season Capture
Regina1	50.371	-104.589	April 28-June 23	May 12-May 19 (n=27)	97
Pelly	51.856	-101.932	April 28-June 9	May 12-May 19 (n=2)	96
Tisdale 1	52.911	-103.981	April 28-June 23	May 19-May 26 (n=1)	73
Ituna	51.171	-103.496	April 27-June 23	May 31-June 7 (n=2)	72
Outlook	51.487	-107.058	April 27-June 28	May 18-May 25 (n=2)	70
Elrose	51.201	-108.033	April 27-June 21	June 8-June 14 (n=2)	38
Grasswood	52.044	-106.670	May 6-June 24	June 14-June 24 (n=30)	30
MeadowLake 1	54.151	-108.832	April 28-June 23	June 2-June 9 (n=1)	30
Cadillac	49.584	-107.902	April 28-June 23	June 9-June 16 (n=16)	26
MeadowLake 2	54.189	-108.290	April 28-June 23	May 26-June 2 (n=1)	23
SwiftCurrent	50.137	-107.848	April 28-June 23	June 2-June 9 (n=1)	23
Baildon	50.266	-105.466	May 4-June 22	May 25-June 1 (n=2)	21
Tisdale 2	52.847	-104.049	April 28-June 23	May 12-May 19 (n=2)	19
Regina 2	50.379	-104.612	May 19-June 25	May 19-June 3 (n=1)	19
Martensville	52.293	-106.658	April 29-June 23	June 2-June 9 (n=1)	18
IndianHead	50.533	-103.670	April 29-June 23	June 9-June 16 (n=2)	13
Southey	50.940	-104.499	April 27-June 21	May 17-May 26 (n=9)	13
Dundurn	52.027	-106.581	May 7-May 18	May 18-May 25 (n=11)	12
Raymore	51.408	-104.529	April 28-June 23	June 2-June 9 (n=2)	12
Rosetown	51.667	-108.101	April 27-May 22	May 26-June 2 (n=7)	12
Craik	51.052	-105.822	May 4-June 15	May 18-May 25 (n=6)	10
Drake	51.746	-105.013	April 27-June 23	May 10-May 18 (n=1)	9
Shaunavon	49.646	-108.422	April 29-June 23	June 9-June 16 (n=8)	8
Bowditch	52.744	-104.078	April 28-June 23	May 26-June 2 (n=4)	7
Kindersley	51.388	-109.426	April 27-June 22	June 2-June 8 (n=4)	7
Lumsden	50.580	-104.709	April 28-June 16	May 12-May 19 (n=1)	7
Rouleau	50.188	-104.907	May 4-June 22	May 18-May 25 (n=1)	5
Kelfield	51.896	-108.602	April 28-June 23	June 16-June 23 (n=4)	4
Saskatoon	52.417	-106.417	April 28-June 23	June 16-June 23 (n=2)	2
Bruno	52.264	-105.628	April 29-June 23		0
Lemburg	50.727	-103.203	April 27-June 21		0
PrinceAlbert	53.203	-105.753	April 29-June 23		0

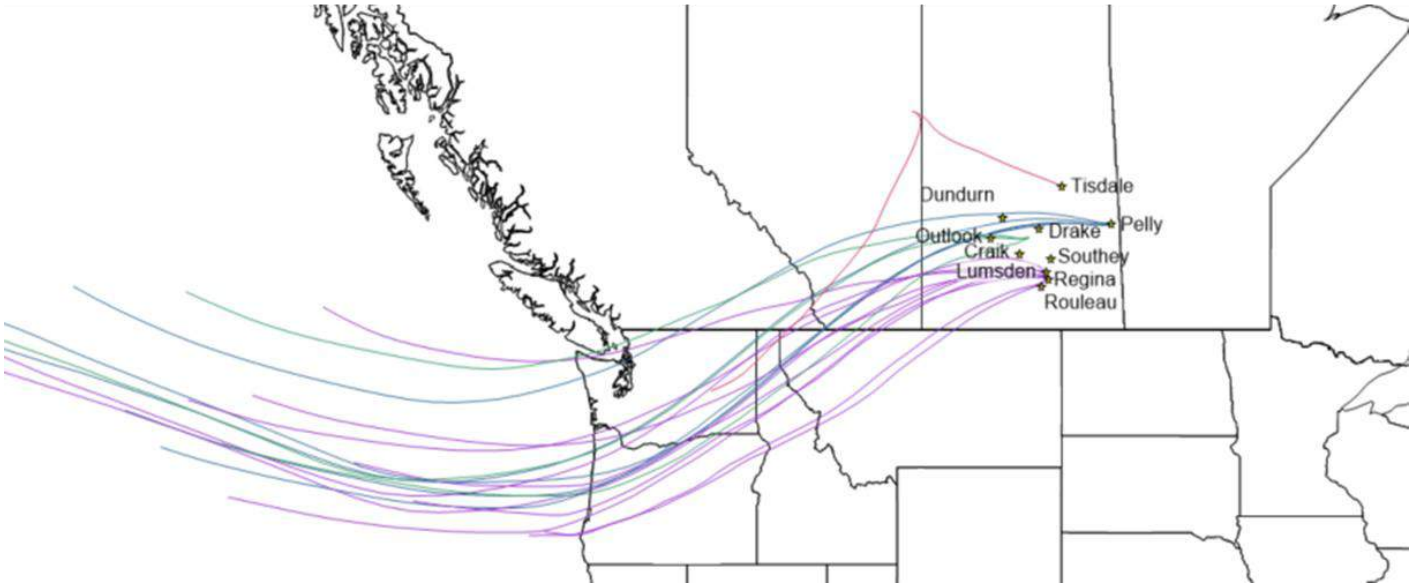
Figure 5.1-9 Maps showing location of sticky card sites and plot of reverse trajectories for first Arrival (May 10-19 2021)

