DISSERTATION

CHARACTERIZING HOST PLANT-VIRUS-VECTOR INTERACTIONS OF THE POTATO VIRUS Y AND APHID PATHOSYSTEM

Submitted by

William Jacob Pitt

Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2023

Doctoral Committee:

Advisor: Punya Nachappa

Amy O. Charkowski
Ian V. MacRae
Frank B. Peairs
Melinda D. Smith

ABSTRACT

CHARACTERIZING HOST PLANT-VIRUS-VECTOR INTERACTIONS OF THE POTATO VIRUS Y AND APHID PATHOSYSTEM

Aphid-transmitted potato virus Y (PVY) is one of the most damaging pathogens of potato worldwide. Plant virus prevalence is influenced by landscape composition, host-use patterns of vectors, and the range of capable vector species. Regarding these influences, there are important knowledge gaps that remain within the PVY-aphid pathosystem. The overall goal of this research was to better understand host-virus-vector interactions within the PVY-aphid pathosystem at multiple levels of ecological organization.

There is limited information on the effect of landscape-scale crop diversity on prevalence of insect-vectored viruses. In my dissertation, I investigate how landscape composition of crops (Shannon diversity of crops, percent crop cover) affects aphid vector communities and prevalence of aphid-transmitted PVY. I conducted a two-year field study in the San Luis Valley in Colorado where I sampled aphid communities with pan traps, quantified PVY incidence in potato crops with ELISA, and determined the association with landscape variables (Shannon diversity index of crops and percent crop cover) surrounding sampling sites. Crop diversity negatively influenced aphid species richness, but positively influenced PVY incidence. The negative association of crop diversity with aphid species richness could have been due to differences in management between crops and/or increased predation/parasitization of aphids. The positive association between crop diversity and PVY is likely because PVY has a wide host

range and increasing crop diversity may positively influence PVY incidence due to increased inoculum in the landscape. Additionally, there was a positive association of potato (virus host) and a negative association of barley (virus non-host) with PVY incidence. In summary, I found that crop species diversity influenced both PVY prevalence and aphid communities, and that the virus host/non-host status of crops likely modulates this effect.

Aphid vectors frequently probe upon various plants within a landscape, but the host use patterns of aphid vectors has not been adequately described. It would be useful to identify plants that aphid vectors are probing upon within a landscape in order to identify 1) vector movement within a landscape and 2) potential sources of aphid-transmitted virus inoculum. I used high throughput molecular gut content analysis (GCA) to characterize plant-aphid vector associations within a major potato producing region, the San Luis Valley, CO, where aphid-transmitted potato virus Y (PVY) is a major issue for potato production. Aphids were trapped weekly with suction traps during the growing seasons of 2020 and 2021. Plant-specific DNA in 200 individual aphids representing 9 vector species of PVY (*Acyrthosiphon kondoi*, *A. pisum*, *Capitophorus elaeagni*, *Diuraphis noxia*, *Hayhurstia atriplicis*, *Myzus persicae*, *Phorodon cannabis*, *Protaphis middletonii*, *and Rhopalosiphum padi*) were amplified by PCR, sequenced with the PacBio sequencing platform, and sequences were identified to genus using NCBI BLASTn. I found that all species of aphid vectors probed on plant genera that are outside of their reported host ranges, with *Solanum, Medicago*, *Populus*, *Brassica*, and *Glycine* as the most frequently detected plant genera. Moreover, aphids probed on many plant genera likely to be PVY host plants. These findings provide information that is essential to develop sustainable and effective management strategies to reduce PVY spread.

With the increasing acreage of hemp (*Cannabis sativa* L.) (Rosales: Cannabaceae) in the United States, I was interested to know whether the cannabis aphid (*Phorodon cannabis* Passerini) (Hemiptera: Aphididae) is a potential vector of PVY. I conducted transmission assays and used the electrical penetration graph (EPG) technique to determine whether cannabis aphids can transmit PVY to hemp (host) and potato (non-host) (*Solanum tuberosum* L.) (Solanales: Solanaceace). I showed, for the first time, that the cannabis aphid is an efficient vector of PVY to both hemp (96% transmission rate) and potato (91%) using cohorts of cannabis aphids. In contrast, individual aphids transmitted the virus more efficiently to hemp (63%) compared to potato (19%). During the initial 15 min of EPG recordings, aphids performed fewer intracellular punctures and spent less time performing intracellular punctures on potato compared to hemp, which may in part explain low virus transmission to potato using individual aphids. During the entire 8-hour recording, viruliferous aphids spent less time ingesting phloem compared to non-viruliferous aphids on hemp. This reduced host acceptance could potentially cause viruliferous aphids to disperse thereby increasing virus transmission. Overall, my study shows that cannabis aphid is an efficient vector of PVY and that virus infection and host plant suitability affect feeding behaviors of the cannabis aphid in ways which may increase virus transmission.

ACKNOWLEDGMENTS

The research in this dissertation was conducted in collaboration with many people, both professionally and personally. I am indebted to my advisor, mentor, and friend, Punya Nachappa, who has believed in me and given me relentless support and encouragement that made this work possible. Punya has helped foster my curiosity of aphids and plant viruses, and I am very grateful to have been able to study under her. I will not forget when I went into Punya's office in 2019 and expressed to her that I desired to continue my education in graduate school, and she generously brought me on in her lab. I would also like to thank my mentor and friend Vamsi Nalam who made the decision to hire me as an undergraduate research assistant, and introduced me to aphids. I am also extremely thankful to my committee members Amy Charkowski, Ian Macrae, Frank Peairs, and Melinda Smith who have provided me with insights into their knowledge and experience. I feel very humbled and grateful to have been able to learn from each of you. I also express sincere gratitude to Rodney Cooper for assisting with gut content analysis of aphids, and for helping me make important connections within the field.

I would also like to thank Jim Ehrlich and the Colorado Potato Administrative Committee who funded much of the research that we conducted. I am grateful for Tess Christensen, Bill Crowder, and the staff at Agro Engineering for their collaboration and assistance with collecting aphids in the San Luis Valley. I would also like to thank Heather Headrick for her assistance with gut content analysis of aphids. Chakradhar Mattupalli, Zack Czarnecki, and the staff and faculty at the San Luis Valley Research Center helped tremendously with field work in the San Luis Valley. I would also like to thank Wylie Keller, Marisa Fricke, and the staff at the Rio Grande Water Conservation District for kindly sharing their landscape data with us.

I am extremely grateful for help from Andy Jensen who not only taught me how to identify aphids, but also how to appreciate their diversity. I would like to thank my mentors Tessa Albrecht and Ana Cristina Fulladolsa Palma, who I have been fortunate to learn from over the past 4 years. I thank my fellow lab members that have helped me along the way, including Lisa Kairy, Emily Villa, Victor Mora, Erika Peirce, Jacob MacWilliams, Jinlong Han, Olivia Carter, Judith Chiginsky, Jen Rushton, Chris Hayes, Henrique Vieira, Joran Withycombe, Max Schmidtbauer, and Luke Deyle. I thank my friends Anna Santos and Caley Morgan who have been so kind to me. My friends Jillian and Neal have helped me for many years, provided me with good advice, and always lent an open ear. I am thankful for you both. I would like to thank my friends from the bands Muck Luck and Ash Redhorse and the Midnight Suns. You guys have helped me learn a lot about music and life and helped me believe in myself.

I am very grateful to my partner, Laine Hackenberg, who has always been there for me. Laine has showed me love and support that has made this achievement possible, and I am excited to begin our next chapter up in Washington.

Finally, I am indebted to my family. My parents, David and Sheila Pitt, showed me love, supported me, and encouraged me every step of the way. Lauren, you've been the best sister to me and I am so grateful for you. You being kind enough to let me take care of Felix way back in the day had such a positive impact on my life. My grandparents, Bill Pitt, Bill LaFavor, and Joyce LaFavor have been the most supportive and encouraging grandparents I could ever hope for. Thank you all so much.

DEDICATION

Dedicated to my parents, David and Sheila Pitt, who have given me undying love, support, and encouragement.

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CHAPTER 1: BIOLOGY AND LANDSCAPE ECOLOGY OF APHID-TRANSMITTED VIRUSES

Introduction

Plant viruses are important components of ecosystems (Bever et al. 2015; Lefeuvre et al. 2019) and reduce agricultural yield worldwide (Rao and Reddy 2020). The latest taxonomic classification of viruses recognizes 2,368 distinct virus species infecting plants (ICTV 2021). The majority of plant viruses are transmitted by insects (Nault 1997), with aphids transmitting approximately one third of all plant viruses studied (Hogenhout et al. 2008; Fereres 2016). Since the first experimental evidence of aphids as vectors of plant viruses in the early 20th century (Allard 1917), significant strides have been made in our understanding of aphid vector biology (e.g., Ng and Perry 2004), virus manipulations on aphid vectors (e.g., Eigenbrode et al. 2018), and landscape ecology and epidemiology of plant viruses (e.g., Plantegenest et al. 2007; Jeger 2020). The goal of this introduction is to provide important background on aphid vector biology, virus manipulations on aphid vectors, and landscape ecology and epidemiology, in addition to providing a brief synopsis of the research described within this dissertation. Characterizing plant-aphid-virus interactions will further fundamental knowledge that is necessary for the sustainable management of plant viruses.

Vector Biology

Plant virus modes of transmission can be categorized as non-persistent, semi-persistent, or persistent (propagative or circulative). These classifications depend on the virus relationship with the vector and the virus relationship with the host plant (Whitfield et al. 2015). Persistent

viruses are phloem-limited, use receptor mediated endocytosis to move through the gut wall and throughout the hemocoel, are acquired by vectors after hours of feeding, and typically remain with the vector for life. Persistent *propagative* viruses replicate within vectors, while persistent *circulative* viruses do not replicate, but circulate throughout the vector. In contrast, non-persistent viruses infect epidermal and mesophyll cells of plants, and bind to receptor proteins at the tips of vectors' stylets, within a structure called the 'acrostyle' (Uzest et al. 2010; Mondal et al. 2021) or within other areas of the stylet (Khelifa 2023). Further, non-persistent viruses are typically acquired by vectors within seconds to minutes, and vectors remain infectious for only a matter of hours. Semi-persistent viruses share qualities of both persistent and non-persistent viruses, and reside within the foregut of vectors. Making these distinctions is important, as the mode of transmission influences the nature of virus manipulations on vectors (Mauck et al. 2012), the effectiveness of management strategies to control virus spread (Boiteau and Singh 1999; Radcliffe and Ragsdale 2002; Carroll et al. 2009), and virus epidemiology within a landscape (Carroll 2005; Kho et al. 2020). Additionally, aphid life history is relevant for virus epidemiology within a landscape. Aphids have complex life cycles, with most species alternating between apterous (wingless) and alate (winged) morphs. Alate morphs are thought to be more important for spread of viruses than apterous morphs due to the greater dispersal activity of alatae (Kennedy 1950; Gadhave et al. 2020). Hence, many field studies use traps designed to capture alate rather than apterous morphs.

Virus Manipulations of Vectors

Plant viruses manipulate vectors in order to enhance virus spread (Ingwell et al. 2012), but the nature of the manipulation depends upon the mode of transmission (Castle and Berger 1993; Mauck et al. 2012). This is thought to be due to co-evolution between host plants, viruses,

and vectors (Ingwell et al. 2012; Mauck et al. 2012). Analysis of the literature has revealed that persistent viruses typically lead to increased vector fitness, while non-persistent viruses typically lead to decreased vector fitness, and both virus modes alter vector behavior in different ways (Mauck et al. 2012; Eigenbrode et al. 2018). From an evolutionary perspective of the virus, a non-persistently transmitted virus would have increased chances of spread from rapid vector dispersal after contacting the host plant, due to the short amount of time needed for transmission. In contrast, a persistently transmitted virus would benefit from immediate host acceptance with subsequent vector dispersal, due to the longer amount of time required for vectors to reach the phloem sieve elements. Indeed, Castle and Berger (1993) showed that *Myzus persicae* performance was significantly increased on potato leafroll virus (persistent virus) infected plants, and performance was decreased on potato virus Y (non-persistent virus) infected plants. Considering vector behavior, Angelella et al. (2018) showed that watermelon mosaic virus (non-persistent virus) increased the number of intracellular punctures performed by the vector *Aphis craccivora*, which suggests that the non-persistent virus modified feeding behavior in a way that would promote virus transmission. Within the potato virus Y (non-persistent virus) – aphid pathosystem, Boquel et al. (2011) showed that viruliferous *Macrosiphum euphorbiae* spent less time ingesting phloem, suggesting that the non-persistent virus reduced host suitability for the aphid. However, in the same study by Boquel et al. (2011), viruliferous *Myzus persicae* spent an increased amount of time ingesting phloem. Indeed, there are many exceptions among patterns of virus manipulations on vectors, suggesting that additional studies involving a wider range of taxa are needed (Eigenbrode et al. 2018).

Landscape Ecology

Studying plant viruses through a perspective of landscape ecology yields potential for the development of strategies to mitigate plant virus spread (Plantegenest et al. 2007). A landscape may be defined as "an area that is spatially heterogenous in at least one factor of interest" (Turner et al. 2001), and landscape ecology may be defined as the study of how spatial heterogeneity influences ecological processes, and/or vice versa (Turner 2005). Within agroecosystems, different crop species in a landscape represent spatial heterogeneity and virus transmission is an economically important ecological process. Within the last few decades, our understanding of how the spatial heterogeneity of crops influences insect-transmitted plant virus prevalence and spread has grown substantially, particularly at landscape scales of 0.2 to 10 km in radius (e.g., Carriere et al. 2014; Delaune et al. 2021). Theoretical evidence suggests that greater pathogen host plant diversity leads to greater pathogen prevalence when the pathogen has a wide host range and many capable vector species (Keesing et al. 2006). Alternatively, the opposite effect can occur, with greater host plant diversity leading to reduced pathogen prevalence, particularly when the pathogen has a limited host range and few capable vector species (Keesing et al. 2006). Indeed, there is empirical evidence that plant diversity and specific crop species influence virus prevalence and vector communities (e.g., Carriere et al. 2014; Liu et al. 2020). However, to my knowledge, there has not yet been a simultaneous assessment of crop diversity effects on potato virus Y (PVY) prevalence and aphid vector communities.

Synopsis of Dissertation Research

My dissertation research focused on non-persistently transmitted PVY, which has a relatively wide host range and is transmitted by many aphid vector species. Potato virus Y has led to reduced yield loss for potato growers globally, and over the past 20 years, has continued to

be a major issue for potato production in North America (Crosslin et al. 2002; Karasev and Gray 2013). The San Luis Valley, CO is a major potato producing region, where many other crop types are grown as well. Increasing cropland within a landscape has been demonstrated to be associated with increased PVY prevalence and decreased aphid vector abundance and richness (Claflin et al. 2017; 2019). The effect of different crop species on aphid vector ecology and behavior and PVY spread at landscape-scales is not known. In Chapter 2, I address questions of how crop diversity and certain crop species within landscapes influence aphid vector communities and PVY prevalence.

Probing, which is a feeding behavior during which aphids puncture a plant's epidermis, is an important step in the host selection process (Powell et al. 2006). While it is known that aphids probe upon many plants within a landscape (Döring 2014), the dietary history of aphid vector populations has not been adequately investigated. Identifying whether aphid vectors frequently probe upon crops or weeds that are capable of sustaining PVY infection would be useful to better understand PVY epidemiology. Molecular gut content analysis (GCA) is a technique that has commonly been used to identify prey species of insects which predators had previously fed upon (e.g., Symondson 2002) but has also been used to identify the dietary history of herbivorous insects through identification of plant DNA that is present within insects' guts (e.g., Matheson et al. 2008). Identifying the dietary history of insects can be useful to describe patterns of plant – insect associations within a landscape. Indeed, in more recent years, GCA, using high-throughput sequencing, has been applied to infer landscape scale movements of phloem feeding insects through reconstruction of their dietary history (e.g., Cooper et al. 2019). In Chapter 3, I utilize high-throughput molecular GCA to reconstruct the dietary history of aphid vector populations in attempt to answer the questions of what PVY-host plants aphid vectors probed upon within the

landscape, and if certain vector species had a higher propensity to probe plants than other vector species.

In Chapter 4, I assess the vector capacity of an invasive aphid species, cannabis aphid (*Phorodon cannabis*), to transmit PVY to both hemp and potato. With the cultivation of hemp in Colorado in recent years (USDA NASS 2023), I was motivated to know whether hemp could serve as a reservoir for PVY. Further, I utilized the electrical penetration graph (EPG) technique to determine changes in cannabis aphid feeding behaviors due to PVY acquisition on hemp (cannabis aphid host) and potato (cannabis aphid non-host). Applied study of the biology and ecology of the PVY – aphid pathosystem will be useful to expand upon theoretical predictions of host-virus-vector interactions, and will provide information that is necessary to develop effective management strategies.

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CHAPTER 2: LANDSCAPE COMPOSITION OF CROPS IS ASSOCIATED WITH APHID VECTOR COMMUNITES AND PLANT VIRUS PREVALENCE

Synopsis

There is limited information on the effect of landscape-scale crop diversity on prevalence of insect-vectored viruses. Here, I investigate how landscape composition of crop species (Shannon diversity of crops, percent crop cover) affects aphid vector communities and prevalence of aphid-transmitted potato virus Y (PVY), one of the most damaging plant viruses for potato production worldwide. I hypothesized that crop diversity would positively influence aphid communities and PVY prevalence. Further, I hypothesized that virus host species would be positively associated with PVY prevalence and virus non-hosts would be negatively associated with PVY prevalence. To test these hypotheses, I conducted a two-year field study in the San Luis Valley in Colorado where I sampled aphid communities weekly with pan traps, quantified PVY incidence in potato crops biweekly with ELISA, and determined the association with landscape variables (Shannon diversity index of crops and percent crop cover) surrounding sampling sites. Crop diversity negatively influenced aphid species richness, but positively influenced PVY incidence. Additionally, there was a positive association of potato (virus host) and a negative association of barley (virus non-host) with PVY incidence. The negative influence of crop diversity on aphid species richness may be due to increased predation/parasitization and/or differences in land management between crops. Since PVY has a wide host range, increasing crop diversity may positively influence PVY incidence due to increased inoculum in the landscape. In summary, I found that crop species diversity influenced both PVY prevalence

and aphid communities, and that the virus host/non-host status of crops likely modulates this effect.

Introduction

Differences in landscape composition can influence presence of insect vectors and thereby prevalence of insect-vectored plant viruses. Indeed, increasing virus hosts in a landscape can lead to higher virus prevalence (Keesing et al. 2006; Carrière et al. 2014; Clemente-Orta et al. 2021), and this has been demonstrated in multiple agroecosystems. For example, host composition within a landscape is positively associated with prevalence of viruses transmitted by planthoppers (Clemente-Orta et al. 2021), aphids (Ingwell et al. 2017), and whiteflies (Carrière et al. 2014). Host composition is also positively associated with prevalence of psyllid-transmitted *Candidatus* Liberibacter solanacearum (Nissinen et al. 2022). However, there is evidence that virus non-hosts may also contribute to increased virus prevalence. For example, presence of cotton (i.e., non-host) was positively associated with incidence of whitefly-transmitted cucurbit yellows stunting disorder virus, likely because cotton is a suitable host for the whitefly vector of CYSDV (Carrière et al. 2014). The percent cover of cereals (i.e., virus hosts) was either positively or negatively associated with prevalence of aphid-transmitted sugarcane mosaic virus depending on the spatial scale analyzed (Clemente-Orta et al. 2020). To summarize, both virus hosts and non-hosts can influence insect-vectored disease prevalence, but additional research is needed to describe consistent patterns of these effects.

An ecologically important component of landscapes within agricultural systems is crop diversity. Crop diversity, at landscape spatial scales (i.e., multiple kilometers), can influence insect vector populations (Gutiérrez Illán et al. 2020) in addition to parasitism and predation by natural enemies (Chaplin-Kramer and Kremen 2012; Bosem Baillod et al. 2017; Redlich et al.

2018). Regarding pathogens, theoretical evidence suggests that greater crop diversity leads to greater pathogen prevalence when the pathogen has a wide host range and many capable vector species (Keesing et al. 2006). However, the opposite effect can occur, with greater crop diversity leading to reduced pathogen prevalence when the pathogen has a limited host range and few capable vector species (Keesing et al. 2006). Indeed, crop diversity at field-level spatial scales (i.e., intercropping, border and trap crops), has been empirically shown to lead to reduced insect-vectored virus prevalence (Power 1990; Matson et al. 1997). However, there appears to be a lack of information on the effects of landscape-scale crop diversity on insect-vectored virus prevalence.

Landscape effects on virus prevalence may be more complex when a virus is transmitted by many different vector species involving a range of host/non-hosts. One such system is potato virus Y (PVY), which has a wide host range (Kerlan 2006; Karasev and Gray 2013), and is transmitted by at least 66 species of aphids (Pelletier et al. 2012; Pitt et al. 2022). Potato virus Y is one of the most damaging plant viruses for potato production worldwide (Karasev and Gray 2013). Previous research showed that percent cropland within a landscape was positively associated with prevalence of PVY, but negatively associated with aphid vector species richness (Claflin et al. 2017; 2019). With some exceptions, aphid species are highly host specific, being able to feed and reproduce on only a few species or genera of plants (Blackman and Eastop 2008). Moreover, aphid species display different, and often unpredictable, population dynamics (Dixon 2012). Aphid vector species are known to differ considerably in virus transmission efficiency, with most aphid species transmitting PVY at low efficiencies (e.g., Halbert et al. 2003; Boquel et al. 2011). And interestingly, non-colonizing aphids (i.e., species which are not able to colonize potato) can be the most important species for PVY transmission (e.g., DiFonzo

et al. 1997; Steinger et al. 2015; Mondal et al. 2016; Galimberti et al. 2020). A high degree of host specificity in aphids, unpredictable population dynamics of aphids, and the importance of the many non-colonizing aphid species underscore the importance of understanding crop species effects on landscape scale aphid-transmission of PVY.

Here, I investigate how landscape-scale crop species diversity and percent crop cover influence aphid vector communities and PVY prevalence. I hypothesize that:

(i) increasing crop (host and non-host) diversity will positively influence aphid communities (abundance, richness, and evenness),

(ii) increasing crop diversity will positively influence PVY incidence,

(iii) the percent cover of each of the three most abundant crops (potato, barley, and alfalfa) will positively influence aphid communities (abundance, richness and evenness), and

(iv) the percent of virus host crop cover (e.g., potato) will positively influence PVY incidence, but percent of virus non-hosts (e.g., barley and alfalfa) will negatively influence PVY incidence.

Methods

A two-year field study (during the years 2020 and 2021) was conducted in the San Luis Valley, Colorado to determine associations between landscape composition and PVY transmission. Potato fields were sampled and tested for the presence of PVY biweekly (i.e., once every two weeks) at 17 fields (eight fields in 2020 and nine fields in 2021), and aphid communities were sampled weekly at 10 sites during both years (**Fig. 2.1**). Potato fields were pivot irrigated and most fields were either ~24 – 25 hectares or ~49 – 54 hectares in area (**Supp. Table A1.1**). The mean distance from sampled potato plants to corresponding aphid traps was 567.4 m. Each sampling site, for both aphids and PVY, was at least 1.5 km away from the next

closest sampling site and was surrounded by a variety of crop species (**Fig. 2.1**). In 2020, one field ("6N6E") was sampled which did not have corresponding aphid traps; this site was included for tests of PVY – landscape associations but not PVY – aphid abundance, species richness, or species evenness associations. One of the fields from 2020 ("RS-1") was composed of the PVY – resistant potato variety, Rocky Mountain Russet, so this field was excluded from PVY analyses. Additional information about potato fields is included in **Supp. Table A1.1**.

Aphid sampling and identification

Three 45.72 x 25.4 x 10.16 cm yellow pan traps (Tamco®, Smyrna, TN) were placed at each of 10 sites from late May to early September. The traps were arbitrarily placed on top of soil within field margins near road intersections and were spaced 14 – 59 m apart at each site. Traps were filled with water and several drops of bleach (in order to break the surface tension of the water so aphids could not escape) until the solution filled the trap approximately 3 cm below the top. Each week, all observed aphids were removed using a paint brush and placed into glass vials filled with ~70% ethanol and transported to a laboratory for identification.

Aphids were examined in 70 – 80% ethanol under a dissecting microscope and identified to the lowest possible taxonomic level using a combination of taxonomic keys and descriptions of species/genus morphology (Blackman and Eastop 2000; 2008; Pike et al. 2003; Jensen 2022; Dransfield and Brightwell 2022). Some aphid specimens were only identified to genus due to unreliability of identification using only morphology without knowledge of the host plant from which the aphid originated, and some aphids (11.74% of total aphids from both years combined) were not identified because of damage to specimens; these aphids were included in statistical tests of aphid abundance, but not species richness or evenness. Occasionally, only 1 – 2 traps were recorded for a particular week due to traps being knocked over from wind or other

disturbances, so total aphid abundance from the three traps at each site were averaged by the number of active traps to provide mean values of aphid abundance. For testing associations with landscape variables, aphid species richness was calculated as the total number of aphid species collected from all traps at a site, as per Claflin et al. (2017). Aphid species evenness was calculated using the E_{var} metric (Smith and Wilson 1996; Avolio et al. 2019). Voucher specimens were mounted on slides in Canada balsam and submitted to the C.P. Gillette Museum of Arthropod Diversity at Colorado State University.

Potato tissue sampling and PVY detection

In each field, 20 plants were systematically sampled in a zig-zag pattern and marked with 36" tall pink or blue flags. The zig-zag pattern was 4 rows wide and 10 steps were taken inwards into the field between sampled plants. Two leaves (to ensure ample tissue for PVY detection) were arbitrarily taken from nodes 1 – 8 of each potato plant and placed in a small plastic bag and stored in a cooler, where they were transported to a refrigerator in a laboratory. The same 20 plants were sampled throughout the season to detect new infections within a field.

A triple antibody sandwich – enzyme linked immunosorbent assay (TAS-ELISA) was used to detect PVY in potato tissue samples (PVY PathoScreen® Kit, AgDia® Inc., Elkhart, IN). Potato tissue samples from each field were pooled (in groups of 10; Chatzivassiliou et al. 2004) and tested for PVY. If PVY was detected from the pooled testing of samples, then samples were tested individually to quantify the number of infected plants per field. For pooled testing of PVY, 0.05 – 0.15 g of tissue was taken from each of 10 samples (tissue from just one of the leaves was used) and homogenized in a general extract buffer (Agdia® Inc.) using an extraction bag and hand homogenizer (Bioreba AG, Reinach, Switzerland). The same steps were followed for PVY detection in individual plant tissue samples, except that tissue was homogenized inside of 2mL

Eppendorf tubes with two 3.97 mm stainless steel beads using a TissueLyser II (Qiagen, Germanton, MD). PathoScreen kit protocol instructions were followed, and optical density (OD) values were observed at 405 nm using a spectrophotometer. Samples were considered positive when OD values were greater than two times the mean OD value for negative controls. PVY incidence was quantified as the number of infected plants divided by the number of plants tested.

Association between PVY incidence and aphid communities

To determine associations between PVY incidence and aphid communities, logistic regression was performed with PVY infection treated as a binomial response. Analysis using data from both years combined showed that year had a significant effect on PVY incidence (Independent variable = "year 2021"; Estimate = 1.60; df = 2; $P < 0.0001$), so data from both years were analyzed separately, similar to Claflin et al. (2017). The values of biweekly PVY incidence and aphid abundance, species richness, and species evenness data from each site were used as a replicate for logistic regression of PVY – aphid associations (30 replicates in 2020; 53 replicates in 2021). Separate models with aphid abundance, aphid species richness, and aphid species evenness as predictors were constructed. Abundance of the five most abundant species/genus groups from both years were also tested for association with PVY incidence. Additionally, in order to determine if associations between PVY incidence and aphid communities varied throughout the growing season, separate logistic models were constructed for each sampling date with biweekly values of PVY incidence as the dependent variable and aphid abundance from the preceding week as the independent variable.

Landscape composition

Landscape composition data from both years were obtained from two ground-truthed 10 x 10 m resolution maps containing all crop types grown in the study area (**Supp. Table A1.2**),

which were provided by the Rio Grande Water Conservation District (RGWCD; https://www.rgwcd.org/subdistrict-no-1-maps). Shannon diversity index (SDI) of crop species and percent cover of each crop species were calculated within three spatial buffers with radii of 1 km, 2 km, and 3 km for each site. Spatial buffers of 1 km, 2 km, and 3 km were selected, as spatial scales of < 5 km are likely where processes of landscape effects on pests and pathogens take place (Delaune et al. 2021). Further, the spatial buffer sizes I selected are within the range of previously reported scales-of-effect of landscape effects on aphid communities (Thies et al. 2005; Claflin et al. 2017; Yang et al. 2019). For aphid sampling sites and potato tissue sampling sites, the centroid of the traps, or potato plants, was calculated and used as the center for the buffers. Landcover types used for calculation of SDI of crops included only those in which an individual crop plant species was able to be inferred (**Supp. Table A1.2**). Additionally, weather parameters were collected from the San Luis Valley Research Center weather station within the study area to qualitatively compare environmental conditions between years (**Supp. Table A1.3**). Landscape analyses were performed in RStudio using R software version 4.1.1 (R Core Team 2022), using the packages 'sf', 'codyn', 'raster', 'exactextractr', 'rgdal', 'rgeos', and 'vegan' (Pebesma 2023; Hallett et al. 2020; Hijmans 2023; Baston 2022; Bivand et al. 2023; Bivand and Rundel 2021; Oksanen et al. 2022).

Association between landscape composition and PVY incidence, and landscape composition and aphid communities

Principal component analysis

I assessed how all the crop species present within 3 km buffers of study sites related to aphid communities and PVY incidence. Because there were many different crop species present within landscapes, assessing relationships between crop species composition, aphid

communities, and PVY incidence required dimensionality reduction. Therefore, principal component analysis (PCA) was conducted with percent crop cover data, aphid abundance and richness, and PVY incidence from each trapping or sampling site. Data from 2020 and 2021 were analyzed together and were centered and scaled before PCA. PCA was conducted in RStudio using the function 'prcomp' (R Core Team 2022) and results were visualized using the function 'fviz_pca_biplot' (Kassambara and Mundt 2020).

Regression analysis

Potato, barley, and alfalfa were selected for regression analysis as they were the three most abundant crop species during 2020 and 2021 and were mostly present at each site. Other crop species were often only present at one or two sites, which posed an issue for analysis using regression models due to an abundance of zeros. To determine associations between landscape variables and PVY incidence, logistic regression was used, again with PVY infection treated as a binomial response (**Supp. Table A1.4**). For logistic regression of PVY – landscape associations, weekly PVY incidence values were summed to the site level (7 replicates in 2020; 9 replicates in 2021). Three separate models (i.e., one model for each spatial buffer) were constructed with PVY incidence as the dependent variable and Shannon diversity index of crops as the independent variable. Additionally, three separate multiple logistic regression models were constructed with PVY incidence as the dependent variable and percent cover of potato, barley, and alfalfa within the three spatial buffers as independent variables. The percentage cover of alfalfa surrounding PVY sites in 2020 was significantly correlated with other landscape variables, so it was not included in PVY incidence models.

To determine associations between landscape variables (SDI of crops and percent cover of crops) and aphid abundance, species richness, and species evenness, linear regression was

used (**Supp. Table A1.4**). A log transformation was performed on aphid abundance, as residuals of models using raw aphid data did not meet the linear regression assumption of normality. Three separate models were constructed to regress log-transformed aphid abundance on the SDI of crops and percent cover of the three crop species (i.e., potato, barley, and alfalfa) within the three spatial buffers; the same was repeated for aphid species richness and aphid species evenness. The percentage cover of barley surrounding aphid sites in 2020 was significantly correlated with other landscape variables, so it was not included in models of aphid abundance, species richness, or species evenness.

Moran's I was used to test for spatial autocorrelation in landscape model residuals. No significant spatial autocorrelation was detected in residuals of any of the landscape models (**Supp. Table A1.5**). Statistical analyses were performed in RStudio using the packages 'stats' and 'dplyr', (R Core team 2022; Wickham et al. 2023b). Figures were constructed using the packages 'ggplot2', 'ggpubr', 'jtools', 'cowplot', 'viridis', and 'RColorBrewer' (Wickham 2023a; Kassambara 2023; Long 2022; Wilke 2020; Garnier 2023; Neuwirth 2022).

Results

Aphid community composition

Aphid community composition was drastically different between 2020 and 2021 (**Fig. 2.2**). In 2020, 17,029 individual aphids were sampled, and 15,114 of these aphids were able to be identified to species or genus (**Supp. Table A1.6**). The three most abundant species/genus groups were *Acyrthosiphon* spp., *Phorodon cannabis*, and *Myzus persicae*, all of which varied in their dates of peak abundance (**Fig. 2.3A**). While *P. cannabis* was recorded from all sites, 67.5% of end-of-season population counts of this species were recorded from one location ("8-7") (**Fig. 2.3A**).

In 2021, a total of 3,169 individual aphids were sampled, and 2,962 of these aphids were able to be identified (**Supp. Table A1.6**). The three most abundant species were *M. persicae*, *Protaphis middletonii*, and *Capitophorus elaeagni*, which again, all varied in their dates of peak abundance (**Fig. 2.3B**). While *M. persicae* was the most abundant species in 2021 but the third most abundant in 2020, total abundance was much greater in 2020 (**Supp. Table A1.6**).

PVY incidence

Potato virus Y incidence varied dramatically between years (**Fig. 2.4**; **Supp. Table A1.1**). Initial infection of PVY during the first sampling date in 2020 was 5% incidence (**Fig. 2.4A**). In 2020, new infections throughout the season were not common (**Fig. 2.4A**) and end-of-season incidence ranged from 0% to 15%, with 5.6% average incidence. In contrast, initial infection of PVY in 2021 was extremely high, with a maximum of 40% incidence (**Fig. 2.4B**). In 2021, new infections within a field were frequently detected each sampling date throughout the season (**Fig. 2.4B**) and end-of-season incidence ranged from 5% to 90% with 34.4% average incidence.

Association between PVY incidence and aphid communities

In 2020, there were no significant associations between PVY incidence and aphid abundance, PVY and aphid species richness, or PVY and aphid species evenness (**Table 2.1**). In contrast, there was a significant association between PVY incidence and aphid abundance, in addition to PVY incidence and aphid species richness during 2021 (**Table 2.1**). Further, there were significant associations between PVY incidence and abundance of *M. persicae* and *C. elaeagni* during 2021 (**Table 2.2**). Analysis of biweekly aphid abundance and PVY incidence in 2021 revealed a significant positive association on June 9 and a significant negative association on July 21.

Landscape composition and aphid communities and PVY incidence

Principal component analysis

A total of 14 and 15 different crop species were present within 3 km buffers surrounding aphid trapping sites and PVY sampling sites, respectively. Together, the first two principal components (PC1 and PC2) from the aphid trapping site PCA explained 38.9% of the variance in crop species composition, aphid abundance, and aphid richness between sites (**Fig. 2.5A**). The highest contributing variables to PC1 were hemp (18.0%), sorghum (18.0%), and rye (16.6%) (**Table 2.3**), all of which were positively correlated with PC1 (**Fig. 2.5A**). The highest contributing variables to PC2 were alfalfa (20.1%), canola (19.3%), and potato (16.2%) (**Table 2.3**). Alfalfa and canola were negatively correlated with PC2, but potato was positively correlated with PC2 (**Fig. 2.5A**). Aphid richness was positively correlated with alfalfa and canola and contributed to 10.5% of variance of PC2 (**Fig. 2.5A**). Aphid abundance showed a weak contribution to explained variance by PC1 and PC2 (**Table 2.3**) but was positively correlated with potato (**Fig. 2.5A**).

Together, PC1 and PC2 from the PVY sampling site PCA explained 38.3% of the variance in crop species composition and PVY incidence between sites (**Fig. 2.5B**). The highest contributing variables to PC1 were peas (16.9%), sorghum (16.2%), and rye (14.6%) (**Table 2.3**), all of which were negatively correlated with PC1 (**Fig. 2.5B**). The highest contributing variables to PC2 were alfalfa (23.0%), canola (19.8%), and potato (13.7%) (**Table 2.3**). Alfalfa and canola were positively correlated with PC2, but potato was negatively correlated with PC2. PVY incidence showed a weak contribution to explained variance by PC1 and PC2 (**Table 2.3**) but was positively correlated with carrots, potato, wheat, and quinoa (**Fig. 2.5B**).

Regression analysis

The virus host, potato and non-virus hosts, barley and alfalfa were the most abundant crops surrounding study sites during both years (**Fig. 2.6**). There was a significant negative association between aphid species richness and SDI of crops within 3 km buffers in 2020 and 2021 (**Table 2.4**). I did not detect a significant association between aphid species richness and percent of potato and barley crop cover across any of the three buffers (**Supp. Table A1.7**). In contrast, there was a significant positive association between aphid species richness and percent cover of alfalfa within a 1 km buffer during 2020 (**Supp. Table A1.7**). A significant positive association between aphid abundance and percent cover of alfalfa within 1 km in 2021 was detected (**Supp. Table A1.8**) but no other significant associations between aphid abundance and landscape composition or aphid species evenness and landscape composition from either year were detected (**Supp. Table A1.8**; **Supp. Table A1.9**).

PVY incidence was significantly positively associated with SDI of crops within a 3 km scale in 2020 (**Fig 2.7A**) and within scales of 2 km (**Fig. 2.7B**) and 3 km ($P < 0.001$) in 2021. In 2020, there were no significant associations between PVY incidence and percent cover of any of the crop species. In 2021, PVY incidence was significantly positively associated with percent cover of potato at all three spatial scales of 1 km ($P < 0.01$), 2 km (**Fig. 2.7D**), and 3 km ($P < 0.0001$). In addition, PVY incidence was significantly negatively associated with percent cover of barley at 2 km (**Fig. 2.7F**) and 3 km in 2021 ($P < 0.01$). No significant associations between PVY incidence and alfalfa were detected.

Discussion

Landscape composition has been shown to impact plant virus prevalence within agroecosystems (e.g., Carriere et al. 2014; Claflin et al. 2017; Clemente-Orta et al. 2020). To

understand these effects, it is important to consider how the landscape modifies not only virus incidence and spread but also vector communities. Here, I show that crop diversity and percent crop cover impacts aphid vector communities and PVY prevalence. Specifically, I find that the SDI of crops was negatively associated with aphid species richness, but percent cover of major crops (potato, barley and alfalfa) did not have a major impact on aphid communities. In contrast, the SDI of crops was positively associated with PVY incidence. Moreover, percent cover of potato (virus host) was positively associated with PVY incidence, but barley (virus non-host) was negatively associated with PVY incidence. This study provides the first empirical evidence that crop diversity, at landscape scales, can modify PVY prevalence and aphid communities.

The aphid community composition in the SLV varied between the two years; however, potato non-colonizing aphids comprised of more than half of the species in both years (75.1% in 2020 and 52.8% in 2021). This is in agreement with previous reports of non-colonizers being the predominant vectors of PVY in potato cropping systems (DiFonzo et al. 1997; Steinger et al. 2015; Mondal et al. 2016; Galimberti et al. 2020). Further, I found a positive association between aphid species richness with PVY incidence in 2021, which emphasizes the importance of non-colonizing aphids for PVY prevalence within landscapes. I also found a positive association between aphid abundance and PVY incidence in 2021, which is in contrast to a study from Zeng et al. (2019) that found no correlation between aphid abundance and PVY incidence across multiple years. While Zeng et al. (2019) conducted their study in the same study region using the same type of traps as the current study, their analysis accounted for temporal, but not spatial variation in PVY incidence and aphid abundance. And while I found a positive influence of the abundance of the colonizing aphid, *M. persicae,* on PVY incidence in 2021, a statistically stronger influence of the non-colonizing, *C. elaeagni* abundance on PVY incidence was detected.

Capitophorus elaeagni host-alternates between Russian olive trees and thistles, is an efficient vector of PVY, and has previously been implicated in PVY transmission in potato fields (Halbert et al. 2003). Overall, I show that both colonizers and non-colonizers were major contributors to the significant positive associations between aphid communities and PVY in 2021 in the SLV.

Potato virus Y is transmitted by over 65 species of aphid vectors; hence, I hypothesized that increasing crop diversity will positively influence aphid communities and thereby PVY prevalence. In contrast, I found a negative influence of crop diversity on aphid species richness in 2020 and 2021. Previous research showed that crop diversity induced reductions in aphid communities from increased predation and parasitism (Bosem Baillod et al. 2017; Redlich et al. 2018). Indeed, Redlich et al. (2018) demonstrated that crop diversification at landscape scales is a promising avenue for optimizing biological control of cereal aphids. But in agreement with my hypothesis, crop diversity was positively associated with PVY incidence. It is possible that reduced aphid species richness was representative of increased aphid movement from non-consumptive effects of predators/parasitoids. Indeed, there is theoretical and empirical evidence that predator/parasitoid-avoidance behaviors lead to increased virus transmission (Hodge et al. 2011; Finke et al. 2012), and this is thought to be via means of increased vector-host encounter rates (Crowder et al. 2019). However, there is also evidence that non-consumptive effects of natural enemies lead to reduced virus transmission by aphids (Lee et al. 2021), and I am not aware of any studies examining the influence of non-consumptive effects on virus transmission by vectors in large-scale field settings. Further, I did not directly measure aphid movement, natural enemy populations, or rates of predation/parasitism. Another consideration is that aphid vector species differ in the efficiency at which they transmit PVY (e.g., Pelletier et al. 2012),

which would likely make possible non-consumptive effects on PVY transmission by aphids dependent on vector community composition.