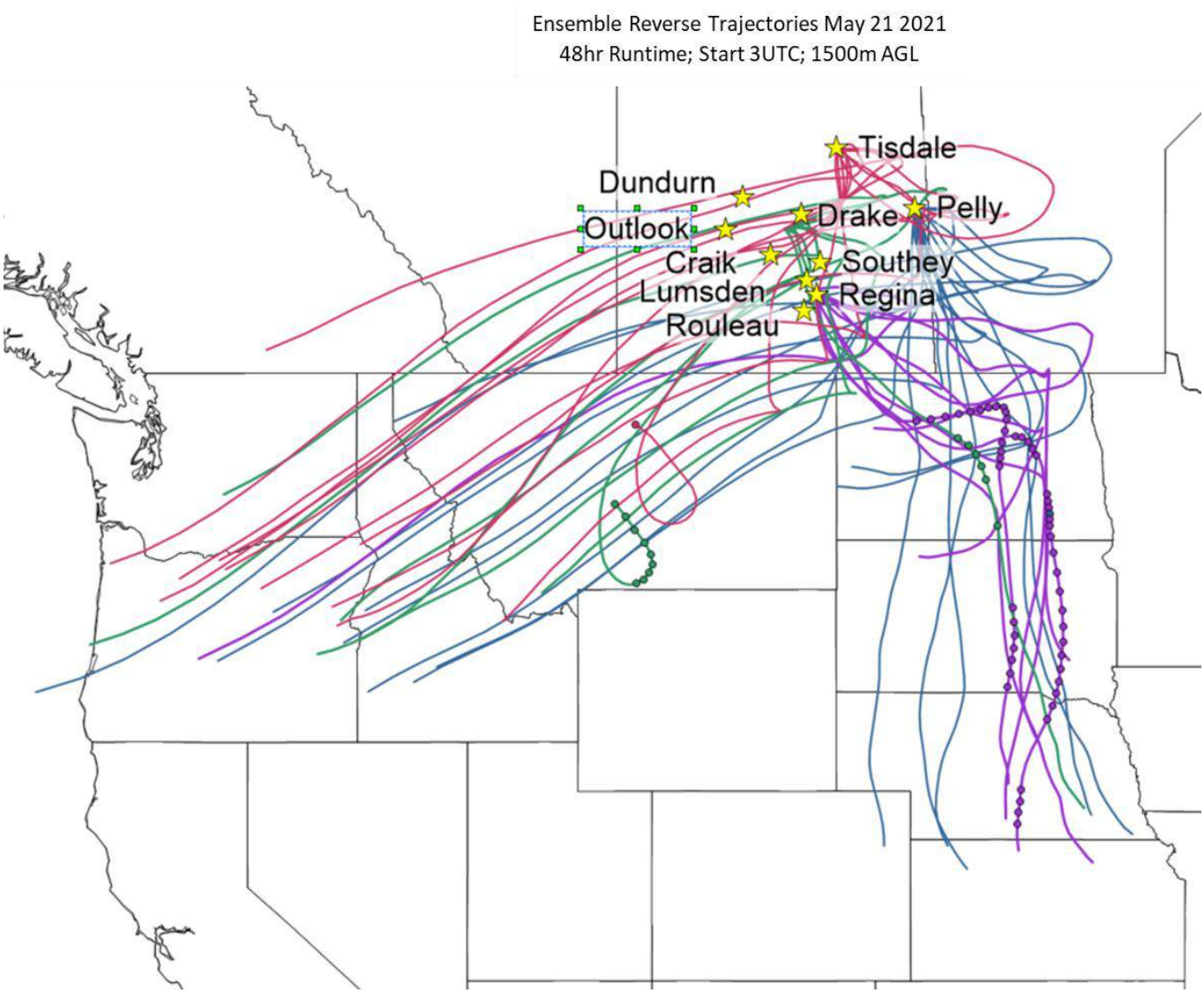


Ensemble Reverse Trajectories May 19, 2021 48hr Runtime; Start 3UTC; 1500m AGL

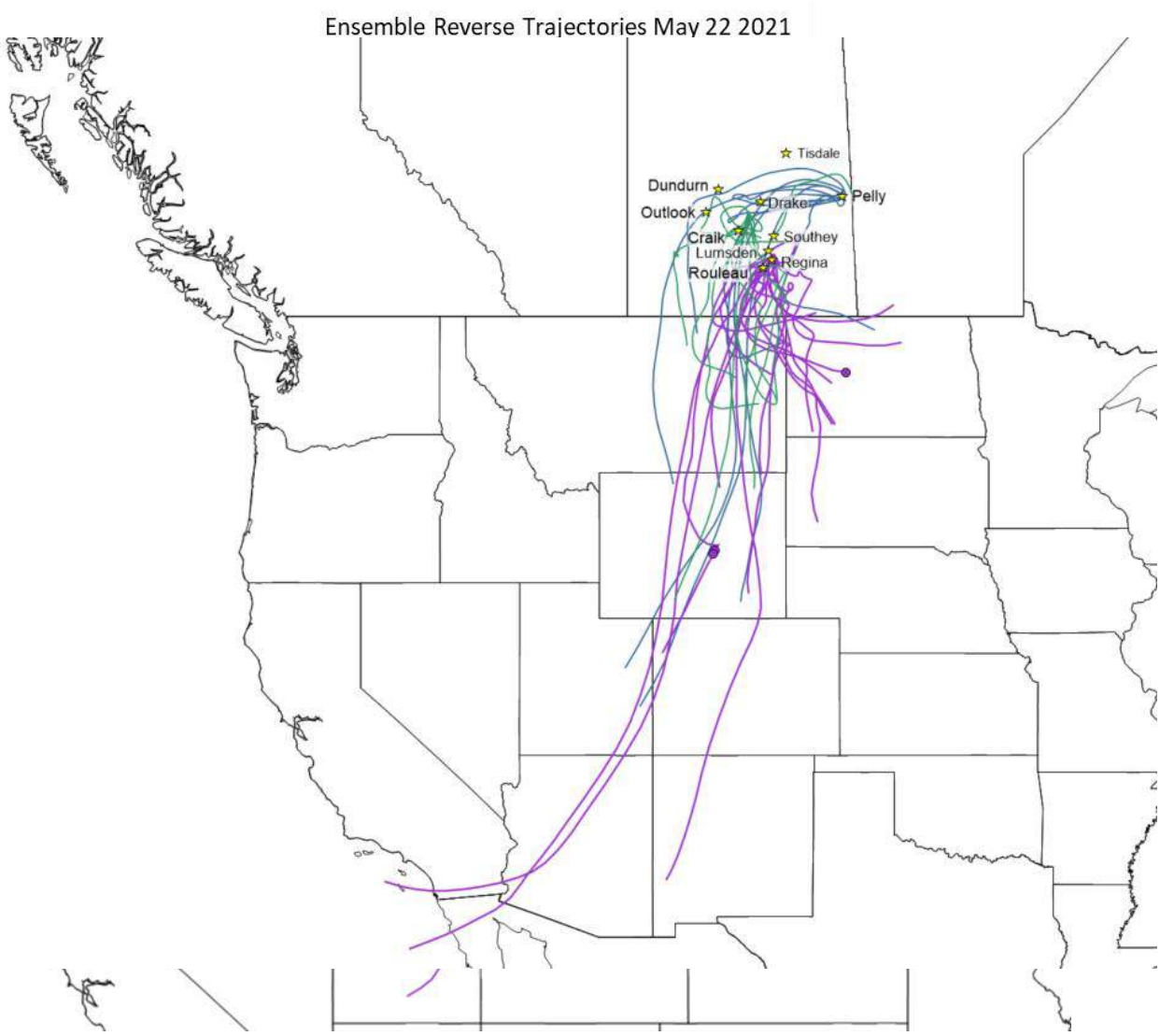


Ensemble Reverse Trajectories May 20, 2021 48hr Runtime;
Start 3UTC; 1500m AGL

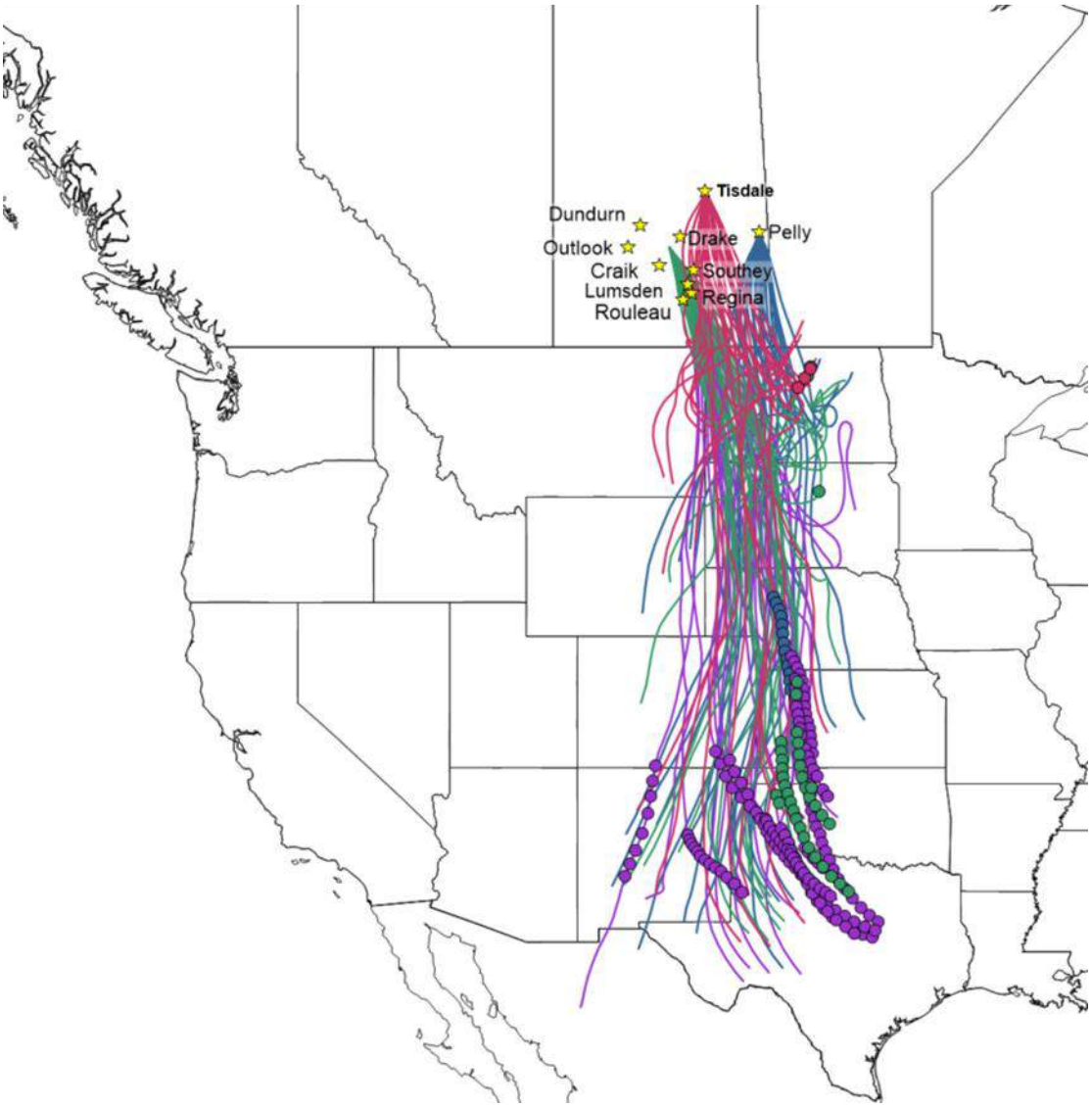



Ensemble Reverse Trajectories May 21 2021
48hr Runtime; Start 3UTC; 1500m AGL
Tisdale
Dundurn
Outlook
Drake
Pelly
Craik
Southey
Lumsden
Regina
Rouleau

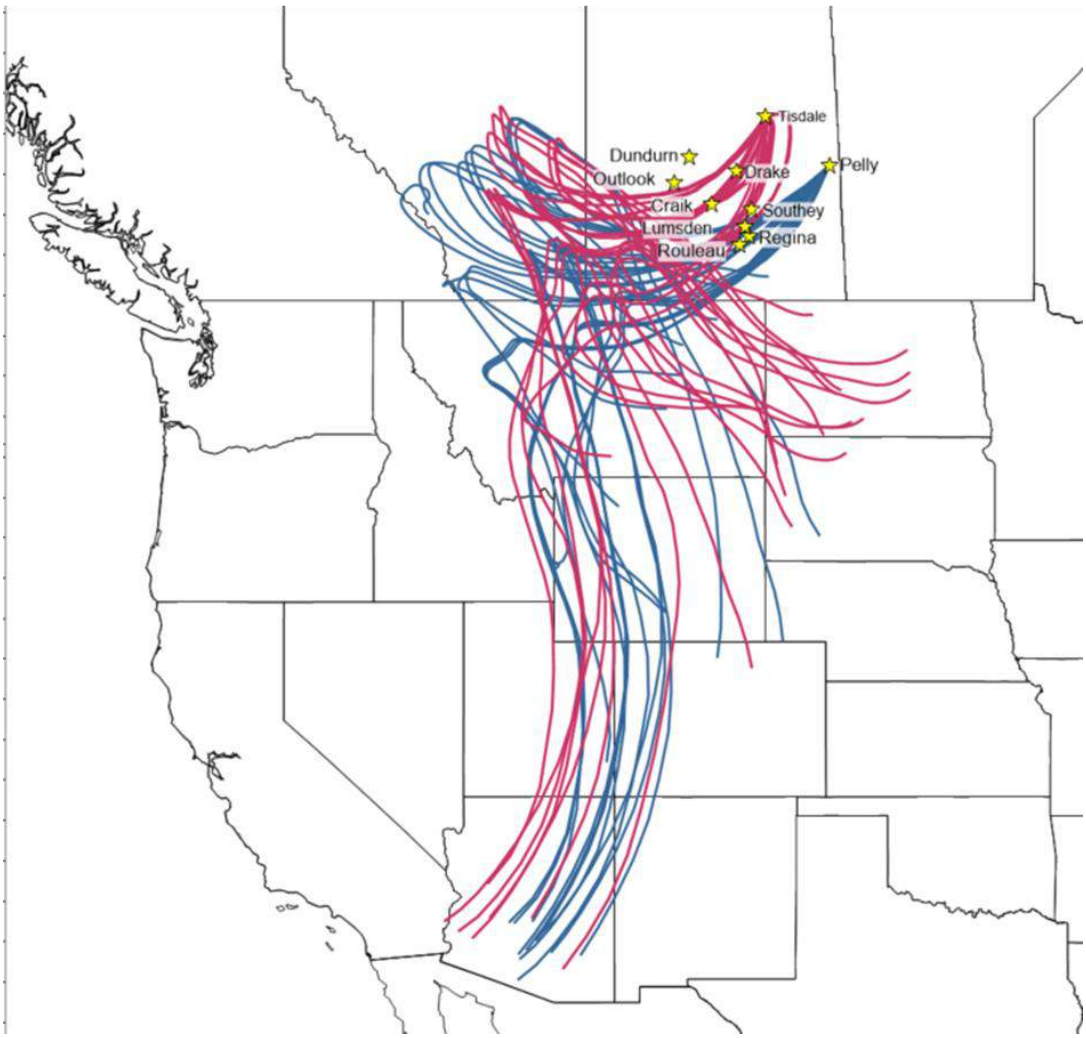
Ensemble Reverse Trajectories May 23, 2021
48hr Runtime; Start 3UTC; 1500m AGL