As PVY has a wide host plant range (Kerlan 2006; Karasev and Gray 2013), it is reasonable to assume that increasing crop diversity will positively influence PVY incidence. In addition to the positive influence of crop diversity on PVY incidence, I found that increasing percent crop cover of potato (virus host) led to increased PVY prevalence, and barley (virus non-host) led to decreased prevalence. This is consistent with previous research showing positive effects of virus hosts on disease prevalence within landscapes (Carriere et al. 2014; Ingwell et al. 2017; Clemente-Orta et al. 2021). In contrast, Carroll (2005) investigated the effects of landscape composition and configuration on PVY prevalence and found that seed potato fields closer to grain crops (virus non-hosts) had higher incidence of PVY. It is likely that the effect Carroll (2005) observed was due to increased activity of cereal aphid vector populations from nearby grain crops. It is intriguing that I observed a negative influence of barley on PVY incidence, given that barley is a host for several aphid vectors which I detected in traps (i.e., *Rhopalosiphum* spp., *S. avenae*, *D. noxia*). However, the current study did not detect an influence of barley on aphid communities. Taken together, these findings suggest that effects of non-host on PVY prevalence within landscapes are variable, and additional studies are required to describe consistent patterns.

The amount of initial inoculum within a landscape is a critical component of disease epidemiology. Indeed, PVY incidence during the first sampling date was much higher in 2021 than 2020, which likely influenced the greater virus spread observed in 2021. The influence of initial inoculum on PVY prevalence and spread underscores the importance of growers sourcing certified seed potato that is below state-designated thresholds for PVY incidence. Another

consideration regarding the influence of crop species diversity on PVY prevalence is that different crop species could represent differences in management techniques that affect PVY epidemiology. For instance, PVY is known to infect various weed species, and herbicide and insecticide use may differ by crop type. Indeed, associations between different crop species and whitefly vector populations were partially attributed to differences in insecticide treatments between crop species (Carriere et al. 2014), and local pesticide use was experimentally shown to mediate landscape effects on biological control of aphids (Ricci et al. 2019). Additionally, alfalfa is harvested multiple times during a growing season, and large populations of pea aphids can move to neighboring crops after harvest (Losey and Eubanks 2000). Indeed, PCA and linear regression analysis in the current study showed that aphid species richness was positively correlated with percent cover of alfalfa, suggesting that alfalfa is likely a source of aphid populations that disperse to potato fields. Further, PCA revealed a positive correlation between canola and aphid species richness. However, it is important to note that the first two principal components from PCA described a relatively small portion of the total variance in landscape composition and aphid communities. It is highly likely that landscape effects on PVY prevalence and vector communities are, to some degree, due to differences in management between crop types within neighboring fields.

There are several important avenues for future research. The specific mechanisms responsible for the effects observed in the current study deserve investigation. This would require examination of how monocultures and polycultures differ in their influence on PVY prevalence and aphid community dynamics, as only monocultures were analyzed in the current study. Further research is also needed to determine the influence of weeds and other non-crop plant species on PVY prevalence and aphid communities. Further, investigating the influence of

landscape configuration, land use/management, and non-consumptive effects of natural enemies on PVY prevalence would likely reveal patterns relevant for understanding how crop diversity influences PVY transmission. Lastly, it should be emphasized that aphid vector species differ in their ability to transmit PVY (e.g., Pelletier et al. 2012). A useful metric to study aphid vector communities in relation to PVY spread is the vector risk index (VRI), which accounts for the PVY transmission efficiencies of vector species (MacRae 2017). The VRI should be incorporated into future studies of landscape effects on aphid vector communities.

In summary, I found that crop diversity influenced both PVY prevalence and aphid communities, and that the host/non-host status of plant species likely mediates this effect. Although, it should be noted that the number of field sites in our study was relatively low, and this could have influenced our findings. Yet, based on knowledge of what crops are in the surrounding landscape, potato growers can evaluate their risk of PVY infection in their fields early in the season well before crops have matured and aphid vector populations are abundant. The findings of crop species effects on PVY prevalence in our study could also provide information to help build spatial risk-assessment models for PVY transmission by aphids during a particular season, similar to the Vegetable Disease and Insect Forecasting Network© developed by the University of Wisconsin-Madison (https://agweather.cals.wisc.edu/vdifn). Moreover, our findings provide additional support for the importance of monitoring non-colonizing aphid vectors to prevent PVY transmission. Furthering ecological understanding of virus-host-vector interactions at landscape scales is beneficial for the effective and sustainable management of plant viruses.

Tables and Figures

Table 2.1: Results from logistic regression of biweekly PVY incidence and aphid species abundance, richness, and evenness at each site from 2020 (n=30) and 2021 (n=53).

	Estimate	df	AICc	P-value
2020				
Aphid Abundance	0.001	2	77.5	0.81
Aphid Richness	-0.06	2	76.8	0.38
Aphid Evenness	-0.47	2	66.7	0.58
2021				
Aphid Abundance	*0.03*	*2*	*481.2*	*< 0.001*
Aphid Richness	*0.10*	*2*	*482.5*	*< 0.001*
Aphid Evenness	-0.42	2	435.3	0.25

Italicized values indicate a significant association (*P < 0.001*)

Table 2.2: Results from logistic regression of biweekly PVY incidence and the five most abundant species/genus groups from 2020 (n=30) and 2021 (n=53).

	2020			
	df	AICc	Estimate	*P*-value
Acyrthosiphon spp.	2	77.57	0.001	0.85
Phorodon cannabis	2	77.57	-0.004	0.85
Myzus persicae	2	77.59	0.002	0.89
Protaphis middletonii	2	77.35	-0.13	0.62
Aphis spp.	2	75.93	-0.39	0.23
2021				
	df	AICc	Estimate	*P*-value
Myzus persicae	*2*	*484.9*	*0.04*	*< 0.01*
Protaphis middletonii	2	493.1	-0.02	0.47
Capitophorus elaeagni	*2*	*472.7*	*0.22*	*< 0.0001*
Aphis spp.	2	492.0	0.16	0.19
Pemphigus sp.	2	490.9	-0.08	0.11

Italicized values indicate a significant association (*P < 0.01*)

Table 2.3: Percent contribution of variables from aphid trapping sites and PVY sampling sites to explained variance by principal component (PC) 1 and PC2.

	Aphid Trapping Sites		**PVY Sampling Sites**	
	Percent Contribution to PC1	Percent Contribution to PC2	Percent Contribution to PC1	Percent Contribution to PC2
PVY Incidence	NA	NA	0.01 (+)	1.2 (-)
Aphid Richness	0.9 (-)	10.5 (-)	NA	NA
Aphid Abundance	0.1 (+)	1.0 (+)	NA	NA
% alfalfa	3.6 (-)	20.1 (-)	2.9 (+)	23.0 (+)
% barley	7.6 (-)	1.5 (-)	5.2 (+)	0.3 (+)
% canola	0.3 (+)	19.3 (-)	3.6 (+)	19.8 (+)
% carrots	0.4 (+)	2.8 (+)	0.5 (+)	10.7 (-)
% hemp	18.0 (+)	4.1 (+)	5.8 (-)	7.6 (-)
% lettuce	4.4 (-)	0.0 (+)	4.4 (+)	0.3 (+)
% peas	16.4 (+)	6.1 (-)	16.9 (-)	0.1 (+)
% potato	0.1 (+)	16.2 (+)	6.0 (+)	13.7 (-)
% quinoa	3.9 (-)	0.9 (+)	9.0 (+)	8.4 (-)
% rye	16.6 (+)	3.8 (-)	14.6 (-)	0.3 (+)
% spinach	2.4 (-)	0.1 (+)	6.0 (+)	0.1 (+)
% sudan grass	6.9 (+)	7.1 (+)	4.0 (-)	2.5 (-)
% wheat	NA	NA	3.3 (+)	7.9 (-)
% sorghum	18.0 (+)	2.4 (-)	16.2 (-)	0.2 (+)
% triticale	0.4 (-)	4.1 (+)	1.4 (+)	3.9 (-)

"NA" indicates absence of crop species surrounding sites or an absence of measurements for that variable

Plus and minus signs within parentheses indicate the direction of the variable's contribution to the principal component

Table 2.4: Results from linear regression of aphid species richness and Shannon Diversity Index of crops for 2020 (n=10) and 2021 (n=10).

	df	AICc	Estimate	P-value
2020				
Aphid Richness				
1 km	3	53.5	-3.7	0.08
2 km	3	55.6	-3.0	0.23
3 km	*3*	*51.5*	*-6.1*	*0.03*
2021				
Aphid Richness				
1 km	3	58.0	-3.6	0.38
2 km	3	54.2	-6.2	0.06
3 km	*3*	*51.5*	*-5.4*	*0.02*

Italicized values indicate a significant association ($P < 0.05$)

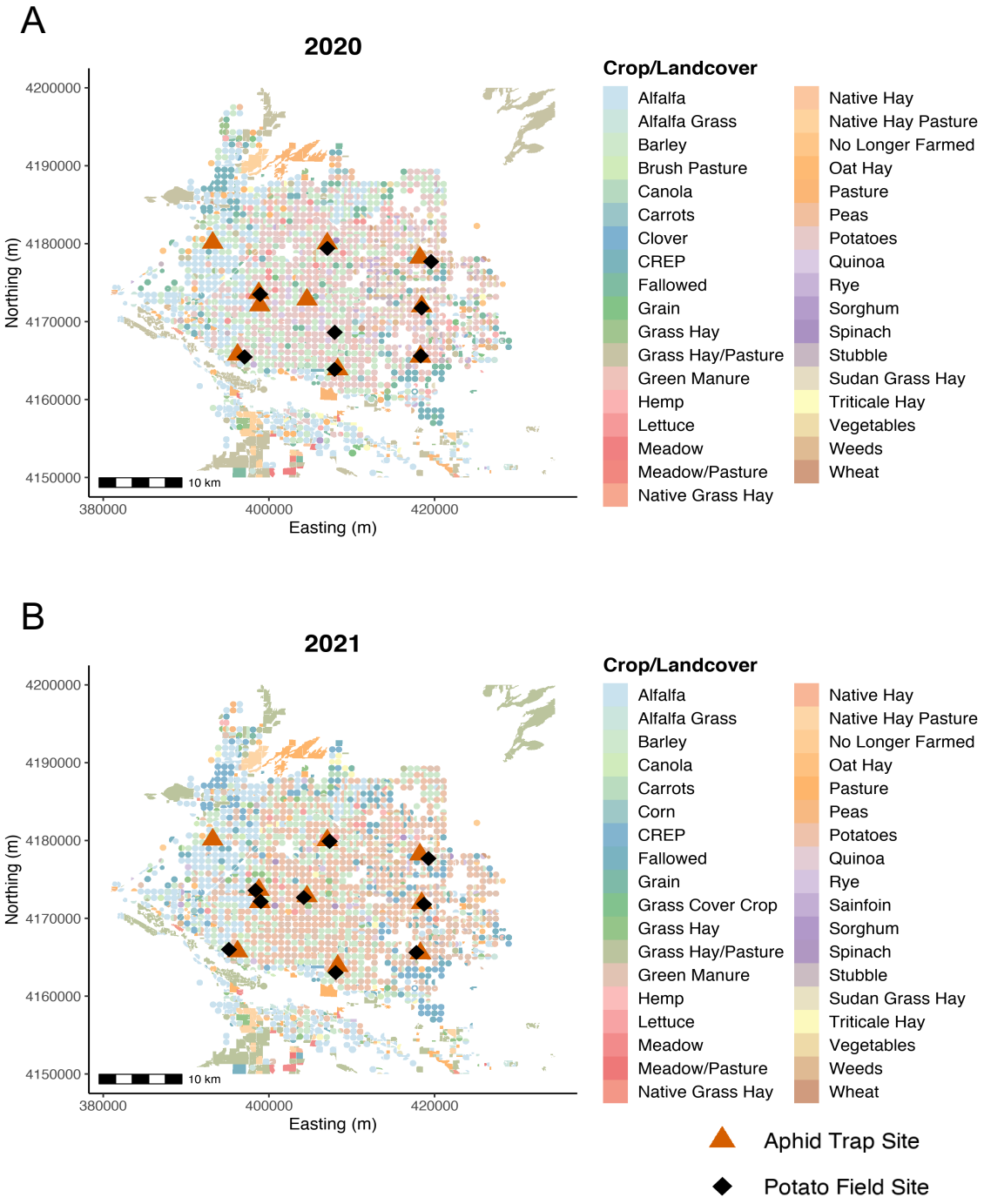

Figure 2.1: Study sites from 2020 (**A**) and 2021 (**B**), with different landcover types represented as different colors.

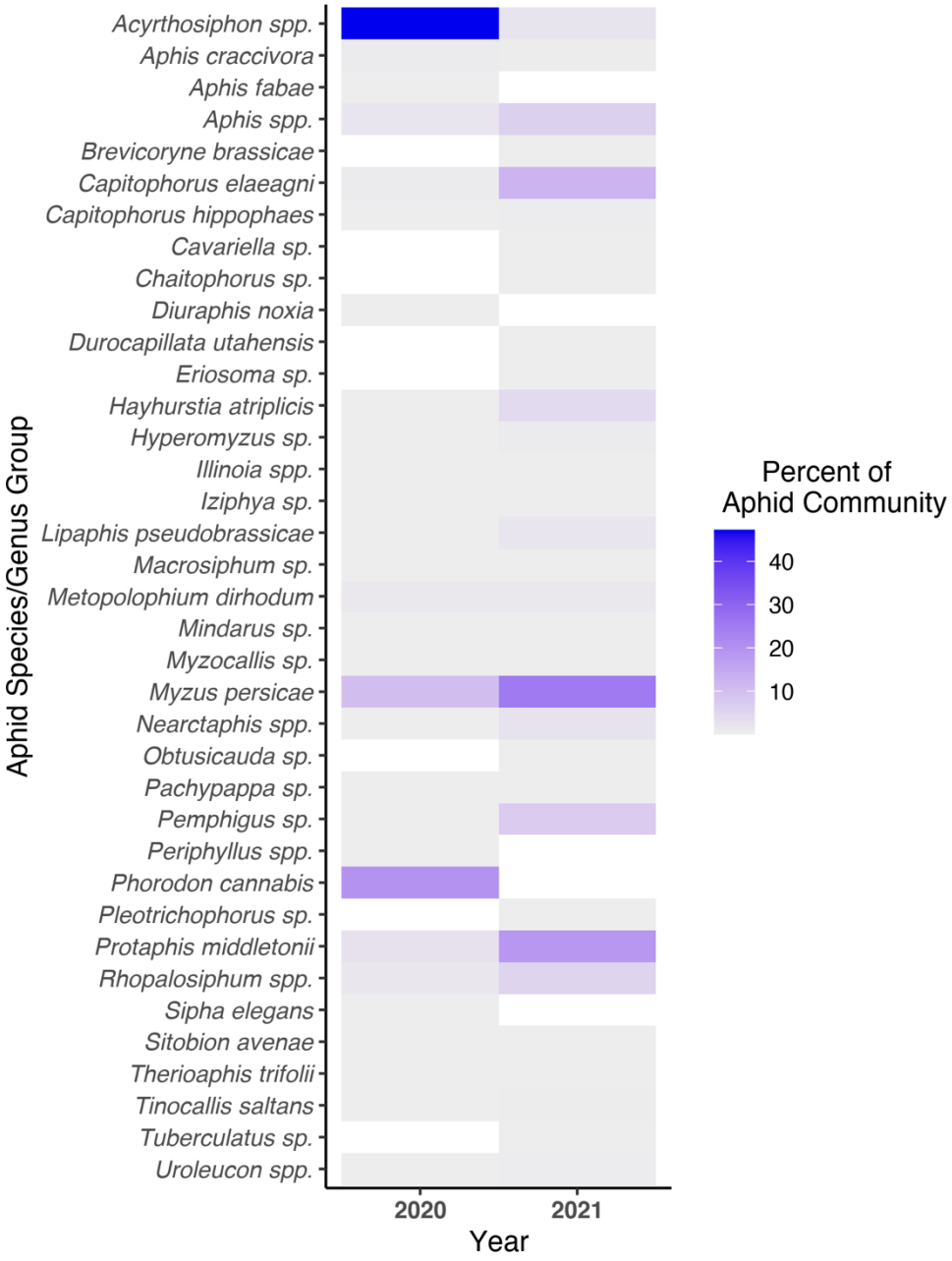

Figure 2.2: Relative abundance of aphid communities from 2020 and 2021. A total of 15,114 aphids from 2020 and 2,962 aphids from 2021 were identified.

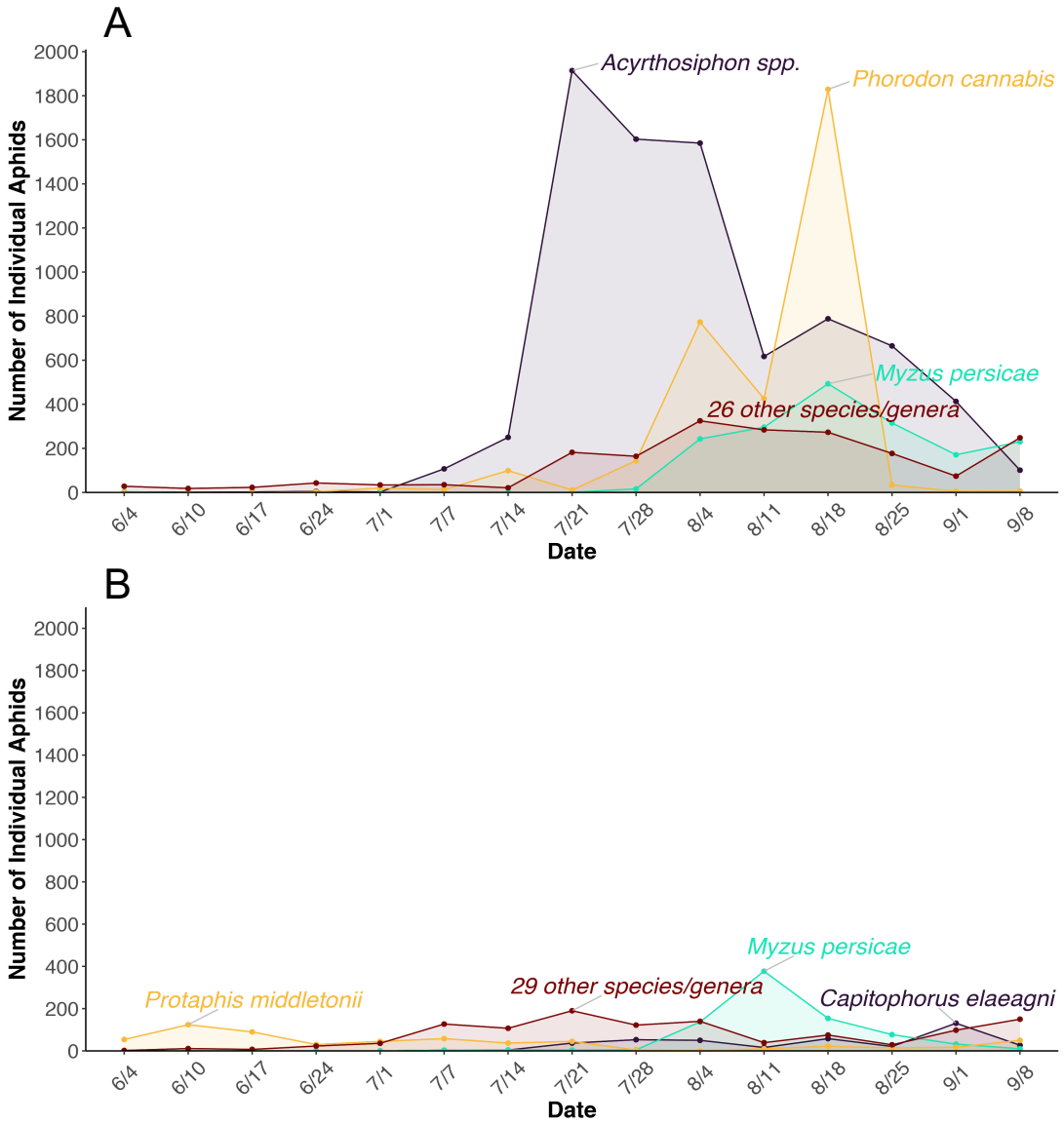

Figure 2.3: Aphid populations by week from (**A**) May 28th – September 8th, 2020, and (**B**) May 27th – September 8th, 2021. Dates indicate aphid populations collected during the previous week. Aphids which were not able to be identified are excluded from the graph.

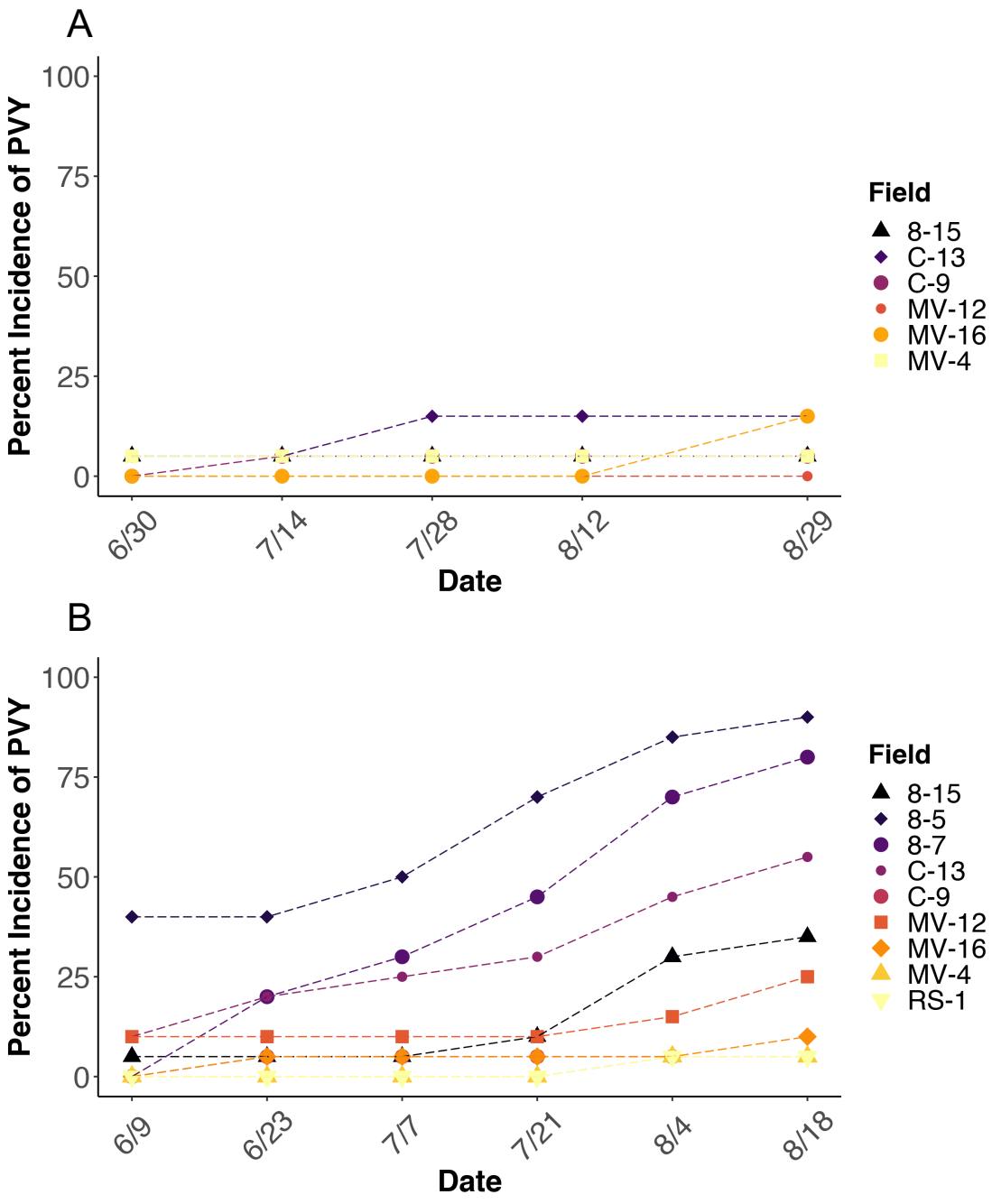

Figure 2.4: Biweekly percent incidence of PVY at each field from June 30th to August 29th, 2020 (**A**) and from June 9th to August 18th, 2021 (**B**).

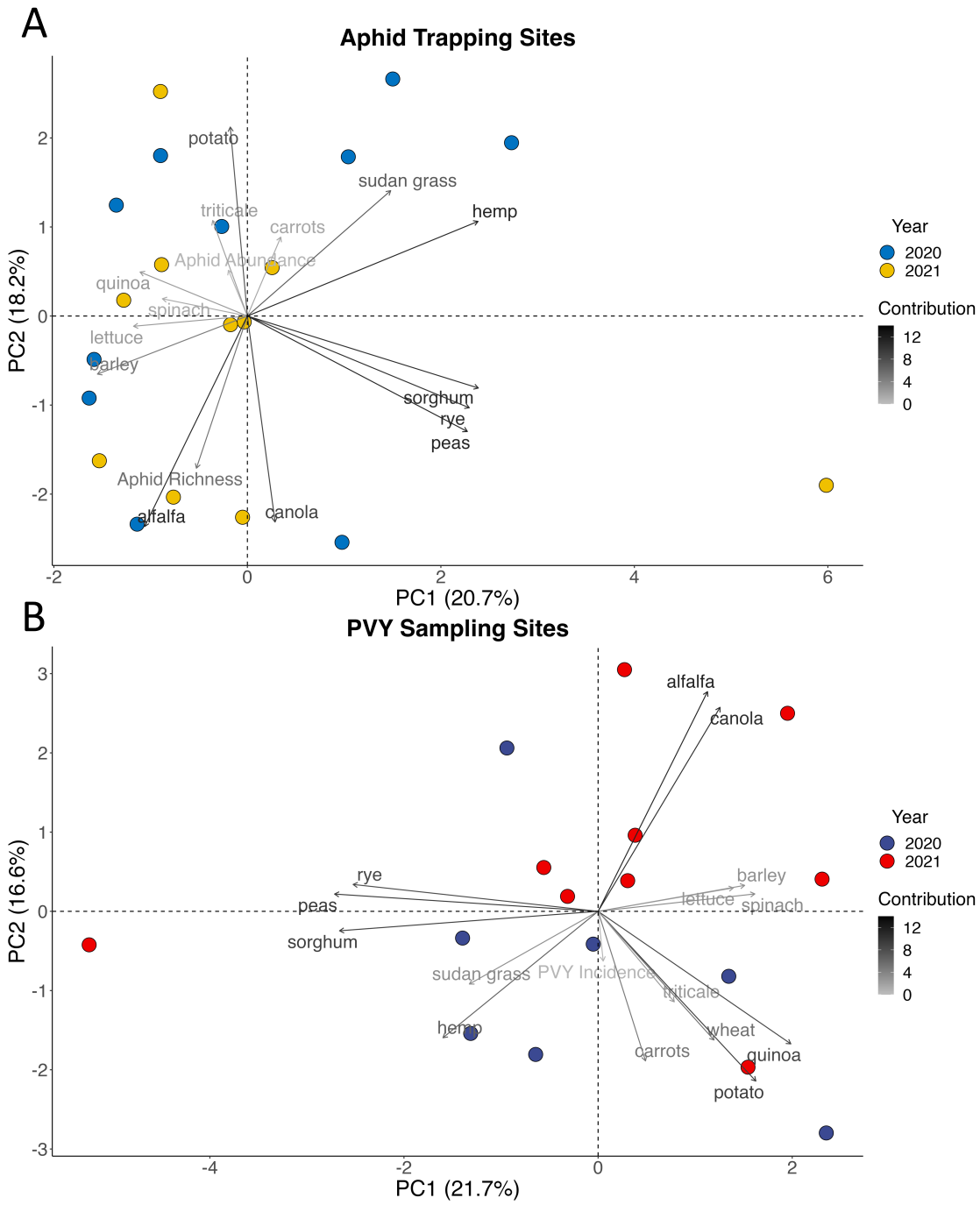

Figure 2.5: Principal component analysis (PCA) biplots of percent cover of crop species within a 3 km buffer surrounding sites, in addition to aphid richness and abundance (**A**) and PVY incidence (**B**). Data points represent each aphid trapping site (**A**) or PVY sampling site (**B**) from 2020 and 2021. Arrows indicate the strength and direction of variable contributions to each principal component. Values in parentheses on each axis depict the total explained variance of the respective principal component.

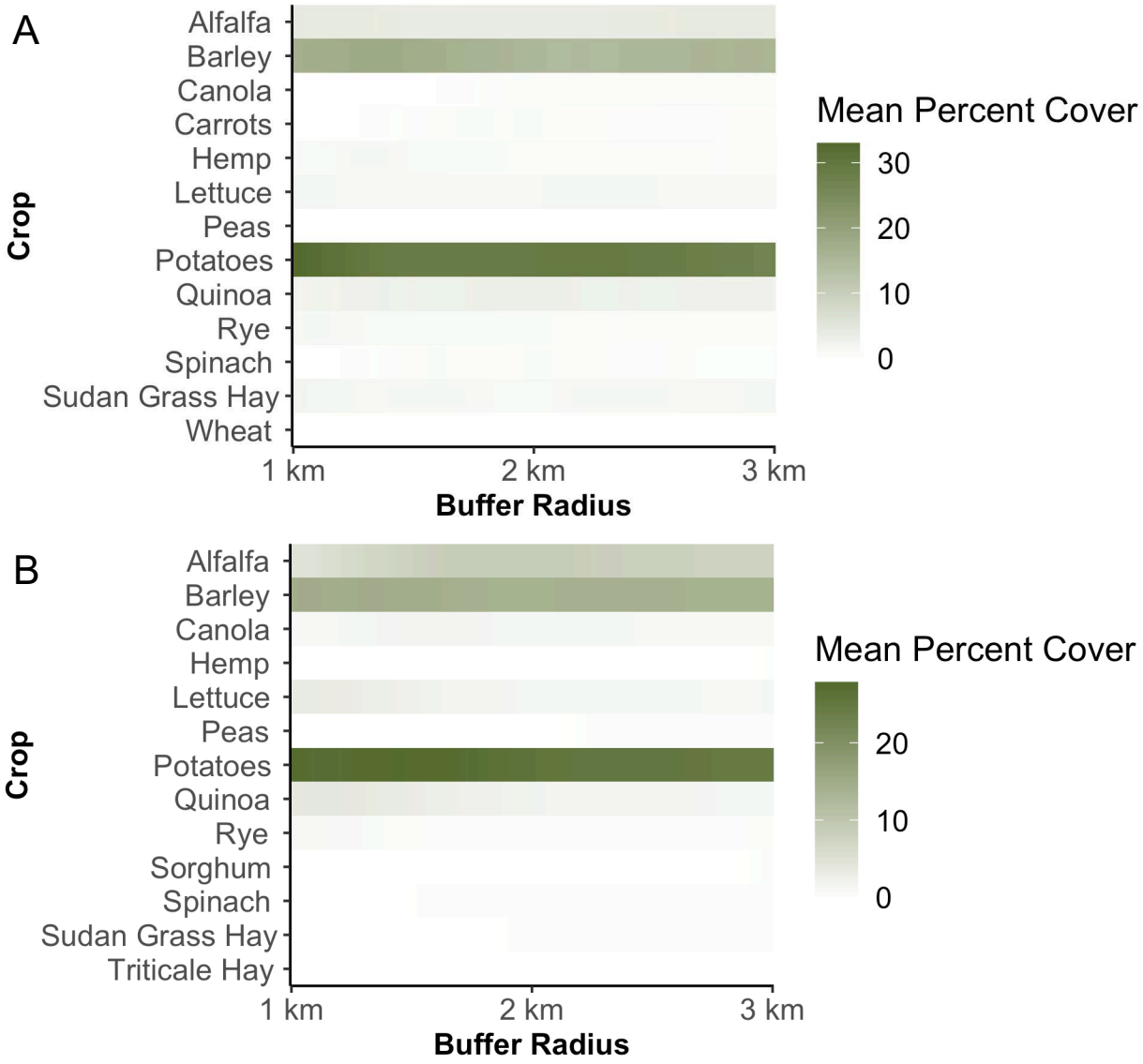

Figure 2.6: Mean percent cover of crop species surrounding potato fields tested for PVY from 2020 (**A**; n=8) and 2021 (**B**; n=9). Percent cover was calculated within spatial buffers ranging from 1 km to 3 km by increments of 10 m (i.e., 1000 m, 1010 m, 1020 m, … 3000m). Crops with no visible percent cover were present within the landscape of at least one field but mean percent cover from all sites was too low to appear on the graph. Only percent cover of crops within spatial buffers of 1 km, 2 km, and 3 km were used for statistical analyses.

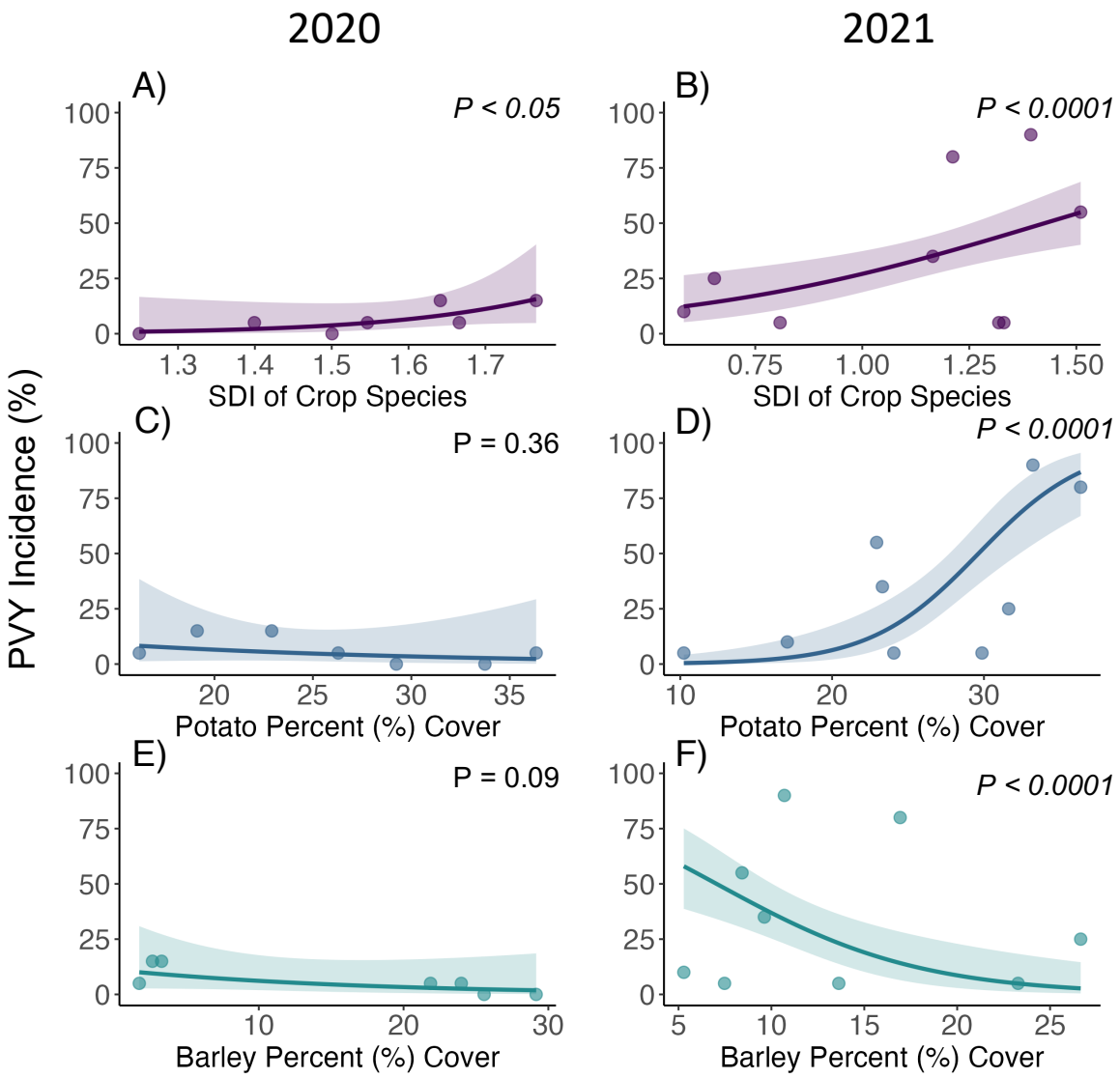

Figure 2.7: Effects plots of logistic regression models of end-of-season PVY incidence and **A)** Shannon diversity index (SDI) of crop species from 2020, **B)** SDI of crop species from 2021, **C)** percent cover of potato from 2020, **D)** percent cover of potato from 2021, **E)** percent cover of barley from 2020, **F)** percent cover of barley from 2021. Landscape data are displayed for 3 km buffers for 2020 and 2 km buffers for 2021, as models with these buffer sizes had the lowest AICc values. Each point displays total PVY incidence (from each of the 20 sampled plants) and landscape values for each site (n=7 for 2020, and n=9 for 2021). P-values are displayed in the upper right corner of each panel. Lines represent the slope and shaded areas represent the 95% confidence from logistic regression.

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CHAPTER 3: MOLECULAR GUT CONTENT ANALYSIS REVEALS PATTERNS OF PLANT-APHID VECTOR ASSOCIATIONS RELEVANT FOR POTATO VIRUS Y EPIDEMIOLOGY

Synopsis

Potato Virus Y (PVY) is an important pathogen of potato and other crops that is transmitted in a non-persistent manner by a diversity of aphid vectors. Winged aphids (alatae) disperse across the landscape to colonize new hosts and will often probe upon a wide variety of non-host plant species. We have a limited understanding of the role in PVY epidemiology by various dispersing aphid species and non-crop plants hosts, in part because of incomplete knowledge of the dietary breadth of dispersing aphid vectors. Here, I use high throughput molecular gut content analysis to characterize plant-aphid vector associations within a major potato producing region, the San Luis Valley, CO, where aphid-transmitted PVY is an issue for potato production. Aphids were trapped weekly with suction traps during the growing seasons of 2020 and 2021. Plant-specific DNA in 200 individual aphids representing 9 vector species of PVY (*Acyrthosiphon kondoi*, *A. pisum*, *Capitophorus elaeagni*, *Diuraphis noxia*, *Hayhurstia atriplicis*, *Myzus persicae*, *Phorodon cannabis*, *Protaphis middletonii*, *and Rhopalosiphum padi*) were amplified by PCR, sequenced with the PacBio sequencing platform, and sequences were identified to genus using NCBI BLASTn. I found that all aphid vector species probed on plant genera that are outside of their reported host ranges, with *Solanum*, *Medicago*, *Populus*, *Brassic*a, and *Glycine* as the most frequently detected plant genera. Moreover, aphids probed on many suspected PVY host plants. Specifically, the aphid vector *Capitophorus elaeagni* probed

upon several weed genera that are presumed PVY host plants. These findings should be considered during the development of PVY management strategies.

Introduction

Aphids are phloem-feeding insects that cause significant crop losses worldwide by directly feeding on host plants and indirectly by transmitting plant viruses. Most aphid species are highly host-specific and are able to reproduce and complete their life cycle on only one or a few plant genera. Other aphid species, such as *Myzus persicae*, the green peach aphid, has a broad host range (Blackman and Eastop 2008). When alate (i.e., winged) aphids disperse to find a new host plant, most individuals are not successful (Ward et al. 1998) but individuals may still probe potential hosts as a critical step in the host selection process (Powell et al. 2006). This exploratory probing by aphids lasts a short amount of time if a plant is not a suitable host for the aphid (Powell et al. 2006), but is still sufficient for transmission or acquisition of non-persistent plant viruses. Indeed, non-persistent viruses are transmitted by aphids during brief punctures of epidermal cells, which occur before an aphid has reached the phloem sieve elements to continuously feed (Martin et al. 1997). Because of the clear implications for disease epidemiology, plant-aphid vector interactions within landscapes have received considerable research attention (e.g., Kennedy 1950; Blackman and Eastop 2000; 2008; Powell et al. 2006; Dixon 2012; Döring 2014).