Ensemble Reverse Trajectories May 24, 2021
48hr Runtime; Start 3UTC; 1500m AGL



Ensemble Reverse Trajectories May 25, 2021
48hr Runtime; Start 3UTC; 1500m AGL



In 2022 we ran an enhanced grid of yellow sticky cards outside of the Provincial network. This was an attempt to pinpoint a closer date of first arrival of leafhoppers with an enhanced ALH monitoring grid by establishing a grid of sticky cards as well as sweep sampling of ditches. We had a migrant wave of aster

leafhoppers in 2022 (Figure 5.10). Sites in the grid were selected close to enough to the Saskatoon Research and Development Centre (SRDC) that the driving distance was not arduous and these were monitored every 1 to 3 days depending on the site, weather and staff availability. The cards were placed at the sites on April 28, 2022 with the first ALH appearing on cards at 2 sites (Aberdeen & Prud'homme) between May 24- 26th. Sweep samples of roadside vegetation were collected on May 27th with a total of 27 individuals being collected. The wings and legs were removed for stable isotope testing and the bodies were forwarded to the molecular lab for PCR testing. Ensemble reverse trajectory models were run using Vonda (roughly equidistant between the 2 sites) as the endpoint. The trajectories were run for a 6 day period May 21st – 26th. Figure 5.11 shows that on May 21st and 22nd the origin of RT was northern Canada; on May 23rd and 24th trajectories were localized within Saskatchewan and on May 25th and 26th trajectories had origin points in northern Montana. Based on these results the Ensemble RT model was run for May 25th for multiple times starting at 0600 UTC (12AM local) and then every 4 hours and for each start time the model was run at heights of 500, 1500, 2000 meters AGL. The geographic spread of the origin points in the Ensemble trajectories varied between start times and height but all ensembles had trajectories with origins in north central Montana. Figure 5.12 shows the trajectory plots of the 30 hour model run with 0300 UTC local end time. This would represent to a flight initiation at 9:00 pm local time in Montana. Dusk in Montana on May 25th is 9:30 pm.

The end point files with ambient air temperature was imported into Excel and trajectories which had both an origin in Montana and ambient air temperature along the trajectory 15C or greater were selected and mapped (Figure 5.11). The map shows that three trajectories within the ensemble at all three heights met the criteria. All had point of origin in close proximity to Glasgow Montana. An Ensemble forward trajectory was run using May 24th as the start date from Glasglow (Figure 5.12) the trajectories travelled in a north eastern direction from Glasgow to Vonda passing east of Regina. This process was repeated with the rainfall file with rainfall. None of the trajectories originating in Montana had any rainfall recorded, however, rainfall was recorded along other trajectories in the ensemble. All of these trajectories recorded 0.7mm/hr rain at Vonda at all three heights. It is unclear if this would trigger cessation of flight or if flight was terminated based on time aloft.

A roadside survey was conducted on June 7 and 8th 2022 with a total of 22 sites being sampled along two loops. These loops were selected prior to the wind trajectory analysis, however, the pathway of the forward trajectories coming from Glasgow aligned well with the sample sites (Figure 5.13). Aster leafhoppers were collected at six of the 22 sites (Chamberland, Tuxford 1, Tuxford 2, Pasqua, Rouleau, Keeler) using 1000 sweeps at each site. The two sites with the highest number of leafhoppers collected were Tuxford 1 (n=75) and Keeler (n=41). The wings and legs from the collected samples were removed for stable isotope analysis and the bodies went for AYp testing.

The results from the 2022 enhanced grid trajectory analysis strongly suggest that the *M. quadrilineatus* that arrived in Vonda on May 24-26th 2022 likely came from the Glasgow area of Montana. We can not say if these leafhoppers were from a generation the developed in Montana or if the generation

originated further south in Kansas or Nebraska with a brief stop in Montana using only the wind trajectories. In 2023, however, a similar pattern with local winds occurred and we are fairly certain that the leafhoppers stopped in Montana and the Dakotas before continuing to move North. Please see the stable isotope portion below, that suggests the origins of the leafhoppers were likely further South in 2022 and 2023. An early study (R. L. Wallis, 1962) that tracked the northward migration of aster leafhoppers through the United States found in early May that leafhopper populations had moved into Nebraska, Kansas and South Dakota and by early June leafhoppers were found in North Dakota and Montana. Another study carried out in the same time period in Canada (Westdal, Barrett, & Richardson, 1961) reported leafhoppers occurring in Saskatchewan on May 12th. While these studies are dated, given the generation time, it does seem unlikely that the leafhoppers collected in 2022 originated in Montana but were part of the same general migration from further South with a stop in Montana. The lack of leafhopper monitoring data from the United States hampers the ability to track the source of the Saskatchewan population. We have attempted to remedy this situation in 2022 by coordinating with entomologists in the Southern US but they failed to find any aster leafhoppers in cereals or forage grasses in seven Gulf States United States at the end of May and early June 2022 which suggests that populations had moved further North prior to the sampling efforts. Trajectory models from 2021-2022 appear to confirm the findings of previous studies (Westdal, Barrett et al. 1961, Nichiporick 1965) that the source of *M. quadrilineatus* arriving in Saskatchewan is the Great Plains area of the United States.

Figure 5.10 Locations of enhanced sticky card monitoring sites.

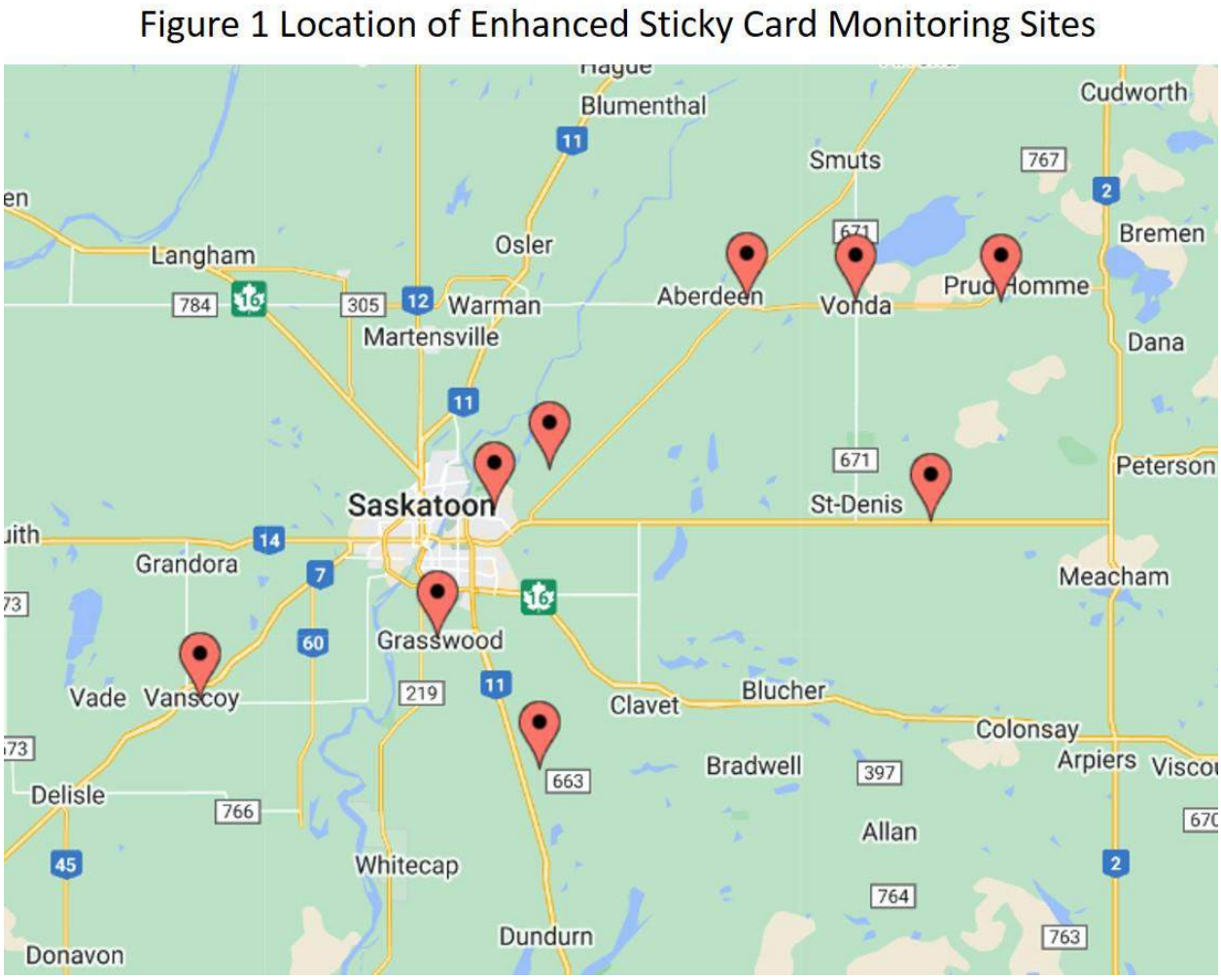


Figure 5.11 Ensemble reverse trajectory model runs May 21-26 2022.