Identifying plants that insect vectors frequently feed upon can be useful to infer vector movement with the landscape (Cooper et al. 2019). Gut content analysis (GCA), using high throughput sequencing, can identify plant DNA present within an insect, thereby reconstructing the insect's dietary history. This is in contrast to GCA using Sanger Sequencing, which cannot detect more than one plant DNA sequence at a time. High throughput GCA has been used to

reconstruct the dietary history of psyllids (Cooper et al. 2019; 2023; Barthel et al. 2020; Reyes-Corral et al. 2021a; 2021b), leafhoppers (Cooper et al. 2022), and the spotted lanternfly (Cooper et al. 2022). Taken together, two key findings have emerged from these studies: 1) insects probed many plants outside their reported host range; 2) insects moved between crop and non-crop habitats. For example, the highly host specific psyllid, *Aphalara loca*, fed upon a wide range of plant families that are outside of the insect's reported host range (Cooper et al. 2019). Moreover, individual tomato/potato psyllid, *Bactericera cockerelli* vectors of *Candidatus* Liberibacter solanacearum (Lso) that causes zebra chip disease of potato, were found to move between potato and non-crop plants within the family Asteraceae (Reyes-Corral et al. 2021b). While high throughput molecular GCA has not yet been used with aphids, Edwards (1965) attempted to identify previous host plants of aphids by identifying amino acids, sugars, and phenolic compounds that were present within aphid bodies, but did not have success with this technique. Vialatte et al. (2006) and Gilabert et al. (2017) took a different methodological approach, and used stable-isotope ratios to identify crop species contained within aphids and found that cereal aphid pest populations in wheat had originated from corn, which is a suitable host plant for these aphids (Blackman and Eastop 2008). Lastly, Matheson et al. (2008) was the first to use Sanger sequencing GCA to show that plant DNA could be recovered from aphids (*Myzus persicae*) and used to identify the plant that an aphid had previously fed upon. These findings suggest that insects often move throughout landscapes to feed on various plant species, and that molecular GCA is a valuable tool with which to study plant-insect vector associations.

Molecular GCA can also help identify potential sources of pathogen inoculum for vectors to acquire and transmit to crop plants. While there may be many capable pathogen host plants within a landscape, virus reservoirs can be identified by determining whether insect vectors are

feeding upon them. For example, Cooper et al. (2023) showed that *Lycium carolinianum*, a native solanaceous plant in potato growing regions of Texas, was capable of sustaining Lso infection, and that the psyllid vector, *B. cockerelli*, frequently fed upon *Lycium* and potato within the landscape. Identifying pathogen host plants that insect vectors are feeding on within a landscape can provide information that is essential to develop sustainable and effective management strategies.

Aphids transmit one of the most economically important plant viruses, potato virus Y (PVY) (Scholthof et al. 2011). Potato virus Y has a wide host range (Kerlan 2006; Karasev and Gray 2013) and is transmitted by ~ 65 species of aphid vectors including potato colonizers that can reproduce and complete their life-cycle on potato, like *M. persicae*, and potato non-colonizers (Pelletier et al. 2012). Studies have shown that non-colonizing aphids are most important for PVY prevalence (DiFonzo et al. 1997; Steinger et al. 2015; Mondal et al. 2016; Galimberti et al. 2020) because these aphids can feed on various plants within a landscape, thereby amplifying populations of non-colonizing vectors and PVY spread. While it is known that aphids frequently probe upon various plants within a landscape (Döring 2014), aphid movement within a landscape and consequences for disease spread is not clear.

In the current study, I used high throughput molecular GCA to identify the dietary history of alate aphid vectors to infer plant species with potential consequences for disease spread. My specific objectives were to 1) determine plants (PVY hosts and non-hosts) probed by aphid vectors, and 2) determine if certain vector species have a higher propensity to probe plants than other vectors. I hypothesized that 1) vectors will probe many weed plants that are PVY hosts, in addition to many PVY non-host plants that are outside of their reported host range and 2) the polyphagous vector species, *M. persicae*, will have a higher propensity to probe multiple PVY

host plants. Identifying patterns of dietary history of aphid vectors within a landscape will provide insight into plant-aphid vector associations that are relevant for PVY epidemiology.

Methods

Study area

A field study was conducted in the San Luis Valley (SLV), CO, USA, which consisted of collecting aphids using suction traps (one in 2020 and four in 2021) located next to potato fields at multiple sites from June 17th to September 22nd, 2020 and from May 27th to September 8th, 2021 (**Table 3.1 and Supp. Fig. A2.1**). The SLV is a major potato producing region that is relatively arid, is at high elevation (~2300 m), and is composed of agricultural, desert, wetland, and forested landscapes.

Aphid collection and identification

The suction traps were constructed out of ~ 20.3 cm diameter PVC pipe, but two different heights of traps were used. Trap A (**Table 3.1 and Supp. Fig. A2.1**), was the only trap used in 2020 but was also used in 2021, was ~ 2.3 m tall and followed the design of Alison of Pike (1988) and Zhu et al. (2006). Traps B, C, and D (**Table 3.1 and Supp. Fig. A2.1**), which were only used in 2021, were ~ 1 m tall, but followed a similar design as Trap A, in that a fan was at the bottom of each trap that forced air downwards, creating a suction of air at the top of the trap, causing insects flying by to be forced downwards into a jar filled with approximately 50% propylene glycol contained in the middle of the PVC frame. Trap B was painted olive green on the outside of the PVC frame, while Traps A, C and D were white. The fan in each trap was powered by using a solar panel which was also connected to a car battery to provide a backup source of power for days with little available sunlight. Occasionally, the fans in traps malfunctioned but were fixed in ≤ two weeks when this occurred. Insects were collected weekly

and were transported to the laboratory for sorting and identification of aphids. Aphids were transferred to a 70% ethanol solution and were identified using taxonomic keys and descriptions of species morphology (Blackman and Eastop 2000; 2008; Pike et al. 2003). Immediately after identification, aphids of the same species, collection date, and location were placed in empty 2mL Eppendorf tubes, in groups of up to 10 individuals, and were stored in a -80°C freezer until extraction of DNA.

DNA extraction

DNA was extracted from individuals representing nine of the most abundant aphid species from 2020 and 2021 that had been confirmed vectors of PVY based on previous literature. The collection date at which trapped aphids were selected for DNA extraction were based on two criteria: 1) the time of peak abundance of a species during the season and 2) the weeks leading up to increases in PVY incidence detected in our study described within Chapter 2, which was conducted simultaneously in the same study region. Before beginning DNA extractions, each specimen was submersed in 70% ethanol for at least 2 minutes, submersed in sterile deionized water for 5 seconds, and individually placed in separate 1.7 mL Eppendorf tubes. Then, 180 uL of phosphate buffer saline (PBS) was added to each tube, and individual aphids were homogenized using a micropestle. Genomic DNA was extracted using a DNeasy® Blood and Tissue Kit, following the protocol specific to insects (Qiagen®, Germantown, MD, USA). Quantity and quality of DNA was assessed using a Nanodrop™ spectrophotometer (Thermo Scientific™, Waltham, MA, USA).

Targeted single molecule real-time (SMRT) sequencing

Polymerase chain reaction (PCR) was used to amplify the *trnF* region of the chloroplast and internal transcribed spacer 2 (ITS2) region, similar to Cooper et al. (2019; 2022). Universal

primers pairs were selected for the *trnF* region (B49873-e: GGTTCAAGTCCCTCTATCCC; A50272-f: ATTTGAACTGGTGACACGAG; Taberlet et al. 1991) and the ITS2 region (ITS2F: ATGCGATACTTGGTGTGAAT; ITS3R: GACGCTTCTCCAGACTACAAT; Chen et al. 2010). As PCR products from different samples would be pooled prior to sequencing, asymmetric barcodes were added to universal forward and reverse primers for each sample in order to create a unique combination of primer pairs for each sample (Pacific Biosciences 2014). Separate PCR assays were performed to amplify the trnF region and ITS2 region using 5µL of DNA from each sample and 5 µM of forward and reverse primers, with a total reaction volume of 50 µL per sample. Cycling conditions for trnF amplification were as follows: 10 min incubation at 94°C, 40 cycles of 30 s denaturation at 94°C, 30 s annealing at 58°C, 45 s extension at 72°C, followed by a final incubation of 7 min at 72°C. Cycling conditions for ITS2 amplification were as follows: 5 min incubation at 94°C, 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 56°C, 45 s extension at 72°C, followed by a final incubation of 5 min at 72°C. Ten µL of PCR products of 400 – 600 bp were visualized on 1% agarose gels stained with ethidium bromide, and the remaining products were purified using the QIAquick® PCR purification kit (Qiagen®, Germantown, MD, USA). Purified PCR products from each sample were pooled; the amount of product used for sequencing was dependent on the visibility of the band, similar to Cooper et al. (2019; 2022; 2023). The volume of purified PCR product used for sequencing was 30 µL if no band was visible, 20 µL if a faint band was observed, 10 µL if a band was clearly visible, and 5 µL if an exceptionally bright band was observed. To monitor contamination and PCR efficiency, a non-template control along with three positive controls consisting of two aphid species and one psyllid species collected directly from their host plants were included with each unique barcoded sample.

Sequencing was conducted in a similar manner to Cooper et al. (2019; 2022; 2023) and Reyes-Corral et al. (2021a; 2021b). Pooled PCR products were shipped to the Washington State University's Laboratory of Biotechnology and Bioanalysis and were sequenced using the Pacific Biosciences® (PacBio) Sequel platform. PCR products were concentrated via vacuum centrifugation and AMPure XP beads (Beckman Coulter Inc. Brea. Ca. USA). Amplicons were ligated to hairpin SMRT bell adapters using the PacBio Express Template kit v 2.0, and libraries were purified using AMPureXP beads (Beckman Coulter Inc., Brea, CA, USA). Purified libraries were quantified, bound to a polymerase, loaded into Sequel 1M v3 SMRT cells, and observed for 10 h on a PacBio Sequel (Pacific Biosciences®). Then, raw movies were processed into reads, reads processed into high quality reads, and barcodes were de-multiplexed using SMRT Link version 8.0. Average read length was 24.6 Kb with average single molecule coverage of 33x. Phred scores ranged from 35 to 45, indicating the data were highly accurate.

Sequence analysis

Sequence analysis was performed using the Geneious Prime® software (version 2023.1.2). Only sequences between 400 – 700 bp in size were analyzed. The de novo assembly tool was used to identify consensus sequences using custom sensitivity settings of minimum overlap identity of 95% and 2% maximum mismatches per read. Consensus sequences were designated as operational taxonomic units (OTUs) and analyzed using the basic local alignment search tool (BLASTn) (Altschul et al. 1990) to compare sequences to known sequences deposited in NCBI GenBank, enabling an assignment of plant taxa to OTUs. Taxonomic classifications were reported to genus level to increase accuracy of reported plant taxa. In order to account for low quality reads and low amounts of contamination, a plant was considered present in a sample if ≥ 6 reads were detected, as in Cooper et al. (2022). As it is not yet known

if a greater number of plant DNA reads is caused by increased probing of that plant, detection of plant genera was analyzed as a binary presence/absence in samples. A high number of reads identified as *Cannabis* and *Beta* were detected in most samples, indicating likely contamination because these plants were housed in our laboratory where the extractions were performed, so those genera were removed from the analysis. Additionally, contamination of various genera were detected in control samples that were not exposed to those plants. In those cases, the mean plus three standard deviations of the number of reads in contaminated samples was used as a threshold for detection of plant genera in field-collected aphid samples. Non-target sequences, including invertebrate, bacteria, or different regions of plant DNA were discarded. Following sequence analysis and plant identification, data processing, data analysis, and figure generation were conducted in RStudio using R software version 4.1.1 (R Core Team 2022) using the package 'tidyverse' (Wickham et al. 2019). In order to identify PVY host plants probed upon by aphids, a literature search was conducted to determine if plant species within the detected genera have been reported to be capable of becoming infected with PVY. If a published report of a plant species tested positive for PVY, then that genus was treated as a presumed PVY host; if a plant genus had tested negative for PVY or if no published reports were able to be found for the ability of that genus to sustain PVY infection, then that genus was presumed to be a PVY non-host (**Table 3.2**).

Results

Sequencing

Plant DNA was detected in 192 out of 200 aphid samples. A total of 354,192 sequences were detected within aphid vectors using trnF and ITS2 primers; the mean E value was 2.15E-104 and mean percent query coverage was 98.01% (**Table 3.3**). After removing contamination, a

total of 72 aphids contained ≥ 6 reads of plant DNA (**Table 3.1; Supp. Table A2.1**), representing 48 different plant genera (**Fig. 3.1**).

Presumed PVY host plants probed upon by aphid vectors

Presumed PVY host plants were probed upon by 34.7% of vectors. The presumed PVY host plants most frequently probed upon by aphid vectors were *Solanum*, *Brassica*, and *Lolium* (**Fig. 3.2**). *Solanum* was detected in the potato-colonizing vector *M. persicae*, in addition to the non-colonizing vectors *D. noxia, P. cannabis,* and *R. padi* (**Fig. 3.2**). Additionally, *C. elaeagni* probed upon multiple weed genera that are presumed PVY host plants, including *Erodium, Brassica, Convolvulus, Plantago, Raphanus, Rumex,* and *Sonchus* (**Fig. 3.2**). Patterns of plant DNA detected within aphid vectors were mostly different between 2020 and 2021 (**Supp. Fig. A2.2**). However, *Solanum* was detected within *D. noxia* and *Acer* and *Populus* within *R. padi* during both years (**Supp. Fig. A2.2**).

PVY non-host plants probed upon by aphid vectors

The PVY non-host genera most frequently detected in aphid vectors were *Medicago* (e.g., alfalfa), *Populus* (e.g., poplar trees), and *Glycine* (e.g., soybean) (**Fig. 3.2**). In addition to *Populus*, 14 other tree genera were detected in aphid vectors, notably *Acer* (maple trees) and *Ulmus* (elm trees) (**Fig. 3.2**). Additionally, several grass genera were detected in aphid vectors, including *Aegilops*, *Secale*, *Triticum*, and *Zea* (**Fig. 3.2**).

Vector species with propensity to probe multiple plants

Aphid vectors probed an average of 1.31 plants (n=72), with relatively similar mean number of plant genera probed upon between aphid species (**Fig. 3.3**). Nineteen percent of aphids probed upon more than one plant (**Supp. Table A2.1**). The vector species with the highest proportion of individuals which probed more than one plant were *A. kondoi* and *M. persicae*

(**Supp. Table A2.1**); however, differences between species were not tested for statistical significance. Of the vectors which probed multiple plants, two individual *D. noxia* and one individual *M. persicae* probed upon *Solanum*; but the other plants these aphids probed upon were presumed PVY non-host plants, notably *Medicago* (**Fig. 3.4**). Interestingly, there was one *C. elaeagni* individual that probed upon five different plants; four of which were presumed PVY host plants (**Fig. 3.4**).

Discussion

Gut content analysis is a useful tool to infer vector movement and identify pathogen reservoirs through the sequencing of plant DNA that is present within insects' guts. Here, I used high throughput molecular GCA to provide the first reconstruction of the dietary history of aphid vector populations within a landscape. I found that two of the most frequently detected presumed PVY host plants within vectors were *Solanum* and *Brassica*, two genera that represent important crop and/or weed species within the SLV (i.e., potato, hairy nightshade, canola, and field mustard). Moreover, I found that 34.7% of aphid vectors probed upon presumed PVY host plants within the landscape and that aphid vectors frequently probed upon plants that are outside of their reported host ranges. Of the four aphid vector species which probed upon *Solanum*, three species were non-colonizing vectors. Further, *C. elaeagni* probed upon several weed genera that are presumed PVY hosts, suggesting that these weeds could potentially be relevant for PVY epidemiology in the SLV. This study provides insights into patterns of aphid vector probing within a landscape, in addition to identifying potentially important PVY reservoirs.

Each aphid vector species probed plants which are outside of their reported host range (Blackman and Eastop 2008), and it is unlikely that many of the plants I detected within aphids are potential sources of vector populations. For instance, tree genera were detected in many

aphids which do not colonize these trees, suggesting that aphids sampled these trees and recognized them as non-hosts. Yet, it is important to note that an absence of information on the specific feeding behaviors during which aphids, and other insects, ingest detectable amounts of plant DNA hinders our ability to infer the specific nature of detected plant-aphid associations. It is not known if aphids ingest plant DNA during phloem feeding or other non-phloem probing behaviors. However, it seems likely that aphids were not feeding from the phloem and probably ingested plant DNA during non-phloem probing behaviors such as intercellular probing or intracellular punctures. During intracellular punctures, aphids can transmit non-persistent viruses like PVY to their non-host plants (Edwards 1963; Martin et al. 1997). Our finding that the dietary history of many aphid vector species consisted mostly of their non-host plants reinforces the concept that aphids disperse within a landscape and probe many non-host plants in search of a suitable host. Aphid dispersal is influenced by complex landscape and atmospheric effects (Pasek 1988; Fabre et al. 2005). Consequently, aphids often have little control of their direction during flight, and frequently alight on and probe non-host plants (Kennedy 1950; Dixon 2012). This type of host selection behavior is thought to be, in part, why non-colonizing aphids contribute to PVY spread (Kennedy 1950; Galimberti et al. 2020).

Two of the five most frequently detected plant genera within aphid vectors, *Solanum* and *Brassica*, are presumed hosts of PVY. *Solanum* was detected within three non-colonizing vectors, and the colonizing vector *M. persicae*. Interestingly, the non-colonizing vector *D. noxia*, which is a serious pest of wheat and barley (Brewer et al. 2019), probed upon *Solanum* during both years of study, suggesting that this aphid species is an overlooked vector of PVY in potato. While the *Solanum* detected in aphid vectors was likely potato, hairy nightshade (*S. sarrachoides* Sendtner) has also been implicated in aphid transmission of PVY (Cervantes 2008; Cervantes

and Alvarez 2010). Hairy nightshade occurs within the SLV (USDA, NRCS 2023; W.J. Pitt personal observation), and it is possible that vectors probed upon this weed and/or potato. In addition to *Solanum*, *Brassica* was also detected in several vector species. However, while canola (*B. napus*) has been reported to be infected with PVY (Korbecka-Glinka et al. 2021), I am not aware of any reports of canola or other *Brassica* being implicated in PVY prevalence within a landscape. But as canola is cultivated within the SLV (RGWCD 2021), this deserves further study. In addition to *Solanum* and *Brassica*, *Lolium* was detected within vectors. But given that PVY apparently does not infect many monocotyledonous plants (Brunt 1996), the report of *Lolium* functioning as a PVY host (Salm and Rey 1992) seems questionable.

Presumed PVY host plants were detected in many aphid vectors, particularly *C. elaeagni. Capitophorus elaeagni* host alternates between Russian Olive trees (*Elaeagnus* spp.) and various thistles (e.g., *Cirsium* spp.). While neither of these plant genera were detected, *C. elaeagni* probed upon many weed genera that are presumed PVY host plants, such as *Erodium, Rumex, and Sonchus*. The propensity of *C. elaeagni* to probe on presumed PVY host plants suggests that these weed genera, and *C. elaeagni,* could be relevant for PVY epidemiology within the SLV. Indeed, this conclusion is supported by the strong positive association between *C. elaeagni* abundance and PVY prevalence detected in Chapter 2. Moreover, *C. elaeagni* was implicated in PVY spread between potato fields in Idaho (Halbert et al. 2003). However, *Solanum* was not detected within *C. elaeagni* in our study.

It is important to note that the suction traps employed in this study are designed to capture dispersing aphids, because of the trap's height above the ground. Indeed, the patterns of plant-aphid associations I found are not entirely encompassing of each particular plant and aphid taxa association, as these associations are affected by many other factors, such as aphid morph

and seasonality. Additionally, while our suction traps were located next to potato fields, our sampling was not designed to systematically capture particular crop or weed genera as traps were placed in arbitrary locations within the study area. Another important consideration is that I removed *Cannabis* and *Beta* from analyses due to contamination, but it is likely that vectors, particularly *P. cannabis*, probed upon *Cannabis*. Further, it is not known how long plant DNA is retained in aphids after probing, and this affects interpretations of plant – aphid associations detected with GCA. Additionally, I did not test aphids for the presence of PVY. Conceivably, future research could identify plants which viruliferous aphid vectors probed upon by utilizing a nucleic acid extraction method (e.g., Gayral et al. 2011) to simultaneously extract RNA to test for PVY and DNA to conduct molecular GCA. An alternative method would be to extract RNA from aphid stylets to test for PVY, while utilizing aphid bodies for DNA extraction and molecular GCA. The use of GCA yields enormous potential to further understanding of host use patterns of aphid vectors that are relevant for virus epidemiology. Specifically, it could be possible to estimate risk of emerging aphid-vectored viruses that are introduced into agricultural systems by predicting patterns of plant – plant virus movement based on long-term datasets of host use patterns of aphid vector species.