Figure 2 Ensemble Reverse Trajectory Model Runs Vonda
May 21-26, 2022

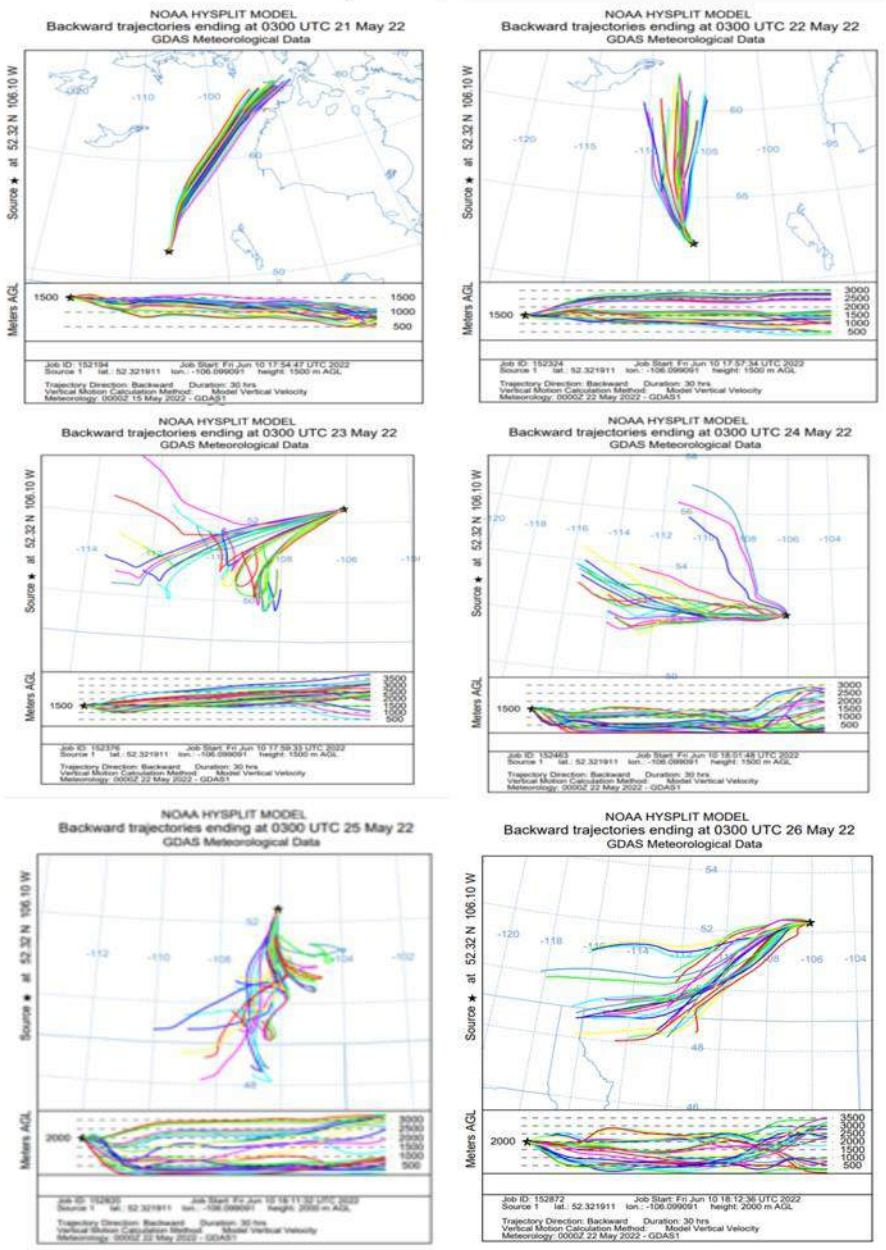


Figure 5.12 Migratory *Macrosteles quadrilineatus* first arrivals at Vonda Saskatchewan at three different heights above ground level, with Reverse Ensemble Trajectories (HYSPLIT) from May 25th 2022 at ambient air temperatures above 15˚C.

Figure 3 Ensemble Reverse Trajectory Model Runs Vonda May 25th 2022

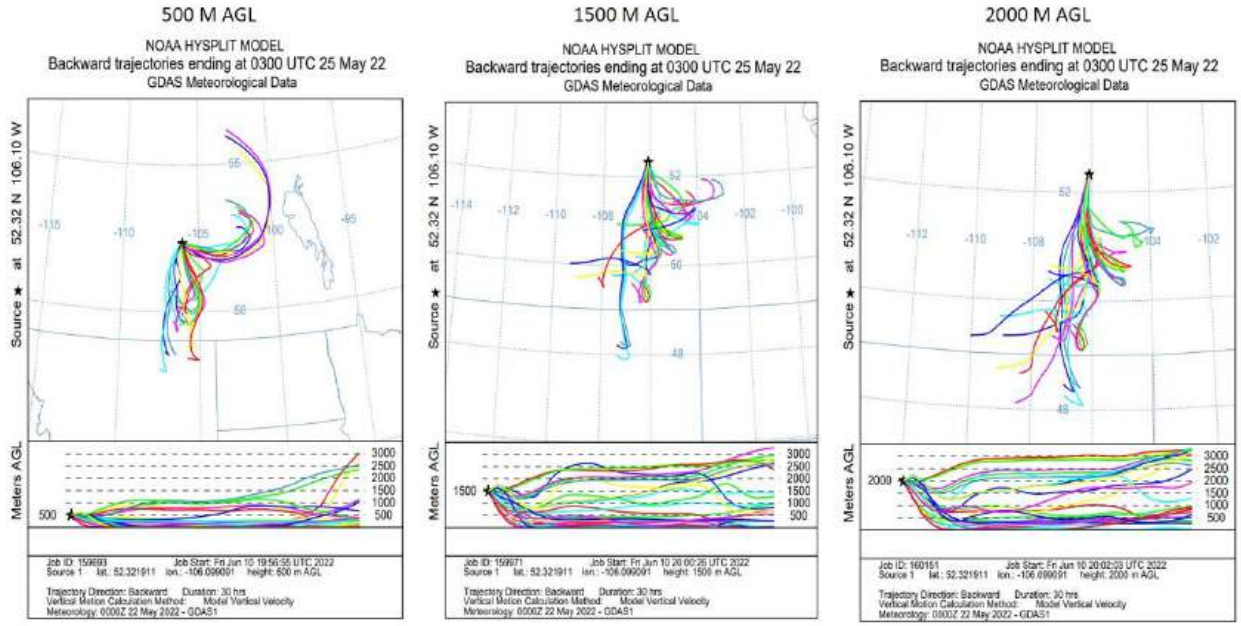


Figure 5.13 Migratory *Macrosteles quadrilineatus* first arrivals at Vonda Saskatchewan, with Reverse Ensemble Trajectories (HYSPLIT) from May 25th 2022 show origins in Montana at ambient air temperatures above 15˚C.

Figure 4 Reverse Ensemble Trajectories May 25th with origin in Montana and ambient air temperature 15C or greater

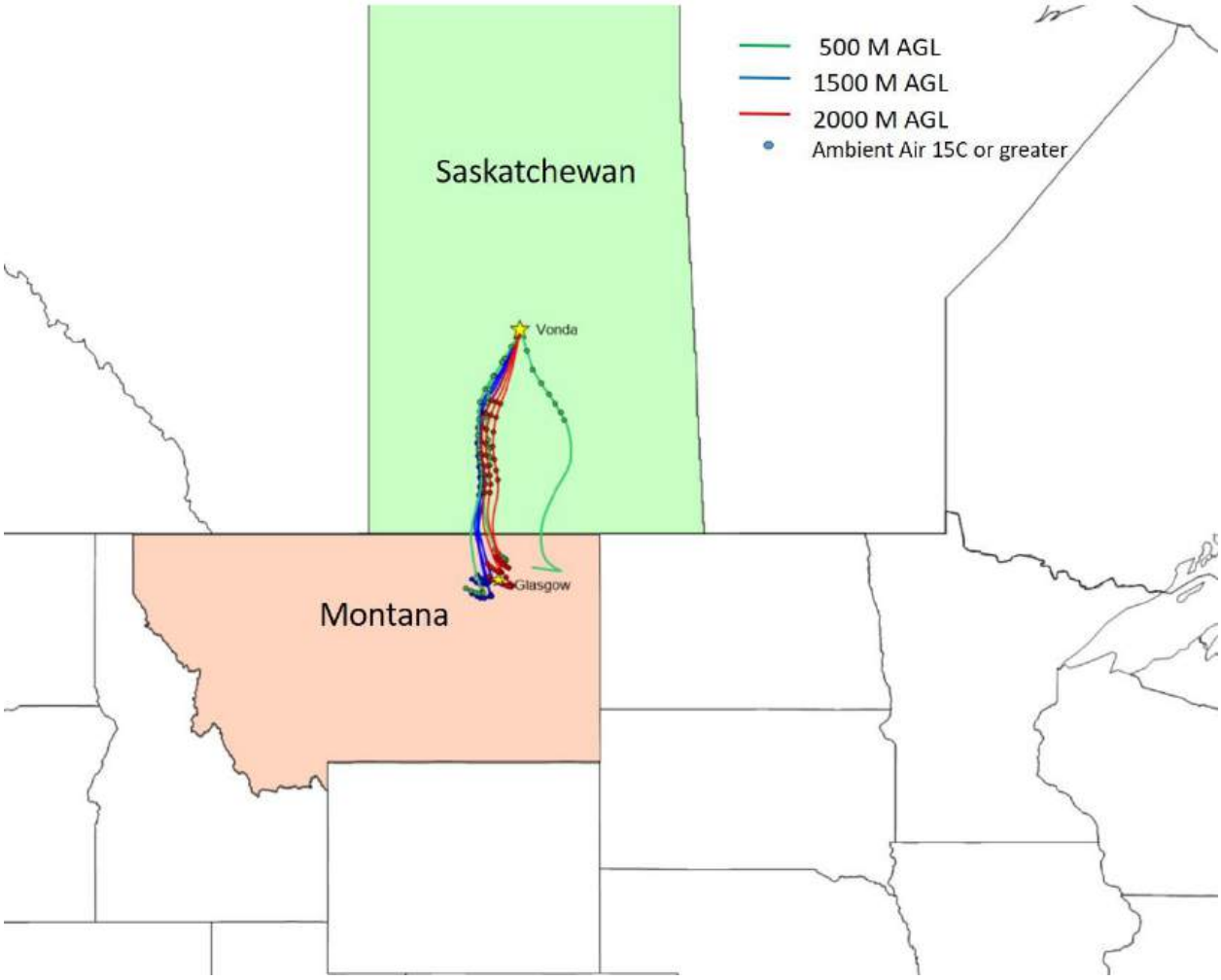
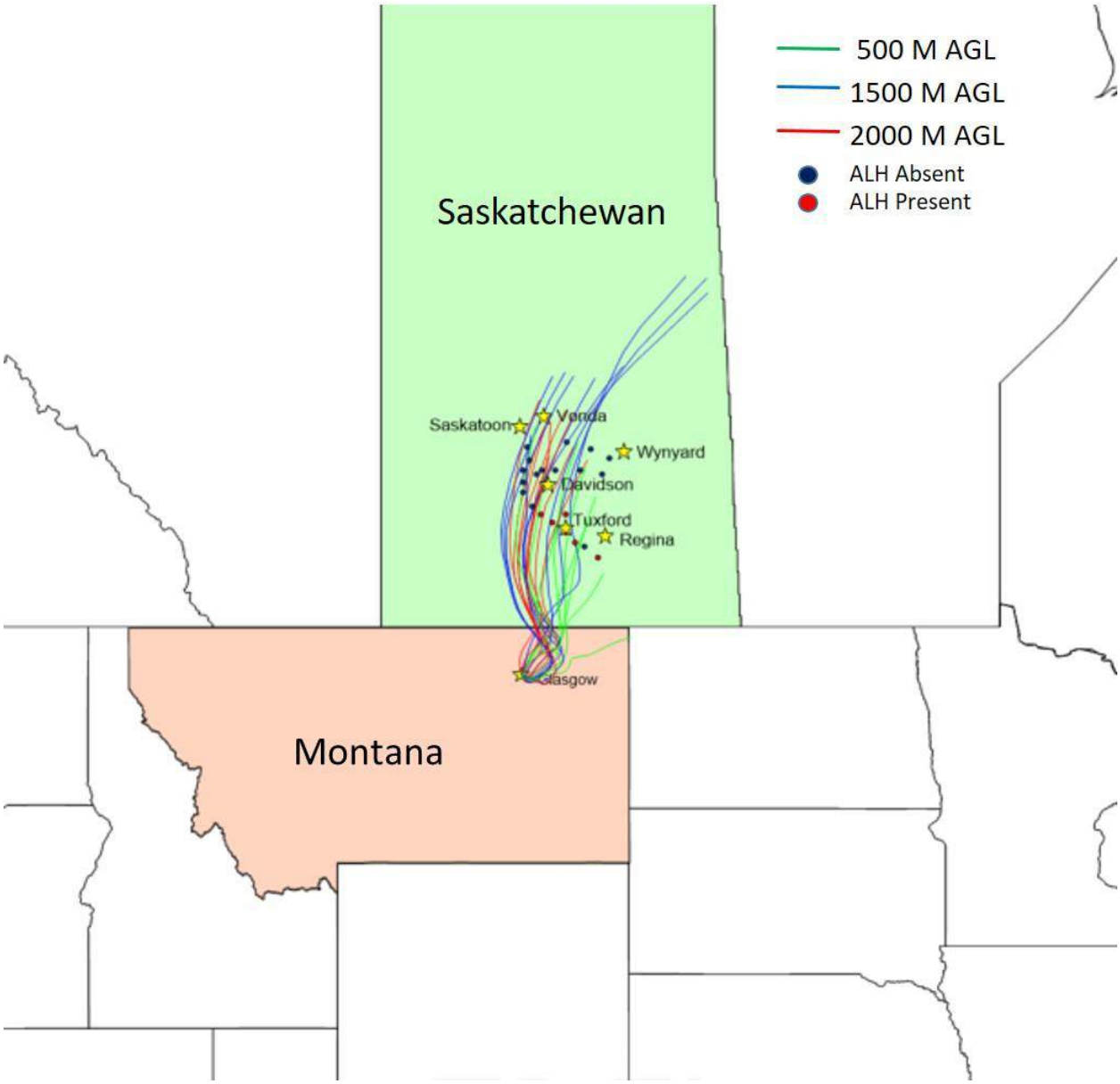


Figure 5.14 Migratory *Macrosteles quadrilineatus* first arrivals across Saskatchewan, with Forward Trajectories (HYSPLIT) starting from Glasgow, Montana from May 24th 2022 at ambient air temperatures above 15˚C.

Figure 5 Forward Ensemble Trajectory Glasgow May 24th 2022
and Roadside Survey Location with Presence-Absence data for Aster Leafhopper



Wind trajectories and 2021 (Diamondback moth) DBM Trap Counts

Diamondback moth (DBM) is another migratory pest but can be monitored with pheromone traps which are better than yellow sticky cards for detecting migrant insects. DBM trap catches from pheromone traps placed at the same sites as yellow sticky cards were examined to determine if arrival dates of ALH and DBM coincided and if these two insects were travelling on the same wind currents. If DBM and ALH were arriving consistently on the same winds, then arrival of DBM could be a proxy for the arrival of ALH.
Possible sources of origin and migration route were examined using Hysplit to calculate Ensemble RTs. The purpose was to determine if there was any similarities in wind trajectory patterns associated with the arrival of DBM and ALH in Saskatchewan. Unlike single trajectory which calculates a single trajectory based on a single meteorological grid cell, Ensemble trajectories calculate 27 trajectories with each trajectory calculated by offsetting the grid cells. Trajectories were run at height of 1500m AGL and for 48 hrs. The run time was selected to coincide with the time that leafhoppers can theoretically remain aloft when in active flight assisted by wind currents. Start time was 3UTC or 9:00pm local time which corresponds to sunset in May. RTs were generated for the locations with the first capture of DBM. DBM were captured 1 week prior to the capture of *Macrosteles quadrilineatus* in 2021.
RT models were run for the trapping period of the first occurrence plus 4 days prior to the card being placed in the field. There were RTs originating in the PNW (Washington and Oregon) associated with the arrival of DBM. PNW states of Washington and Oregon fits as potential sources of migrating DBM given at that point of the growing season these areas would be producing cool season Brassica crops. The sample period used for trapping was one week so we cannot definitively say if the number of days between trapping the 2 migratory species was 1 day or 7 days. This situation does present as a possible confounding factor where ALH and DBM migrations occurred with one week of each other so it is possible that difference in occurrence date could be more related to differences in trapping efficiency (pheromone trap, higher efficiency vs. the visual yellow sticky card) than wind patterns.

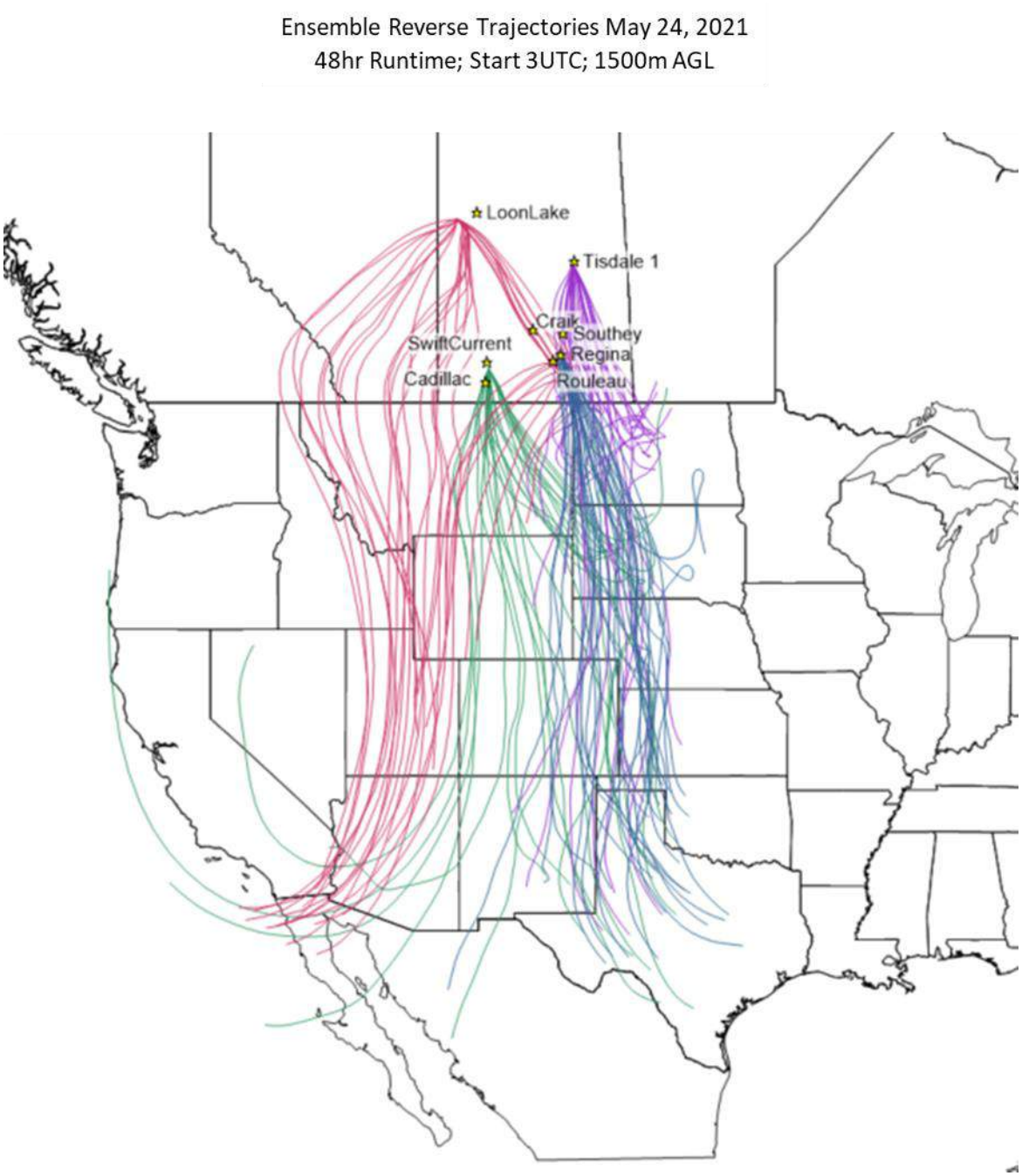
2021 DBM Catch

Fifteen out the 32 trap sites caught DBM with a total of 35 DBM captured and this was the lowest number in the past four years of monitoring DBM. Eight of the 15 sites where DBM were captured only had a single moth collected over the trapping period. To follow up with the low numbers of DBM that migrated, few DBM larvae were reported from fields until very late in the season when some larvae were detected feeding on canola regrowth following harvest. The first capture of DBM male moths occurred **May 5-12** 2021 at 3 sites : Pelly (n=1), Raymore (n=1), and Shaunavon (n=1). The majority of the DBM captured (69%) occurred May 26- June 2 (n=24) at 7 sites: Cadillac, Craik, Loon Lake, Regina, Rouleau, Southey, Swift Current and Tisdale. Some of the RTs for May 1 passed over or originated in Washington. May 1 was the only day between May 1-12 when RTs did not originate in northern Canada (Figure 15.13). Also of note prior to May 26 – June 2, when most of the DBM were captured, there were RTs from the PNW originating or passing Washington on May 26 and on May 24th there were RTs that originate throughout the southern USA reaching as far as Mexico (Figure 5.14). These RTs are also a possible migration source location for *Macrosteles quadrilineatus*. In 2021 the first capture of *M. quadrilineatus* occurred at 5 sites between **May 10-19**. Those sites were Drake (n= 1), Lumsden (n=1), Pelly (n= 2), Regina 1 (n= 27), and Tisdale (n=2). As of the first interim report, 2022 DBM trap data had not been received from the Saskatchewan Ministry of Agriculture

with the trapping period extended by several more weeks in 2022 to encompass more of the DBM migratory season. We thank the Saskatchewan Ministry of Agriculture for all of their help in trapping leafhoppers and diamondback moths.