Consistent with previous studies utilizing GCA, I found that aphid vectors frequently probed upon plants that are outside of their reported host ranges, providing an additional piece of evidence for the importance of non-colonizing vectors for PVY spread. Indeed, many of these genera are presumed to be PVY host plants. Further research should be conducted to assess the importance of these plants, particularly *Brassica* and *Erodium*, on PVY prevalence within landscapes. Moreover, I found evidence for the likely importance of *C. elaeagni* and *D. noxia* as

vectors of PVY, suggesting that weed host plants of these aphid species, in addition to weeds that are PVY host plants, should be considered when developing strategies for PVY management.

Tables and Figures

Table 3.1: Collection date and location information for aphid vectors that were trapped and sequenced.

Year	Trap ID	Collection Date	Number of aphids sequenced	Number of aphids that probed
2020	A	6/17 - 6/23	1	1
		6/23 - 6/30	1	0
		7/21 - 7/28	10	4
		7/28 - 8/4	13	3
		8/4 - 8/12	79	30
		8/12 - 8/18	5	2
2021	A	6/30 - 7/7	1	0
		7/21 - 7/28	17	7
		7/28 - 8/3	9	4
		8/3 - 8/12	2	1
		8/12 - 8/18	10	2
	B	6/16 - 6/23	1	1
		6/23 - 6/30	1	1
		6/30 - 7/7	1	0
		7/7 - 7/14	2	0
		7/14 - 7/21	11	3
		7/21 - 7/29	9	4
		7/28 - 8/3	5	3
		8/3 - 8/12	5	2
	C	8/11 - 8/18	16	4
	D	7/21 - 7/28	1	0
Total			**200**	**72**

Table 3.2: Presumed host and non-host genera of PVY.

Plant Genus	Presumed PVY Host	Reference
Achillea	+	Korbecka-Glinka et al. (2021)
Brassica	+	Korbecka-Glinka et al. (2021)
Convolvulus	+	Chatzivassiliou et al. (2004)
Crepis	+	Korbecka-Glinka et al. (2021)
Daucus	+	Crowe et al. (1988)
Erodium	+	Kaliciak and Syller (2009)
Helianthus	+	Chod et al. (1990)
Lolium	+	Salm and Rey (1992)
Picea	+	Cooper and Jones (2006)
Plantago	+	Chatzivassiliou et al. (2004)
Raphanus	+	Machado-Assefh et al. (2023)
Rumex	+	Crowe et al. (1988)
Sambucus	+	Chatzivassiliou et al. (2004)
Setaria	+	Korbecka-Glinka et al. (2021)
Solanum	+	Smith (1931)
Sonchus	+	Machado-Assefh et al. (2023)
Acer, Aegilops, *Allium, Aucuba*, *Carthamus*, *Celtis*, *Cicer*, *Citrullus*, *Citrus*, *Coriandrum*, *Cucumis*, *Descurainia*, *Ficus*, *Fraxinus*, *Glycine*, *Juglans*, *Kochia*, *Lonicera*, *Malus*, *Medicago*, *Morus*, *Pinus*, *Pistacia*, *Poa*, *Populus*, *Pyrus*, *Salix*, *Secale*, *Triticum*, *Ulmus*, *Vitis*, *Zea*	-	-

Table 3.3: Summary statistics of sequencing results obtained from NCBI BLASTn analysis using Geneious Prime®.

Number of sequence reads[a]	354,192
Number of OTUs[b]	17
E Value	
Min	0
Mean	2.15E-104
Max	2.68E-101
Mean Query Coverage	98.01

[a]After filtering out *Cannabis*, *Beta*, and samples with < 6 reads, a total of 11,671 reads remained.
[b]After filtering out *Cannabis*, *Beta*, and samples with < 6 reads, a total of 13 OTUs remained.

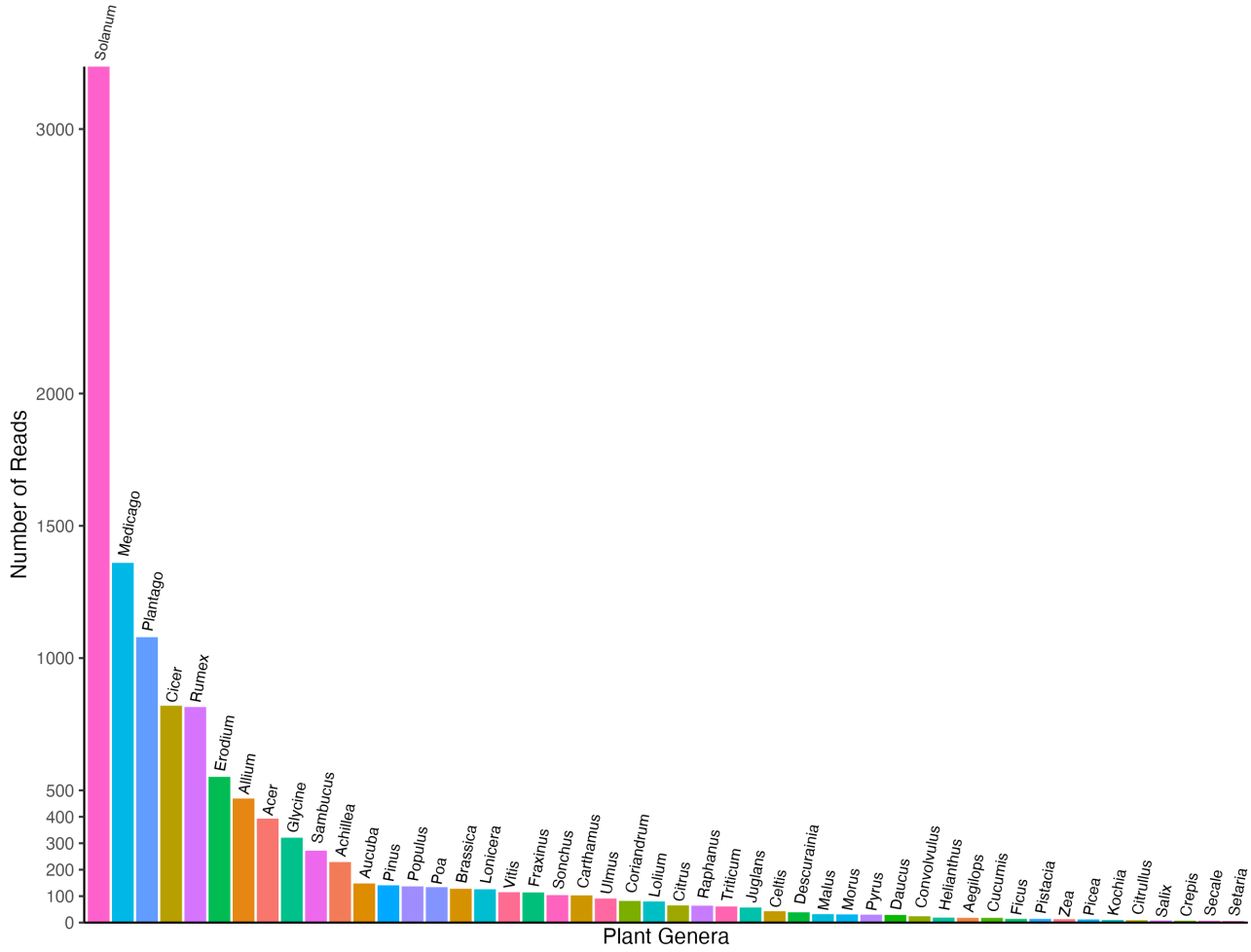

Figure 3.1: Total number of reads of DNA identified as plant genera across all individual aphids, after removing contamination.

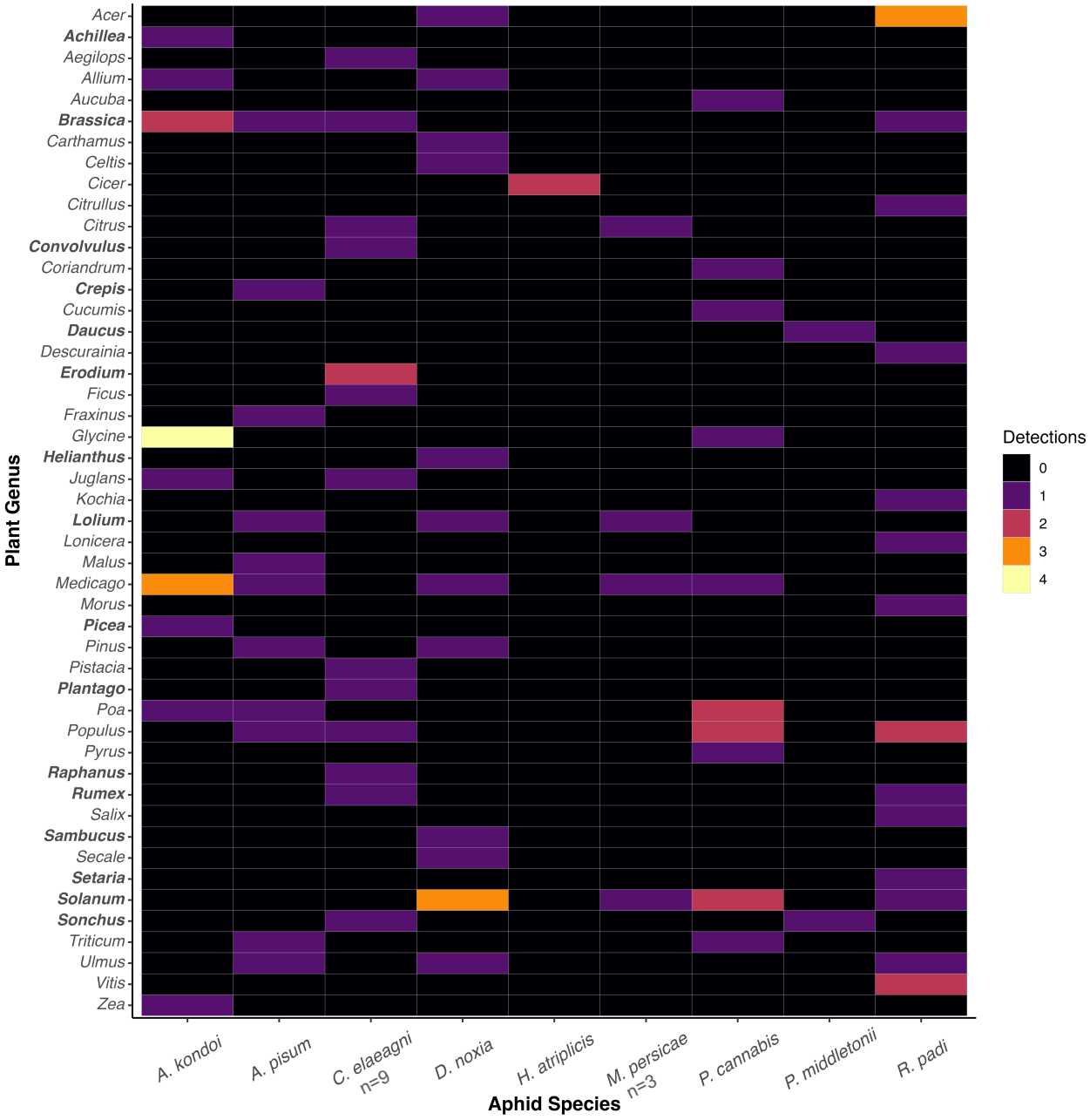

Figure 3.2: Heatmap displaying sum of binary detections of each plant genus for each aphid vector species. Sample size refers to the number of individual aphids that returned sequences. Plant genera that are bolded are presumed host plants of PVY (**Table 3.2**).

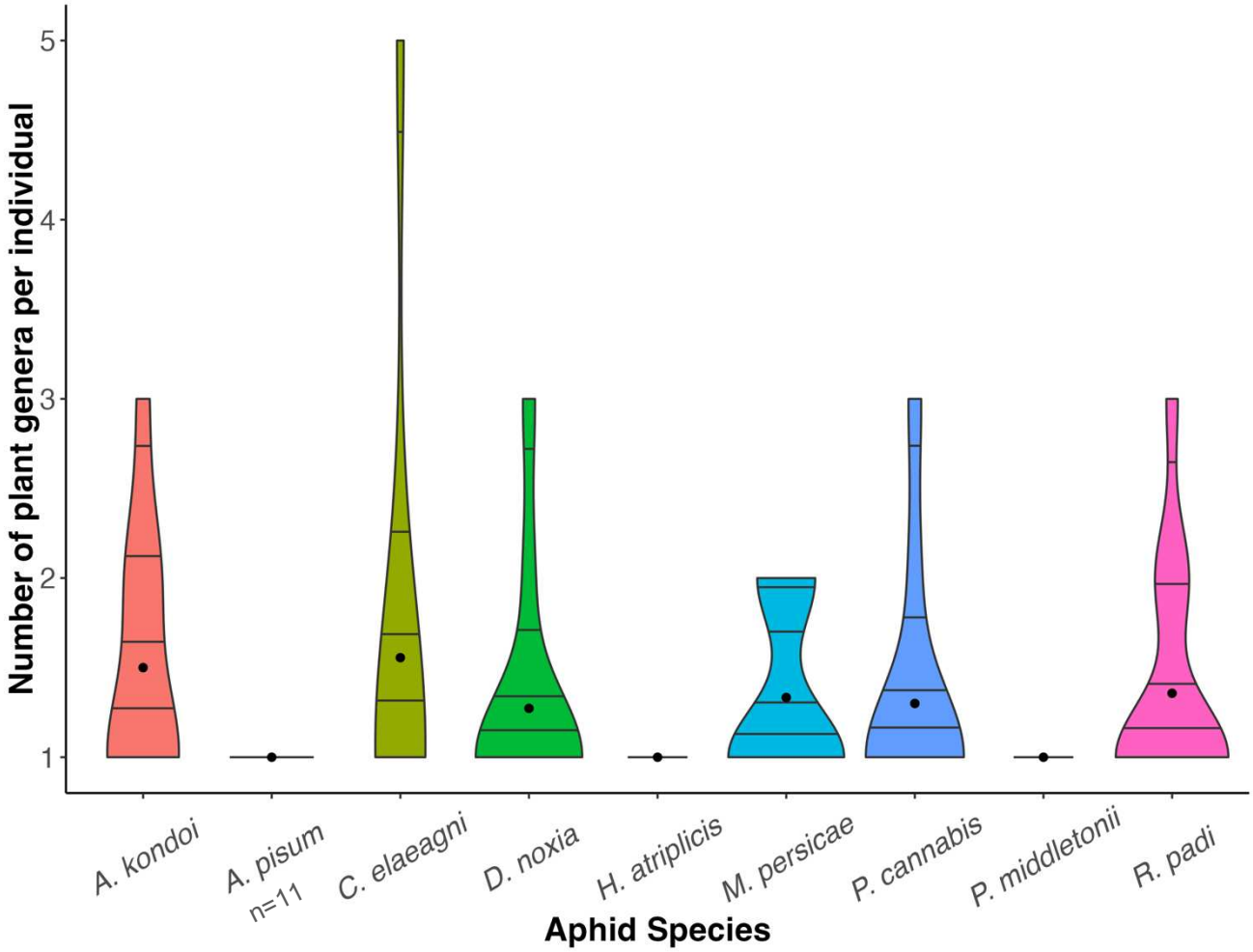

Figure 3.3: Violin plot displaying the number of plant genera probed upon by individual aphids for each species. Black dots indicate means and solid lines indicate quantiles.

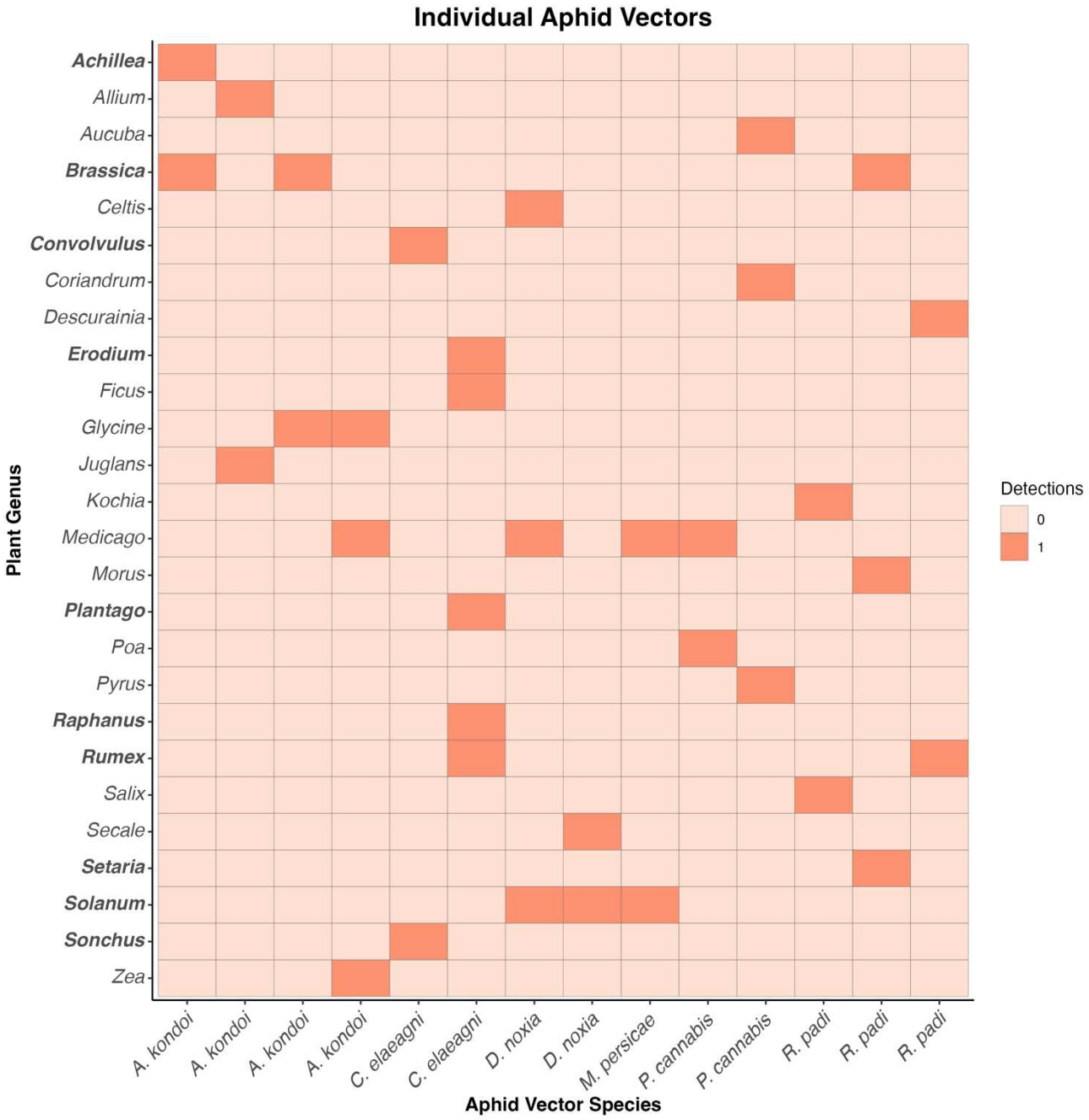

Figure 3.4: Heatmap displaying plant genera detected within individual aphid vectors, representing several species, that probed on more than one plant (n=14). Plant genera that are bolded are presumed host plants of PVY (**Table 3.2**).

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CHAPTER 4: VIRUS INFECTION AND HOST PLANT SUITABILITY AFFECT FEEDING BEHAVIORS OF CANNABIS APHID (HEMIPTERA: APHIDIDAE), A NEWLY DESCRIBED VECTOR OF POTATO VIRUS Y[1]

Synopsis

Aphids are the most prolific vectors of plant viruses resulting in significant yield losses to crops worldwide. Potato virus Y (PVY) is transmitted in a non-persistent manner by 65 species of aphids. With the increasing acreage of hemp (*Cannabis sativa* L.) (Rosales: Cannabaceae) in the United States, I was interested to know if the cannabis aphid (*Phorodon cannabis* Passerini) (Hemiptera: Aphididae) is a potential vector of PVY. Here, I conduct transmission assays and utilize electrical penetration graph (EPG) analysis to determine whether cannabis aphids can transmit PVY to hemp (host) and potato (non-host) (*Solanum tuberosum* L.) (Solanales: Solanaceace). I show for the first time that the cannabis aphid is an efficient vector of PVY to both hemp (63% transmission rate) and potato (19%) using individual aphids. During the initial 15 min of EPG recordings, aphids performed fewer intracellular punctures and spent less time performing intracellular punctures on potato compared to hemp, which may in part explain low virus transmission to potato using individual aphids. During the entire 8-hour recording, viruliferous aphids spent less time ingesting phloem compared to non-viruliferous aphids on hemp. This reduced host acceptance could potentially cause viruliferous aphids to disperse, thereby increasing virus transmission. Overall, this research shows that the cannabis aphid is an

[1]Published as: Pitt, W. J., Kairy, L., Villa, E., Nalam, V. J., & Nachappa, P. (2022). Virus infection and host plant suitability affect feeding behaviors of cannabis aphid (Hemiptera: Aphididae), a newly described vector of potato virus Y. *Environmental Entomology*. https://doi.org/10.1093/ee/nvac001

efficient vector of PVY, and that virus infection and host plant suitability affect feeding behaviors of the cannabis aphid in ways which may increase virus transmission.