Fig. 5.14 Ensemble Reverse trajectory winds and three first appearances of DBM males on pheromone traps at Shaunavon, Raymore and Pelly from May 1st 2021 back track over the Pacific Northwest.

Fig. 5.15 Ensemble Reverse trajectory winds and appearances of DBM males on pheromone traps at seven sites in Saskatchewan from May 24th 2021 back track as far South as Mexico.



2022 Winds

The aster leafhopper migration in 2022 was not significant. Southern winds through the great plains corridor on May 25th 2022 brought first catches of leafhoppers on yellow sticky cards and in sweeps from several locations from May 25th to 27th 2022 including Vonda, Lumsden and at the Saskatoon Research Farm plots. The leafhopper population remained small through the year and there did not appear to be any further Southern migrations this year.

2023 winds

The first collection of ALH in the Saskatoon area was made on May 23rd 2023. Aster leafhoppers (ALH) were collected in roadside sweeps at 6 out of 8 sentinel sites and at the SRDC farm plots. Reports of ALH from roadsides were received from Manitoba on May 23rd 2023 as well. This migration prompted additional roadside surveys to be carried out in SK: May 29 – West central and Lake Diefenbaker, June 7 – SW, June 8 – East of Saskatoon. In total 31 sites were sampled with ALH collected at 27 (87%). Hysplit Ensemble RT model was run for 3 locations: Kindersley, Saskatoon, and Swan River. It was run for the May 23rd the 3 days prior (May 20-22nd) and then for the days following until last survey date of June 8th. Model Parameters used were a start time of 3UTC (9pm CST), 72hr runtime, and with three wind heights of 500m AGL and 1500m AGL. In the 3 days prior to the May 23rd first arrival, the Reverse Trajectory (RT) pattern for Saskatoon and Swan Lake, Manitoba were very similar with trajectories originating in northern Canada. Kindersley also had RTs originating in northern Canada but on both May 20th and May 22nd there were also trajectories with origins in the Pacific North West (PNW) and western USA. The first collection in the Kindersley area was not until May 29th 2023. The RT pattern on May 25 and 26thrd 2023 are very similar for Saskatoon and Swan River with likely RT origins in the great plains (Fig. 5.16 a,b). The RT pattern for Kindersley, however, in this time point is the PNW. Given the first sample date for Kindersley area that we have are the west central sweeps on May 29th I ran RT between May 23rd and May 29th. Once again the predominate Rt pattern was PNW but May 25th had several possible origins in great plains and on the following days there were a few additional origin points. The RTs run with Kindersley as the destination, up until the June 7 2023 sample dates in SW Sask, showed that on June 4th to 6th there were RT pattern from the great plains region and from eastern Canada (Fig. 5.17). The stable isotope ratios from both of the West Central sample dates (-144, May 29 and -147, June 7 2023) were nearly identical indicating that they all had the same geographic origin. The trajectories for Saskatoon from May 23rd 2023 to May 31st 2023 at 500 Above ground level (AGL). Saskatoon had multiple consecutive days with RT coming from the great plains regions of the United States.

Figure 5.16. Single reverse trajectory runs over 72 hours to Saskatoon and Swan River on May 25th 2023 (A) and May 26th 2023 (B).

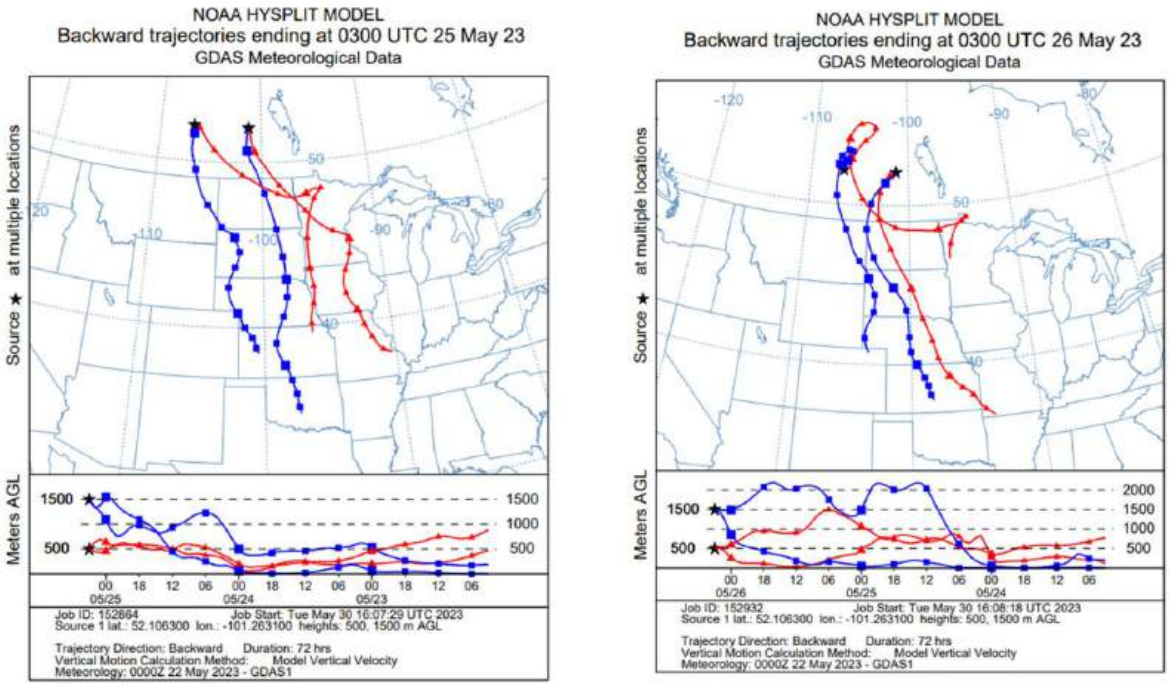
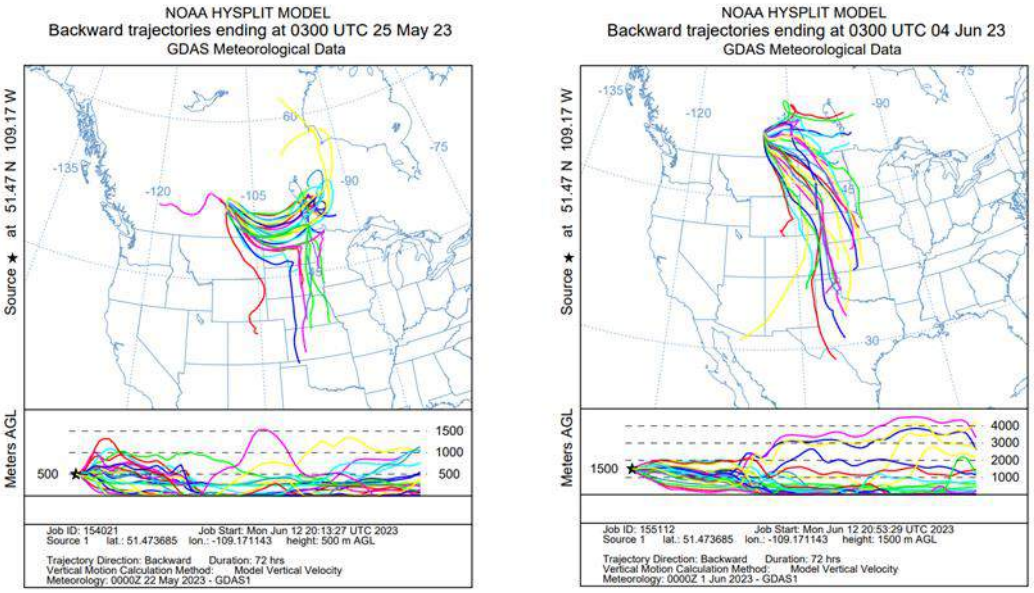


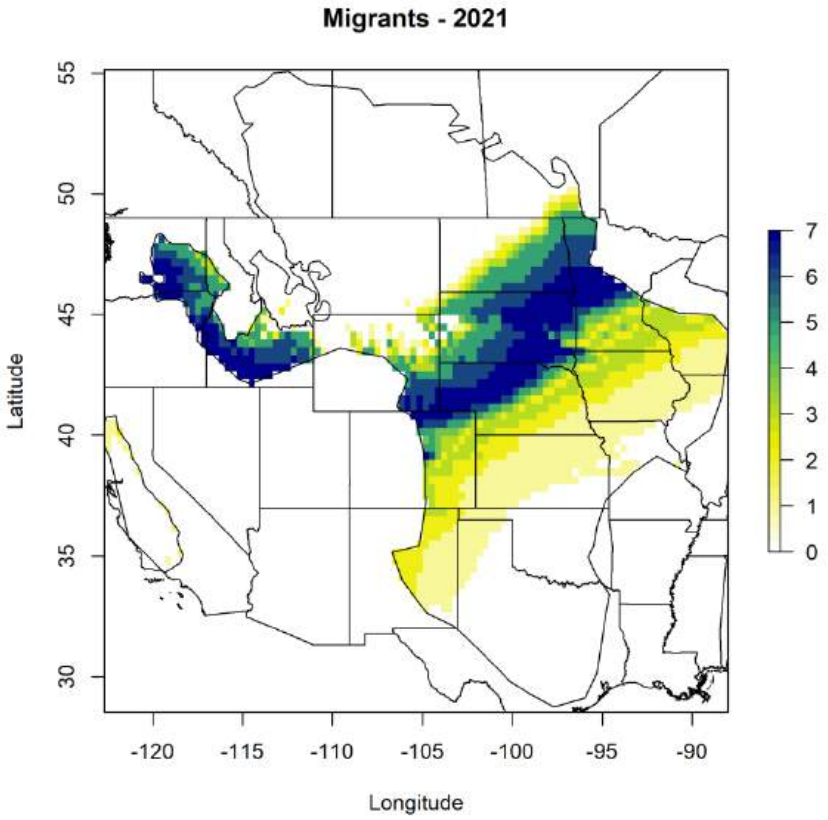
Figure 5.17 Ensemble reverse trajectory runs over 72 hours to Kindersley May 25th 2023 (A) and June 4th 2023 (B) that originated over the Great Plains region of the United States.



Stable Isotopes determination of origins.

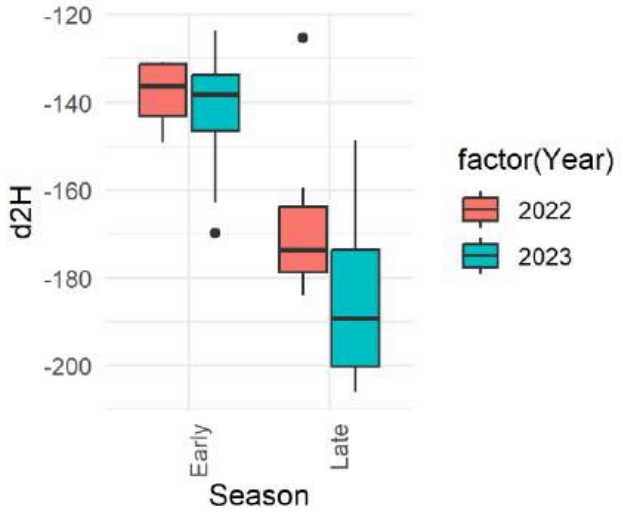
Wings and legs are metabolically stable and their tissues are laid down when the insect moults to its adult form. The ratio of heavy hydrogen (deuterium) to hydrogen varies geographically by precipitation and, when insects feed on plants watered by that precipitation, the insects incorporate these ratios into their body tissues. So, the ratio of heavy hydrogen (deuterium) to hydrogen in tissues that are metabolically inert after moulting, like wings and legs, differs depending upon where in North America an insect originates. This "isoscape" was constructed in our previous project using leafhoppers from various locations in the United States as well as the migrant leafhoppers and the offspring of the migrant leafhoppers from cereal crops in Saskatchewan, which have a lower deuterium ratio than the migrants and the source leafhoppers. In all project years, the leafhoppers used for isotope analysis were swept in roadside ditches and alfalfa first, and later on in the years, in crops, once crop plants were tall enough to sweep. The stable isotopic profile of the 2021 migrant leafhoppers match the deuterium isoscape through the Great Plains Corridor (GPC) but also the Palous growing region of Washington and Oregon (Figure 5.18). This match is likely exacerbated by the Washington/Oregon, or Pacific Northwest region (PNW) growing region sharing the same latitude as the Dakotas. There were however, winds that originated from the PNW crossing Saskatchewan during the first week of leafhopper arrival in 2021. A more likely migratory source location is the Great-Plains corridor based on the number of days with winds through that corridor and the wind's tendency to stay low to the ground for an extended length of time through the GPC, especially on the 24th of May 2021 (Figure 5.15). The dark blue band indicates the highest probability of origin is Northern Nebraska and all of Eastern South Dakota with some probability, and with winds that originated in those regions, of Oklahoma, the Northern tip of Texas and Eastern New Mexico. The California growing region is an unlikely source for 2021 and the Pacific Northwest winds from that region did not originate from that far South in the period of time that leafhoppers arrived.

Figure 5.18. Deuterium isoscape map showing the most probable origins of migrant aster leafhoppers from all of North America in 2021 using the growing season deuterium (GSD) calibration.



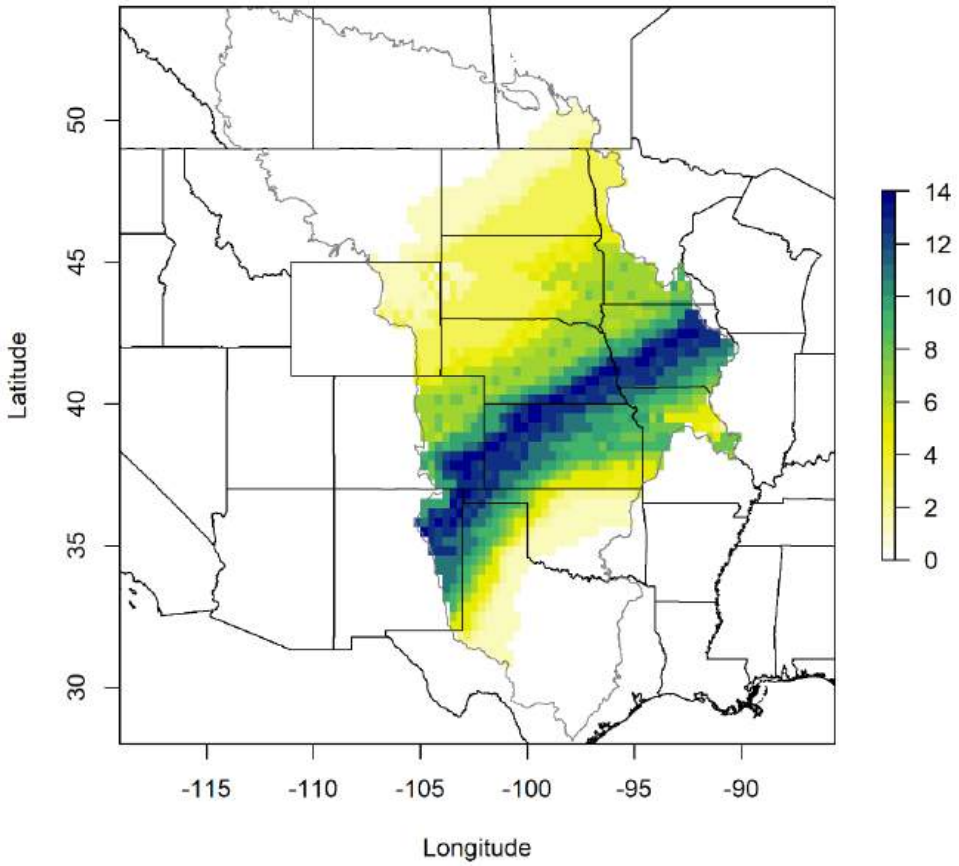
In 2022,first arrival of leafhoppers was likely May 27th, but the migration was not a large one, and only enough leafhoppers for four samples (around 30 LH/sample) of the migrant generation were collected. In 2023, the first migrants arrived on May 23 2023, and were caught in alfalfa fields and roadside ditches with sticky cards. Yellow sticky cards corroborated the first arrival dates. 2023 had a large migration of aster leafhoppers, and in 2023, 77 total samples, were submitted for isotopic analysis. The amount of tissue from the approximately 30 leafhoppers amounted to a mean of 0.3388 mg (range 0.21-0.38 mg). The probabilistic origins of leafhoppers from 2022 and 2023 using the GSD calibration for deuterium isotopes are detailed in Figures 5.19-22. The migrant ALH population in 2022 and 2023 had nearly identical deuterium (dh_2) ratios (Figure 5-17) as are indicated by the overlapping boxplots of early season migrants in 2022 and 2023. We split the data into three sets because of the difference in values between the seasons and years: 1) early (May/June) 2022 and 2023; 2) late (July/August) 2022; 3) late 2023 (Fig 5.19). The boxplots results (Fig 5.19) suggest a common geographic origin for both year's (2022 and 2023) ALH migrations.

Figure 5.19. Boxplot shows the deuterium (DH2) values of early (migrant) and late season (resident) aster leafhoppers in 2022 and 2023.



The probabilistic origins of aster leafhopper migrants in 2022 and 2023 were similar enough to be graphed together (Fig. 5.20) with Kansas and Nebraska as the mostly likely origins of the leafhoppers that migrated. The most likely origin band (blue) is further South than in 2021, but also tighter than the 2021 band, potentially due to increased samples used to create the 2022-2023 map from the large number of samples collected in 2023. In the 2022-2023 map, no probabilistic origins occurred from anywhere but the Great Plains corridor of the US.

Figure 5.20 Deuterium isoscape map showing the most probable origins of migrant aster leafhoppers from all of North America in 2022 and 2023 using the growing season deuterium (GSD) calibration.



In addition to testing the deuterium (heavy hydrogen) content of the migrant aster leafhoppers, we also tested the stable Carbon ratios which indicate if an insect was feeding on a C3 or a C4 photosynthetic plant. All of the 2021 leafhoppers had carbon ratios (δ13C between -25.12 and -27.33) which indicates that all of the migratory leafhoppers that were caught had matured on C3 plants, like wheat or winter wheat or other cereal crops, during their nymphal and then adult stage prior to migrating. Aster yellows does not replicate well in cereals and is a poor host for transmission of aster yellows to aster leafhoppers (Bahar et al. 2018). This result further supports our hypothesis that winter wheat maturation in the Upper mid-West US states is likely the driver and probable host crop that produces the adult generation that migrates into Canada. This result also helps to explain the typically low infection rate of the migratory leafhoppers that arrive each year. So, what is the driver of these aster yellows outbreaks? I've done some investigative work and have a theory, now that we have had the opportunity to catch and test leafhoppers through two projects, and using outbreaks from the past. During the course of this project, we have collected several years of migratory leafhopper and infectivity percentage data. In 2019 and 2021, there were few migrant leafhoppers and barely any AYp infected migrants and no drought 2020, there were many migrant leafhoppers but they were barely infected and there was no drought in the Great Plains corridor. The only years, including the last big outbreak years of 2012 (Olivier et al. 2017), and 2023 where the infection rate of aster leafhoppers migrants were high and led to aster yellows outbreaks in canola, had widespread drought in the Kansas/Nebraska

(Great Plains corridor) winter wheat growing regions suggesting that spring drought and failure of their host cereals is the driver of aster yellows outbreaks in Western Canada. The mechanism then would be: failure of their reproductive and favoured food host, winter wheat, which is a poor host for transmission of aster yellows phytoplasma. Dying winter wheat forces the leafhopper population onto the remaining green plants, which are weedy reservoirs of broad-leafed plants where they acquire aster yellows. Migration occurs as usual around the May long weekend when the Southern winds travel North and the migrant leafhoppers arrive more infected with aster yellows phytoplasma than typical.

References (Objective 5)

Olivier, C., T. Dumonceaux, E. Pérez-López, T. J. Wist, B. Elliott and S. Vail (2017). Detection, symptomatology and management of aster yellows disease in canola. In. *Integrated management of insect pests on canola and other Brassica oilseed crops* . 1 . ed. Gadi Reddy.