Introduction

Aphids are herbivorous insects that are the most common vectors of plant viruses resulting in economic losses to crops globally (Ng and Falk 2006, Blackman and Eastop 2008). Vector-borne plant viruses are known to affect both the behavior and performance of insect vectors either directly by manipulating vector physiology (Ingwell et al. 2012) or indirectly by manipulating host plant physiology (Mauck et al. 2010, Eigenbrode et al. 2018, Nachappa et al. 2020). Changes in insect vector behavior and performance are thought to provide an advantage for virus dissemination and/or persistence within vector communities. Factors such as the mode of transmission can also influence virus effects on vector behavior (Mauck et al. 2012). Non-persistent viruses are acquired and released relatively quickly (seconds to minutes) from the vector. These viruses affect vector feeding at plant epidermal layers of plants, the sites of where non-persistent virus acquisition and inoculation occur (Carmo-Sousa et al. 2014). In contrast, persistent viruses, which take longer to acquire (minutes to hours) from the phloem and stay with the vector for its entire lifespan, have been shown to affect vector feeding within phloem sieve elements (Moreno-Delafuente et al. 2013). These contrasting manipulations are thought to be strategies developed by viruses through coevolution with vectors and host plants (Mauck et al. 2018, Chesnais et al. 2019).

Most aphids are highly host-specific, i.e., they reproduce on only one or a few species or genera of plants (Blackman and Eastop 2008). However, they are still able to settle and probe non-host plants during host plant selection and, in this process, aphids can vector non-persistent viruses to their non-host plants (Edwards 1963). Aphids often display different feeding behaviors

when feeding on non-host plants, and these differences can have implications for virus transmission. For instance, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae), which does not colonize potato (*Solanum tuberosum* L.) (Solanales: Solanaceace), spent less time probing and transmitted potato virus Y (PVY) less efficiently to potato than did *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Pelletier et al. 2008). Moreover, viruses can affect feeding behaviors of aphids on non-host plants. *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae), which also does not colonize potato, performed more intracellular punctures on PVY-infected potato plants compared to non-infected potato plants (Boquel et al. 2012). Hence, it is important to understand a vector's ability to transmit a virus to both host and non-host plants since non-host plants may serve as virus reservoirs, which can lead to a higher probability of virus incidence in crop plants.

Potato virus Y (genus *Potyvirus*, family *Potyviridae*) is one of the most economically damaging pathogens of potatoes in the U.S and worldwide (Karasev and Gray 2013). In 2021, approximately 943,000 acres of potatoes were planted in the US, with Idaho and Washington as the states with the highest acreage (National Agricultural Statistics Service 2021). Symptoms of PVY infection in potato can include leaf chlorosis, crinkling, and tuber necrosis, and vary depending on the virus strain, potato variety, environmental conditions, and whether the infection was tuber-borne or transmitted by aphids (Karasev and Gray 2013). Potatoes are at a very high risk for PVY infection and major yield reduction resulting from infection. For example, in 2014 the yield losses to PVY were estimated at roughly $34 million per year in the state of Idaho alone (McIntosh 2014). Other solanaceous crops (i.e. tobacco, peppers, tomatoes, eggplants) also have a high risk of infection and can experience serious yield loss (Karasev and Gray 2013). PVY is transmitted in a non-persistent manner (seconds to minutes) by approximately 65 different aphid species (Pelletier et al. 2012). The potato-colonizing green

peach aphid is widely considered as the most efficient vector, however, non-colonizing aphid species (those that probe but do not colonize potato) can be important for PVY prevalence within a landscape (Steinger et al. 2015, Mondal et al. 2016, Galimberti et al. 2020). In addition to reducing initial inoculum through the planting of non-infected seed potatoes, controlling aphid vector populations can be an effective management strategy (Ragsdale et al. 2001). While insecticides are effective in controlling the vectors, they seems to have a low impact on the spread of PVY because of the short time needed to transmit the non-persistent virus. Moreover, since insecticides may encourage vector movement, they have potential to increase spread of non-persistent viruses by aphids (Rolot et al. 2021).

The recent legalization of industrial hemp (*Cannabis sativa* L.) (Rosales: Cannabaceae) in the United States has introduced a "new" crop into the agricultural landscape. In the U.S., hemp is legally defined as *C. sativa* plants with less than 0.3% Δ9-tetrahydrocannabinol (THC) on a dry weight basis. According to the "U.S. Hemp Crop Report", in 2020 there were 336,655 acres licensed to grow hemp in 34 states (Vote Hemp 2021). Colorado is the leading state in production of the crop with 36,225 licensed acres in 2020. Hemp production is expected to keep increasing as more growers enter the lucrative market. There are numerous arthropod pests and diseases that affect hemp (McPartland 2000). Indeed, cannabis plants can be infected with PVY via mechanical inoculation, and light green mosaic symptoms have been observed (Kegler and Spaar 1997).

The cannabis aphid, *Phorodon cannabis* Passerini was recently discovered colonizing hemp in North America in 2016 (Cranshaw et al. 2018). It became one of the most abundant piercing-sucking insects found on hemp in the U.S. (Cranshaw et al, 2019; Pitt 2023, Chapter 2). The cannabis aphid is a known vector of alfalfa mosaic and cucumber mosaic viruses (Schmidt

and Karl 1970). There are published reports ascertaining that *P. cannabis* has vectored hemp mottle virus (Schmidt and Karl 1970, McPartland et al. 2000), hemp streak virus (Goidanich 1955), and hemp mosaic and hemp leaf chlorosis viruses (Ceapoiu 1958). However, the existence of these viruses is not clear, and none are recognized by the International Committee on Taxonomy of Viruses (Walker et al. 2019). Additionally, it is not known whether the cannabis aphid can transmit PVY.

In the current study, I had two specific goals: 1) determine PVY transmission efficiency of cannabis aphid to hemp and potato and 2) determine effects of virus infection and host plant suitability on cannabis aphid feeding behaviors. I conducted transmission assays and utilized the electrical penetration graph (EPG) technique. Electrical penetration graph analysis is a commonly used technique to study feeding behaviors of insects with piercing/sucking mouthparts (Tjallingii 1988). Therefore, it can be used to study the basis of plant virus acquisition and transmission and host plant selection by insects and the way in which insects can feed from the phloem of the plant. I hypothesized that 1) the cannabis aphid will successfully transmit PVY to hemp and potato with differing efficiencies and 2) virus infection of aphids and host plant suitability will affect feeding behaviors in ways which influence transmission. Knowledge of vector transmission efficiencies and feeding behaviors will provide valuable insights into the patterns of vector movement and virus spread within landscapes.

Methods

Plants, Insects, and Virus

Hemp seeds (variety: Elite™) were obtained under material transfer agreement from New West Genetics©, Fort Collins, CO. Potatoes (variety: CO07015-4RU), which have no known

resistance to PVY, were obtained from the San Luis Valley Research Center in Center, CO. Prior to use in the experiments, the potato plants were tested for presence of PVY with an ImmunoStrip® (Agdia®, Elkhart, IN) and they were PVY-free. Plants for all experiments and for cannabis aphid colonies were grown at the Colorado State University Plant Growth Facilities in a greenhouse, with a daytime temperature of 21 ± 1 °C and a nighttime temperature of 16 ± 1 °C under a photoperiod of 16:8 (L:D) hours at ambient relative humidity. All plants were fertilized with Osmocote® (Scott's Company, Marysville, OH) 15-9-12 N:P:K ratio time-released fertilizer as per label instructions and watered ad libitum. Two to three-week-old hemp plants (with three to four fully-expanded leaves/leaf nodes) and potato plants (with four to eight fully-expanded leaves/leaf nodes), grown in circular one gallon plastic pots (16 x 16 x 17.5 cm) containing Pro-Mix® HP potting mix (Halifax Seed, Halifax, Nova Scotia, Canada), were used for all experiments.

Cannabis aphids were obtained from hemp plants at an indoor hemp facility in Loveland, CO. Insects were reared and maintained on Elite™ variety hemp plants under the greenhouse environmental conditions described above. Hemp plants for cannabis aphid colonies were infested with aphids after approximately four to six weeks (five to ten leaf nodes) and were grown in a 45.7 x 45.7 x 76.2 cm cage (BioQuip Products Inc., Rancho Dominguez, CA).

The PVY inoculum was originally obtained from PVY^{NTN} potato plants from a farm in the state of New York. The virus was maintained via mechanical inoculation on *Nicotiana tabacum* under greenhouse conditions. *Nicotiana* is the most widely used experimental host in plant-virus research mainly because of the large number of diverse plant viruses that can successfully infect it (Goodin et al. 2008). For mechanical inoculation, approximately one to two PVY^{NTN} infected tobacco leaves were ground in 0.01 M potassium phosphate buffer at a 1:10

tissue weight to buffer volume ratio and 0.5 – 1 teaspoon of 320 grit silicon carbide powder was stirred into the solution. Cotton swabs were dipped in the solution and gently rubbed on young leaves (nodes one to two) to mechanically inoculate tobacco plants to serve as a virus source for all experiments. Virus presence in tobacco tissue was confirmed using an ImmunoStrip® for potato virus Y (PVY) (Agdia®, Elkhart, IN), and leaves with observable veinal chlorosis were selected for use in experiments.

Population Growth Experiments on Potato

Although the cannabis aphid is only reported to colonize cannabis (Blackman & Eastop 2008), I conducted population growth experiments to confirm that potato is a non-host for cannabis aphids. Ten two- to three-week-old potato plants (four to eight leaf nodes) were placed individually in separate cages. A single one- to seven-day old female parthenogenetic apterous adult aphid was placed on the adaxial leaf surface of plants with one to three leaf nodes using a paint brush. The aphids were confined to the leaf using a clip cage (36.5 x 25.4 x 9.5 mm) (BioQuip Products Inc). The adult aphid was given 24-76 h to reproduce and was then manually removed from the plant, where any resulting offspring remained within the clip cage. Numbers of individuals, longevity, and time to maturity were recorded every 24 h. In total, 10 replicates were performed at two different time points (two biological replicates). A single two- to three-week-old hemp plant (three to leaf four nodes) was used as a control for all population growth assays.

Transmission Assays

Assays were conducted in mesh "Bug Dorm" cages 60 x 60 x 120 cm (MegaView, Taiwan). A random mix of all life stages of aphids were used for transmission assays, as nymphs and adults both potentially can transmit the virus (Cunningham and Schulz 1963). Aphids were

starved for two hours and then placed on PVY^{NTN} infected tobacco plant, using a paint brush, for an acquisition access period (AAP) of 30 minutes. Groups of twenty aphids were moved with a paint brush onto the adaxial leaf surface of hemp plant (one-two leaf node) and potato (one - three leaf node) and allowed to feed for an inoculation access period (IAP) of 24 hours. Following the IAP, aphids were removed with a paint brush and plants were sprayed once with Final Stop® Vegetable Garden Insect Killer (Dr. Earth®, Winters, CA), which contains a mixture of essential oils as active ingredients, to achieve a thorough coverage of all leaf and stem surfaces, as per label instructions. Plants were then maintained for two weeks, and approximately 50 mg of leaf tissue was collected from nodes three - four for hemp (n=22), and nodes one - three for potato (n=22), at 14-15 days post inoculation (dpi). Tissue was collected in 2 mL Eppendorf tubes, flash frozen in liquid nitrogen, and stored in a freezer at -80° C until analysis. Additionally, 10 apterous adult female aphids were collected and stored individually in 2 mL Eppendorf tubes immediately after the initial AAP of 30 minutes and placed in a -80°C freezer until they were tested for the presence of PVY by RT-PCR (described in detail below).

Electrical Penetration Graph (EPG) Analysis

Cannabis aphid feeding behaviors on hemp and potato were monitored using the electrical penetration graph technique (EPG) (Tjallingii 1978, 1988), on a GIGA 8 complete system (EPG Systems, Wageningen, Netherlands) (Tjallingii and Esch 1993). Aphids were starved for one to two hours prior to recording. Using silver glue, aphids were tethered to a 1.5 – 2 cm long piece of 18 µm diameter gold wire, which was connected to a copper wire, brass pin, and insect electrode which was linked to the GIGA 8 machine. A plant electrode connected to the GIGA 8 was placed into the soil of each of the eight potted plants, which were all housed inside of a Faraday cage. Wired aphids were placed on the adaxial leaf surface of nodes one -

three for hemp or potato, and feeding behaviors were recorded for eight hours during daytime in a room with ambient sunlight and a temperature of 21 ± 1 °C. Four treatments were performed: viruliferous aphid on hemp; viruliferous aphid on potato; non-viruliferous aphid on hemp; non-viruliferous aphid on potato. For the viruliferous aphid on hemp and potato treatments, aphids were placed on PVY^{NTN} infected tobacco tissue for 30 minutes after the starvation period, immediately prior to recording. Seven to 14 days after recording, all plants were tested for virus presence by RT-PCR (described in detail below) to confirm that the aphid had successfully acquired and transmitted the virus. This information was used to quantify transmission efficiency of individual aphids to hemp and potato. Plants associated with aphids that did not probe during the 8 h recording period were discarded. Only EPG recordings in which the plants tested positive were used for analysis (confirming that the aphid transmitted PVY and was viruliferous). EPG waveforms were analyzed using Stylet+ software (EPG Systems, Wageningen, Netherlands) to determine the amount of time aphids spent in the four main feeding phases: non-probing phase (NP); pathway phase (PP); sieve-element phase (SEP); and xylem phase (XP). The Excel workbook developed by Sarria et al. (2009) was used to calculate parameters of EPG data. Data were analyzed for three-time intervals for aphids which probed; the initial 15 minutes and 30 minutes of recording (short-term feeding behaviors) and the entire 8 h recording period (long-term feeding behaviors). The initial 15 minutes and 30 minutes were chosen because these time intervals have been shown to be relevant for non-persistent virus manipulations on aphid feeding behavior (Carmo-Sousa et al. 2014). The entire 8 h recording period was chosen as this is a typical amount of time analyzed in EPG experiments with aphids (Nalam et al. 2018), and effects of PVY on aphid feeding behavior can occur within this time of feeding (Boquel et al. 2011a, 2012). Twelve parameters related to pathway phase (C) and intracellular punctures (pd) were

analyzed for the initial 15 minutes and 30 minutes of recording. The sub-phases of II-1, II-2, and II-3 contained within a pd are when non-persistent virus inoculation and acquisition occur and were analyzed for the first 15 minutes and 30 minutes. Twenty-three parameters related to non-probing (np), C, pd, stylet difficulties (F), phloem salivation (E1), phloem ingestion (E2), and xylem ingestion (G) were analyzed for the entire 8 h of data.

Detection of PVY in Aphids and Plants Using Reverse Transcriptase PCR (RT-PCR)

Total RNA was extracted from ~50 mg of leaf tissue from hemp and potato, and from <5mg tissue from 10 individual aphids. Two stainless steel beads (3.97mm) were placed into 2mL microcentrifuge tubes containing leaf tissue samples, and tissue was disrupted for one to three minutes at a frequency of 30 Hz with the TissueLyser II (Qiagen, Germanton, MD). The same tissue disruption protocol was used for aphid tissue, except that ~250µL of silica beads (800µm) were used. Total RNA was extracted from leaf tissue using the Quick RNA Miniprep Kit (Zymo Research, Irvine, CA) or a non-kit method from Yockteng et al. (2013). Total RNA was also extracted from individual aphids using the RNeasy Micro Kit (Qiagen, Germanton, MD). After extraction, RNA was checked for quality and quantity using a Nanodrop ND 100 (Thermo Scientific, Pittsburgh, PA). Two micrograms of RNA from aphid and leaf tissues were used as a template for cDNA synthesis using the first strand cDNA synthesis kit (GoldBio St. Louis, MO, USA). The cDNA was tested for PVY with RT-PCR using universal PVY primers 'ATACTCGRGCAACTCAATCACA' 'CCATCCATCATAACCCAAACTC' (Du et al. 2006) and the following cycling conditions: 2 min incubation at 95°C, followed by 35 cycles of 30 sec denaturation at 95°C, 30 sec annealing at 58°C, and 1min extension at 72°C, and a final 10 min incubation at 72°C. The PCR product of 166 bp was sequenced (Genewiz, NJ) and compared with PVY sequences deposited in GenBank.

Statistical Analysis

For transmission assays (with cohorts of 20 mixed life stages of aphids) and EPG assays (with individual adult aphids), the infection status of hemp or potato plants was treated as a binomial response (positive or negative) and analyzed using χ^2 test for association. In EPG assays with potato, there was low virus transmission by cannabis aphids; hence there were only 2 replicates for 15- and 30-minute time intervals and 3 replicates for the 8 h time interval for PVY-positive potato. The sample size is different between short term and long-term time intervals due to an aphid that did not probe until after 30 minutes had passed (i.e. EPG data were analyzed only for aphids which probed).

In EPG assays, there were different sample sizes for the number of plants that tested positive for PVY versus the number of EPG recordings for viruliferous aphids (Hemp: n=25 PVY infected hemp plants, n=22 viruliferous aphid EPG recordings; Potato: n=5 infected potato plants, n=3 viruliferous aphid EPG recordings). The reason for the discrepancy is because I tested plants for virus infection even if: 1) viruliferous aphid became disconnected from the gold wire; 2) some channels had waveforms that were not discernible (too much noise or not enough gain); 3) waveforms were out of range for a long period of time. EPG data were not normally distributed, so comparisons were made between treatments using the non-parametric Mann-Whitney *U* test to determine differences between means displaying a p-value ≤ 0.05. All statistical analyses were conducted using R software (version 4.0.2) and the 'stats' package (R Core Team 2022). Figures were constructed using the packages 'ggplot2', 'ggsignif', and 'ggpattern' (Wickham 2011, Ahlmann-Eltze and Patil 2021, FC et al. 2022).

Results

PVY transmission to hemp (host) and potato (non-host)

Cannabis aphids were not able to develop or reproduce on potato (**Supp. Table A3.1**), suggesting that potato is a non-host for the cannabis aphid. Hence, for the rest of the chapter I describe hemp as a host and potato as non-host.

Cannabis aphids were tested for their ability to transmit PVY using cohort transmission assays (20 aphids of mixed life stage per plant) and individual assays (one individual adult aphid per plant). Cannabis aphids had a 70% (7/10) virus acquisition rate from PVY-infected tobacco, which was used as the virus source for all experiments. In cohort transmission assay experiments, 95.5% (21/22) of hemp plants and 90.9% (20/22) of potato plants tested positive for PVY. No significant difference was found in PVY infection between the two host plants (χ^2=0.36, P=0.55). In individual experiments, 25 out of 40 hemp plants (62.5%) and 5 out of 26 potato plants (19.2%) tested positive for PVY after feeding by viruliferous aphids. A significantly higher proportion of hemp plants tested positive for PVY compared to potato plants (χ^2=11.90, P<0.001).

Cannabis aphid feeding behavior waveforms

The EPG technique was used to determine cannabis aphid feeding behaviors related to virus transmission on the host (hemp) and non-host (potato) and were the first known depictions of feeding behaviors for the cannabis aphid. Examples of recorded EPG waveforms on hemp are shown in **Fig. 4.1**. Cannabis aphids displayed the typical EPG waveforms observed from aphids, which include non-probing (np), stylet pathway (C), potential drop (pd), stylet difficulties (F), xylem ingestion (G), phloem salivation (E1), and phloem ingestion (E2).

Short-term feeding behaviors in hemp and potato (initial 15 and 30 minutes)

Virus effects

When feeding on hemp, viruliferous aphids probed fewer times than non-viruliferous aphids (U=257, P=0.04) (**Table 4.1**). There were no other significant differences in parameters related to virus acquisition and transmission between viruliferous and non-viruliferous aphids on hemp during the initial 15 minutes of recording (**Table 4.1**). No comparisons were made between viruliferous and non-viruliferous aphids feeding on potato because of the small sample size of viruliferous aphids. Data of waveforms from the initial 30 minutes of recording were also analyzed which showed that viruliferous aphids took more than twice the amount of time to probe from the start of the EPG compared to non-viruliferous aphids (U=140, P=0.04) (**Supp. Table A3.2**).