Wallis, R. (1962). Spring migration of the six-spotted leafhopper in the western Great Plains. *Journal of Economic Entomology, 55*(6), 871-874. doi:https://doi.org/10.1093/jee/55.6.871

Wallis, R. L. (1962). Spring Migration of the Six-Spotted Leafhopper in the Western Great Plains. *Journal of Economic Entomology, 55*(6), 871-874. doi:10.1093/jee/55.6.871 %J Journal of Economic Entomology

Westdal, P. H., Barrett, C. F., & Richardson, H. P. (1961). THE SIX-SPOTTED LEAFHOPPER, MACROSTELES FASCIFRONS (STÅL.) AND ASTER YELLOWS IN MANITOBA. *Canadian Journal of Plant Science, 41*(2), 320-331. doi:10.4141/cjps61-044

7. Conclusions and recommendations:

The 2021, 2022 and 2023 field seasons were completed and bonus 2024 content was included in this report when were able to complete that work. The no cost extension of the project into 2023 was serendipitous, as 2023 was an aster yellows out break year. Wind, and stable isotope results indicate a great plains migration event for the aster leafhoppers while winds indicate that diamondback moth came from the Pacific Northwest, and likely the states of Oregon and Washington in both years. The migrations of these two insect pests do not seem to be closely linked. Molecular analysis of the aster leafhoppers revealed no useful genetic population structure for determination of origins, likely due to the mixing of genes by migrating aster leafhoppers each year. Isotopic analyses indicated that aster leafhoppers were all feeding on monocot plants as their body tissue was being laid down, and the timing of the migrations, suggests that the reproductive plants that produced the migratory leafhoppers was winter wheat. The heavy hydrogen and wind trajectory results support this story, by putting an origin region of Nebraska and South Dakota as a probable migration point in all of the project years. Migrant leafhoppers have not been very infected when we have caught them just after migration, which suggests that in most years, aster yellows is not brought into Canada in any significant way in the migrant leafhoppers and that is reflected in the intervening years between “outbreak” years. The low aster yellows results on the Provincial survey supports this story as well, with spring migrations of leafhoppers that are small in number and/or barely infected with aster yellows phytoplasma in the spring linked to low levels of symptomatic aster yellows plants in the fields on the late summer survey.

Several perennial and biennial plants, including alfalfa, were identified as positive with aster yellows prior to any aster leafhopper activity in Saskatchewan in 2021 indicating that these had been infected with AYp the

previous year and carried over the infection from one growing season to the next as a "green bridge" between seasons. AYp testing of the F1 generation of leafhoppers (offspring of the migrant generation) typically has been showing a higher percentage of infected leafhoppers than the migrants (Olivier et al. 2017), which also supports the reservoir plant theory where AYp is being harboured by a weedy reservoir in Saskatchewan, that the leafhoppers pick up as they reproduce in Saskatchewan. Lab experiments revealed that in a ditch setting with two dominant plants, aster leafhoppers chose the brome grass almost exclusively over the alfalfa for feeding and reproduction. When seedling-stage canola plants are present, many of the leafhoppers will move into the canola field and begin to feed on canola, without any mechanical disturbance in the ditch area. As the infected percentage of leafhoppers went up, so did the number of aster yellows symptomatic plants. Outbreaks of aster yellows disease in Western Canada are likely linked to drought in winter wheat in the leafhopper source areas of Kansas and Nebraska. We have demonstrated that the aster leafhopper migration into Western Canada usually occurs around the May long weekend in the third or fourth week of May and it is then that we have the opportunity to test leafhoppers with the new molecular tools and determine what their risk to the crops that year are. Alfalfa fields and roadside ditchs provide habitat for migrant leafhoppers where they are concentrated and can be trapped and tested for AY before the crop has emerged. That being said, future research should tie the leafhoppers in these areas and their infectivity, through the AY Risk numbers, to AY infection in nearby fields to understand what the local AY risk means for AY infections in the future.

7. **Administrative and other aspects:** Report extension meetings; papers produced; conference presentations made; personnel involved; acknowledgements; equipment bought; photos; project materials developed.

Results of the molecular analysis of aster leafhoppers have been presented virtually by our graduate student Karolina Pusz-Bochenska at the 2021 ESC-ESO Joint Annual Meeting in October 2021 and were presented at the ESC-ESA Joint Annual Meeting in Vancouver Nov 13-16. Many Illumina and DNA extraction kits plus reagents were purchased and used over the course of the 1.5 years of this project. We have paid our PhD Karolina Pusz-Bochenska with these funds. She was co-supervised by Drs. Wist and Jack Gray and has developed the molecular microsatellite approach to analysing aster leafhopper genetics under the tutelage of Dr. Jose Andres (Cornel University). She successfully defended her PhD in May 2024 and is going to Standford School of Medicine for a Post-doctoral opportunity. In the laboratory, Jennifer Holowachuk (Dr. Chrystel Olivier's indeterminate technician, and now Doug (2022 samples) Dr. Martin Erlandson's (now retired) technician worked on molecular diagnostics of plants and insects for aster yellows. FSWEP student Rebecca Green was also paid out of the project funds to help with molecular tests for the presence of AYp in leafhoppers and plants. Nancy Melnychuk, Mozhgan Mousavi and Haroon Andkhoie (Dr. Wist's indeterminate technicians) conducted much of the field surveys for aster leafhoppers. Dr. Ruwandi Andrehennadi (Chrystel Olivier's biologist) was working on the DBM molecular study, but Dr. Wist's technician, Jennifer Holowachuk took over. Thompson Hygen, COOP student, worked on the alfalfa to canola green bridge lab project. We thank Carter Peru and Dr. James Tansey and their regional specialists of the Saskatchewan Provincial Ministry for adding aster leafhopper trapping and providing results of the diamondback moth pheromone trap results.

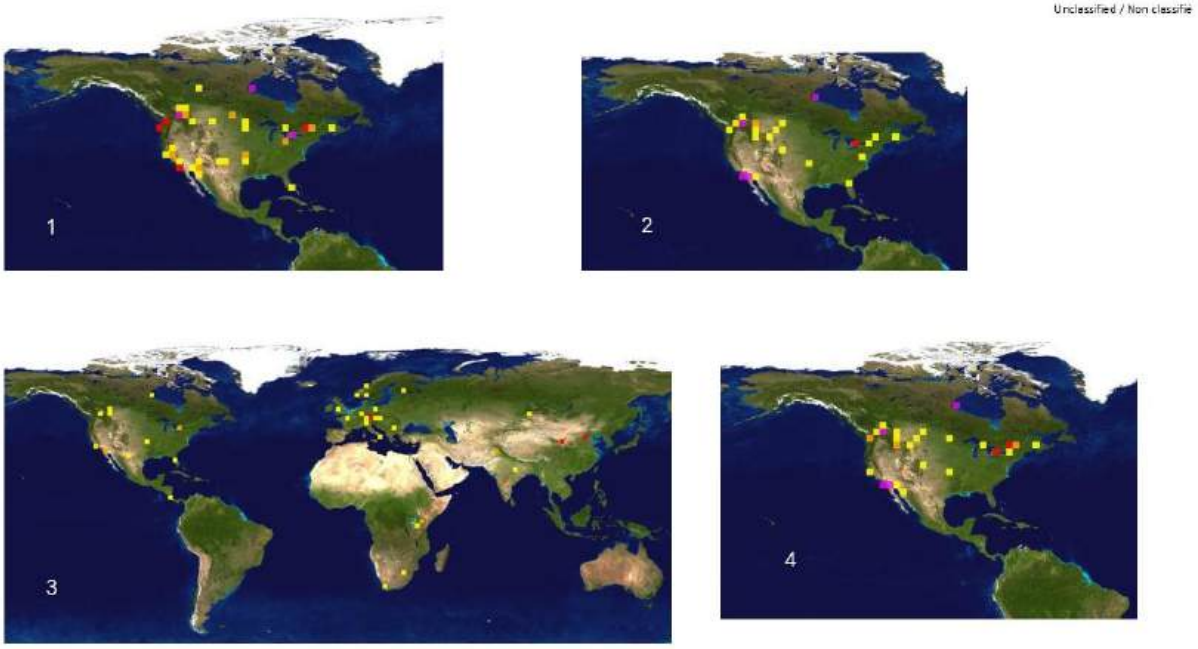
The Western Producer (reporter Robert Arnason) wrote a story (June 13th 2023) on the finding of this project about the aster leafhopper migration and levels of infection in 2023, that triggered a CFIA warning brief about the potential of an aster yellows outbreak in 2023. A manuscript on the population genetic structure of the aster leafhoppers is nearly ready for submission and manuscript on the wind patterns and stable isotopes is being prepared. Dr. Wist presented this project in symposiums at the 2023 and 2024 Entomological Society

of Canada meetings (Saskatoon Oct 2023, Quebec City Oct 2024) and the 2023 Entomological Society of America meeting in Nov 2023 in Maryland. He was an invited speaker for CanolaWeek 2023 where he presented this project in Calgary in December 2023.

8. **Financial statement** including an indication of any carryover.
    - Please see financial statement included with this scientific report.
9. Appendix

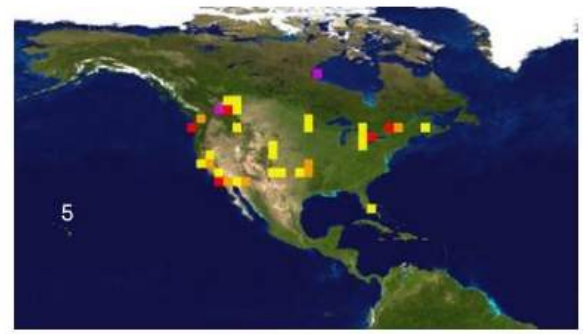
Figure A1. Haplotypes of Diamondback moths (DBM) and their collection locations and intensities (yellow to red) posted in the Barcode of Life Database. A Haplotypes 1-4, b 5-8, c 9-11, d. 12-15.

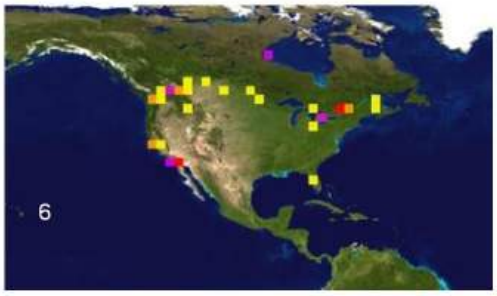
Unclassified / Non classifié

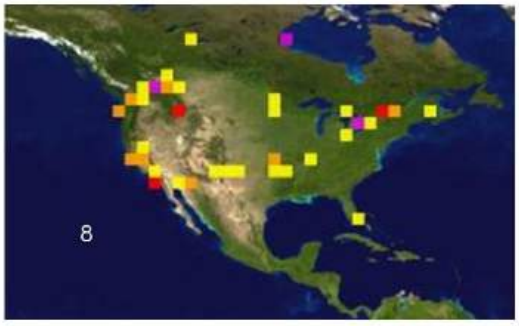


B.

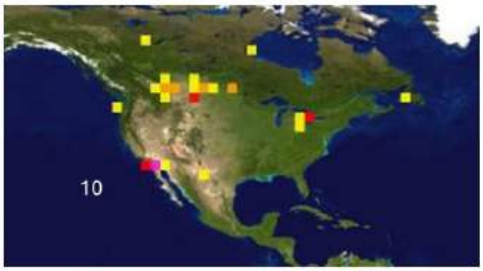
Unclassified / Non classifié







Unclassified / Non classifié



C.

D.