Non-host effects

During the initial 15 minutes of recording, non-viruliferous aphids on potato had a lower number of intracellular punctures (potential drops, pd) compared to non-viruliferous aphids on hemp (U=306.5, P=0.01) (**Fig. 4.2a**). Non-viruliferous aphids feeding on potato spent less time in pd compared to non-viruliferous aphids on hemp (U=279.5, P<0.001) (**Fig. 4.2b)**. The mean duration of pd was shorter for aphids on potato compared to aphids on hemp during the initial 15 minutes of recording (U=225, P=0.047) (**Fig. 4.2c).** Non-viruliferous aphids on potato spent significantly less time in pd subphases II-1 (virus inoculation behavior), II-2, and II-3 (virus acquisition behavior), compared to aphids on hemp (U=271, P<0.001; U=288, P<0.001; and U=277, P<0.001, respectively) (**Table 4.1**). There were no significant differences in any other selected behaviors during the initial 15 minutes of recording between non-viruliferous aphids feeding on hemp and potato.

Long-term feeding behaviors in hemp and potato (entire 8 hours)

Virus effects

Over the 8 hours of recording on hemp, viruliferous aphids spent significantly greater time non-probing (np) compared to non-viruliferous aphids (U=149, P=0.02, **Fig. 4.3a**). On hemp, viruliferous aphids spent more time in ingesting xylem (G) (U=78, P=0.04, **Fig 4.3d**); in contrast, viruliferous aphids spent less time ingesting phloem (E2) compared to non-viruliferous aphids (U=212, P=0.02, **Fig. 4.3f**). Total duration spent in stylet pathway (C), stylet difficulties (F) and phloem salivation (E1) were not significant (**Fig 4.3b, c**, and **e**).

There were no significant differences in feeding behaviors associated with virus transmission, i.e. intracellular punctures, between viruliferous and non-viruliferous aphids on hemp over the 8-hour recording period (**Table 4.2**). However, viruliferous aphids spent significantly less time probing compared to non-viruliferous aphids on hemp (U=357, P=0.02) (**Table 4.2**). Viruliferous aphids spent a significantly smaller percentage of probing time ingesting phloem (E2) compared to non-viruliferous aphids on hemp (U=340, P= 0.048) (**Table 4.2**). Viruliferous aphids displayed a lower number of sustained phloem ingestion (E2) events compared to non-viruliferous aphids on hemp (U=346, P=0.03) (**Table 4.2**). I did not compare feeding behaviors between viruliferous and non-viruliferous aphids on potato because of the small sample size of viruliferous aphids.

Non-host effects

There were many differences between feeding behaviors of non-viruliferous aphids on hemp and potato during the 8-hour recording period. Aphids feeding on potato showed a lower number of pd and average number of pd per probe compared to aphids on hemp during the 8-hour recording period (U=329, P=0.02; U=324, P=0.02, respectively) (**Fig. 4.4a** and **b**). The first probe was significantly longer for aphids on potato compared to hemp (U=126, P=0.01) (**Fig. 4.4c**). Aphids feeding on potato showed a greater number of stylet difficulties (F) and

greater total time spent non-probing (np) compared to aphids on hemp (U=106.5, P= 0.002; and U=116, P= 0.005, respectively) (**Fig. 4.4d** and **4e**). On potato, aphids spent less time feeding from the phloem (E) compared to aphids on hemp (U=57, P=0.001) (**Fig. 4.4f**). All the other selected parameters for phloem related behaviors for non-viruliferous aphids feeding on potato were significantly lower than non-viruliferous aphids on hemp, with the exception of percent contribution of E1 to the phloem phase, which was significantly greater for aphids on potato (**Table 4.2**).

Discussion

Here, I demonstrated that the cannabis aphid (*P. cannabis*) is an efficient vector of PVY to hemp, its natural host and potato, a non-host. This is the first known report of the cannabis aphid as a vector of PVY and is the first known recording and annotation of EPG waveforms produced by the cannabis aphid (**Fig. 4.1**). I found that cannabis aphid feeding behaviors were altered on host versus non-host plants which presumably affected PVY transmission. Further, our data suggest that PVY altered feeding behaviors of aphids on host plants in ways that facilitate virus spread.

In the PVY-potato system, there are approximately 65 known aphid vector species (Pelletier et al. 2012). While they are not all as efficient in virus transmission as the main vector, *M. persicae*, they are the main contributors to PVY spread (Steinger et al. 2015, Mondal et al. 2016, Galimberti et al. 2020). Hence, knowing which species transmit PVY and their transmission efficiencies are important from a management perspective. In our study, cannabis aphids transmitted PVY to 96% of host plants (hemp) and 91% of non-host plants (potato) in transmission assays with cohorts of 20 aphids. However, using individual aphids, I found that the

PVY transmission decreased to 63% in hemp and 19% in potato. It is not surprising that decreasing the number of potentially viruliferous aphids from 20 to 1 per plant led to reduced virus transmission efficiency. Indeed, altering the number of aphids used in assays can alter transmission efficiencies of PVY (Boquel et al. 2011b). Individual *M. persicae* have been shown to transmit PVY to potato plants with 83% efficiency (Boquel et al. 2011b), which is notably higher than the transmission to potato by individual cannabis aphids observed in our study (19%). However, when the aphid's host plant range is considered, transmission to hemp plants by the cannabis aphid is remarkably efficient.

It is likely that the difference in transmission efficiency between host and non-host plants in individual assays is due to differences in aphid feeding behaviors, particularly those associated with intracellular punctures (pd) that occur shortly (within 3-6 s) after the aphid initiates probing on the plant. These behaviors are associated with efficient acquisition and transmission of non-persistent viruses (López-Abella and Bradley 1969, Powell 1991). Indeed, PVY transmission efficiency is positively associated with feeding behaviors related to intracellular punctures (Powell et al. 1992, Collar and Fereres 1998, Fernández-Calvino et al. 2006, Boquel et al. 2012, Boquel et al. 2014). I expected that viruliferous aphids would show an increased number of intracellular punctures compared to non-viruliferous aphids, however, I found no significant differences in any parameters related to intracellular punctures between viruliferous and non-viruliferous aphids on hemp and potato (**Table 4.1** and **2**; **Supp. Table A3.2**). In contrast, I found that non-viruliferous aphids on potato performed significantly fewer intracellular punctures compared to non-viruliferous aphids on hemp during the initial 15 minutes of recording (**Fig. 4.2a**). Specifically, aphids feeding on potato spent lower amount of time in

subphase II-1, during which aphids secrete saliva releasing the virions (Tjallingii and Esch 1993). This may in part explain the lower transmission efficiency of PVY to potato.

It is possible that during intracellular punctures, cannabis aphids perceived cues related to unsuitability of potato as a host thereby rejecting the host and not sampling the plant further. Indeed, aphids often leave non-host plants after short probes of the epidermis (Powell et al. 2006). In addition, aphids use other non-gustatory host suitability cues from plants, such as tactile, visual, and olfactory cues during the initial host plant selection prior to probing (Dixon 2012, Powell et al. 2006). So, it is possible that cannabis aphids recognized the non-host status of potato prior to EPG recordings thereby deterring further intracellular punctures. It is important to recognize that the lower transmission efficiency to potato observed does not necessarily equate to lower incidence in field settings. It is possible that the reduced suitability of potato would increase vector dispersal in search of suitable hosts thereby increasing virus transmission (Powell et al. 2006).

I found that, on host plants (hemp), viruliferous aphids spent less time ingesting phloem (waveform E2) compared to non-viruliferous aphids over the entire 8-hour recording period (**Fig. 4.3f**). Our findings are consistent with previous research showing potyvirus-induced reductions in phloem ingestion (Blua and Perring 1992, Boquel et al. 2011b, Boquel et al. 2012, Wosula et al. 2014), but these effects can differ between aphid species (Boquel et al. 2011b; Boquel et al. 2012). Moreover, aphids showed a lower number of sustained phloem ingestion events, spent a lower percentage of probing time ingesting phloem, and spent more time non-probing compared to non-viruliferous aphids on hemp (**Table 4.2**; **Fig. 3a**). Viruliferous aphids showed patterns of feeding behavior which indicate reduced host acceptance on hemp. Previous research has shown that decreased host acceptance for a viruliferous vector, if perceived after initiating probing,

would create more opportunities for non-persistent virus transmission, as vectors would be encouraged to disperse in search of a more suitable host plant (Mauck et al. 2012, Eigenbrode et al. 2018).

This study has some limitations that should be considered when interpreting the results. Importantly, it should be noted that I determined PVY transmission efficiency of apterous cannabis aphids, although alate morphs are thought to be more important for potyvirus spread than apterous morphs (Gadhave et al. 2020). Further, cohorts of 20 cannabis aphids were used for cohort transmission assays, although 20 apterous cannabis aphids feeding upon a potato plant is unlikely to occur in field settings. When performing EPG experiments, aphids are tethered to an electrical probe and are not able to leave the plant which they are on. This is in stark contrast to natural movement where the aphid can successfully disperse from the plant (Caillaud 1999). I did not measure quantities of phloem sap consumed by viruliferous and non-viruliferous aphids, so I do not know if viruliferous aphids actually ingested smaller quantities of phloem in potato plants. Lastly, statistical comparisons were not made between non-viruliferous and viruliferous aphid feeding behaviors on potato because of the small sample size of viruliferous aphids owing to low virus transmission to potato.

This work highlights that PVY infection in hemp has the potential to pose a significant risk for potato production via virus transmission by cannabis aphids. Indeed, the cannabis aphid was among the dominant aphid species collected from the San Luis Valley, CO in Chapter 2. In addition, generalist aphid species such as *M. persicae* and *M. euphorbiae* could likely transmit PVY to hemp from infected potato plants and vice versa. Future research should investigate effects of PVY infection on hemp yield and cannabinoid levels as this information will be critical for understanding the risk of PVY infection in hemp.

Tables and Figures

Table 4.1. Feeding behaviors (intracellular punctures/potential drops, pd and probing behaviors) (mean ± SE) pertaining to virus transmission by *Phorodon cannabis* on hemp and potato in the initial 15 minutes of recording.

Parameters	Hemp				Potato	
	Non-viruliferous (n=21)	**Viruliferous (n=18)**	***U***	***P***	**Non-viruliferous (n=20)**	**Viruliferous (n=2)[a]**
Probing behaviors						
Time to first probe from start of EPG (sec)	209.2 ± 28.34	355.07 ± 68.11	140	0.17	230.05 ± 30.54	205.25 ± 77.84
Duration of first probe (sec)	247.16 ± 53.35	252.74 ± 64.60	187	0.97	465.62 ± 62.43	557.96 ± 214.63
Number of probes	**2.29 ± 0.24**	**1.61 ± 0.16**	**257**	**0.04**	1.5 ± 0.18	1.5 ± 0.5
Intracellular punctures						
Mean duration of pd (sec)	5.50 ± 0.29	4.96 ± 0.17	171	0.37	4.88 ± 0.31	4.34 ± 0.003
Average number of pd per probe	3.39 ± 0.60	3.14 ± 0.45	144.5	1	2.26 ± 0.26	3.50 ± 1.50
Number of pd	5.38 ± 0.82	4.5 ± 0.82	223	0.34	2.65 ± 0.39	4.5 ± 0.5
Total duration of pd (sec)	33.13 ± 3.65	25.42 ± 4.12	187	0.14	14.25 ± 2.01	19.55 ± 2.19
Time from the beginning of the 1st probe to first pd (sec)	149.18 ± 38.33	92.80 ± 39.66	176	0.28	142.77 ± 42.85	27.42 ± 10.18
Time from the end of the last pd to the end of the probe (sec)	88.07 ± 25.31	85.54 ± 36.27	166	0.46	184.47 ± 42.91	116.14 ± 64.69
Total duration of subphase II1 for the pd (sec)	11.55 ± 1.49	10.06 ± 1.71	154	0.51	5.28 ± 0.75	8.26 ± 1.08
Total duration of subphase II2 for the pd (sec)	7.22 ± 0.93	5.59 ± 0.97	171	0.2	2.55 ± 0.37	4.76 ± 0.21
Total duration of subphase II3 for the pd (sec)	14.17 ± 1.56	10.24 ± 1.78	184	0.08	6.00 ± 1.04	6.54 ± 1.32

[a]Due to the low sample size of viruliferous aphids on potato, statistical comparisons were not made
P-values calculated according to the Mann-Whitney *U* Test, with bolded values indicate significant differences ($P<0.05$)

Table 4.2. Feeding behaviors (mean ± SE) of *Phorodon cannabis* on hemp (host plant) and potato (non-host plant) over the entire 8-hour recording.

Parameters	Hemp				Potato	
	Non-viruliferous (n=23)	**Viruliferous (n=22)**	***U***	***P*-value**	**Non-viruliferous (n=20)**	**Viruliferous (n=3)[a]**
General probing/non-probing behaviors						
Time to 1st probe from start of EPG (min)	9.66 ± 4.44	9.43 ±2.07	184	0.12	3.83 ± 0.51	15.74 ± 12.34
Number of np	18.61 ± 2.76	16.86 ± 2.32	266	0.78	16.65 ± 2.22	17.33 ± 10.84
Number of C	22.09 ± 3.16	21.82 ± 2.77	246.5	0.89	23.45 ± 2.22	24.67 ± 16.29
Number of F	2.09 ± 0.68	3.05 ± 0.90	226	0.53	5.55 ± 1.05	5.67 ± 4.70
Number of probes	18.35 ± 2.78	16.55 ± 2.32	266	0.78	16.15 ± 2.17	16.67 ± 11.17
Number of short probes (C<3 min)	7.52 ± 1.65	7.55 ± 1.93	264	0.82	5.80 ± 1.27	7.67 ± 6.17
Total probing time (min)	**398.24 ± 18.59**	**344.60 ±21.58**	**357**	**0.02**	314.80 ±25.15	158.26 ± 67.25
Duration of 1st probe (min)	10.57 ±4.13	21.83 ±9.35	228	0.58	64.19 ±27.74	16.50 ± 11.89
% of probing spent in C	39.64 ± 4.74	42.23 ± 4.16	232	0.64	61.50 ± 5.57	89.46 ± 8.80
% of probing spent in F	3.78 ± 1.05	6.43 ± 3.03	237.5	0.72	10.30 ± 3.02	1.70 ± 1.61
Intracellular punctures						
Number of pd	109.13 ± 13.96	107.18 ± 9.52	245	0.87	63.05 ± 12.31	31.33 ± 13.97
Average number of pd per probe	8.10 ± 1.21	8.71 ± 1.34	241	0.79	5.04 ±1.08	2.92 ± 1.88
Phloem related behaviors						
Number of E1	4.22 ± 0.72	3.00 ± 0.81	319	0.13	0.30 ± 0.18	0
Number of E2	3.52 ± 0.64	2.50 ± 0.67	316	0.15	0.20 ± 0.12	0
Number of sustained E2 (>10 min)	**1.78 ± 0.29**	**0.95 ± 0.23**	**346**	**0.03**	0	0
Duration of the longest E2 (min)	146.50 ± 23.46	105.74 ±28.70	186	0.14	1.12 ± 1.03	-[b]

Parameter	Hemp				Potato	
	Non-viruliferous (n=23)	**Viruliferous (n=22)**	***U***	***P*-value**	**Non-viruliferous (n=20)**	**Viruliferous (n=3)**
Phloem related behaviors						
Contribution of E1 to phloem phase (%)	14.45 ± 3.97	31.79 ± 8.96	114	0.34	71.79 ± 18.53	-
Potential E2 Index	68.19 ± 7.11	54.88 ± 10.83	160	0.55	0.44 ± 0.35	-

Percent of probing spent in E1	6.27 ± 1.99	6.25 ± 3.07	312	0.18	0.10 ± 0.07	0
Percent of probing spent in E2	**40.13 ± 5.88**	**22.22 ± 5.62**	**340**	**0.05**	0.05 ± 0.046	0
Percent of E2 >10 min	63.66 ± 6.75	47.22 ± 10.01	180.5	0.19	0	-
Xylem related behaviors						
Number of G	1.09 ± 0.20	1.86 ± 0.32	175.5	0.07	2.40 ±0.56	3.00 ± 1.53
Percent of probing spent in G	10.18 ± 2.41	22.87 ± 4.82	177.5	0.09	28.05 ± 5.20	8.84 ± 7.20

[a]Due to the low sample size of viruliferous aphids on potato, statistical comparisons were not made.

[b]Dashes indicate an absence of values for that parameter

P-values calculated according to the Mann-Whitney *U* Test, with bolded values indicating significant differences ($P<0.05$).

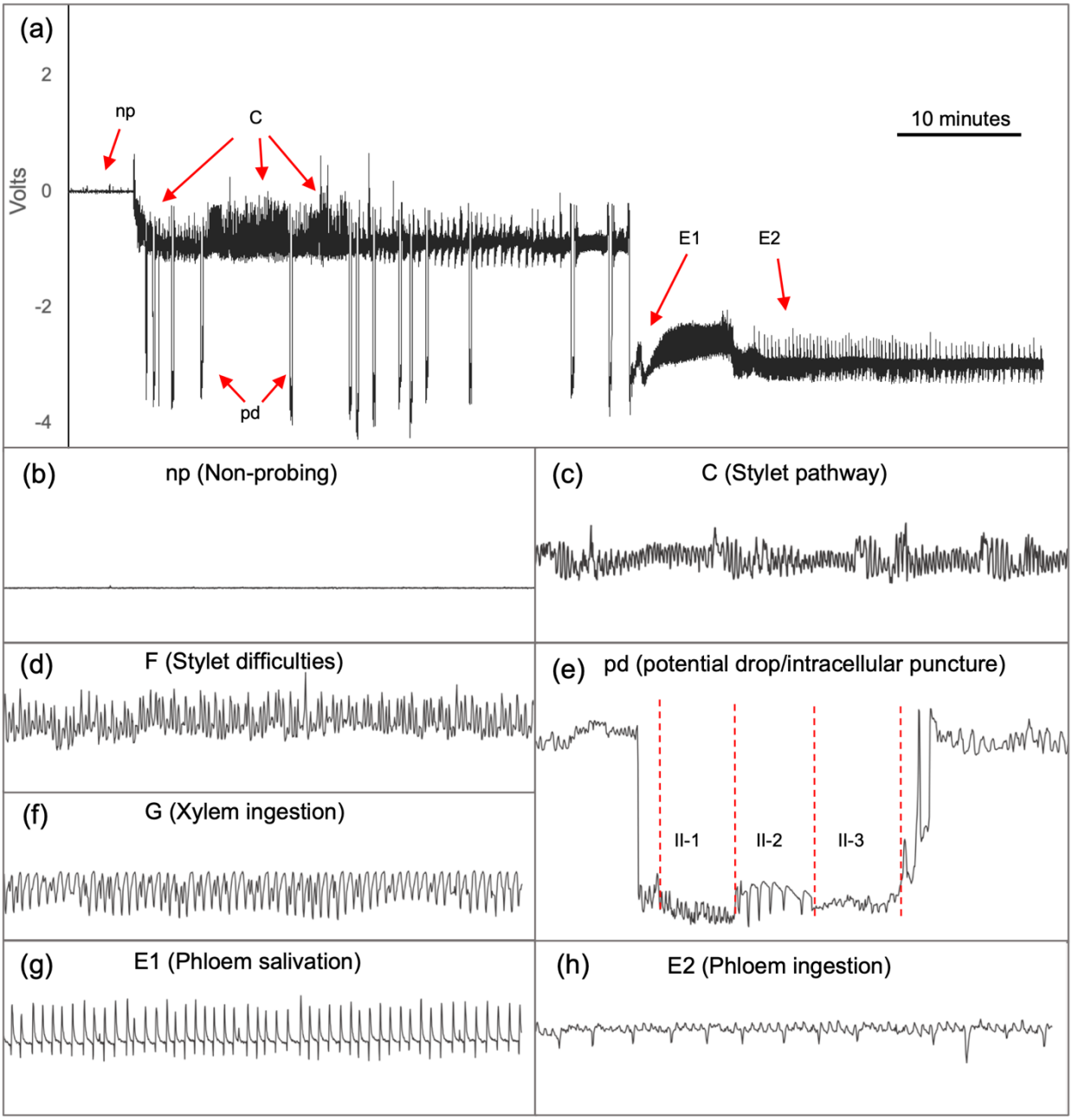

Figure 4.1. (a) Overview of waveforms observed from *Phorodon cannabis* feeding on hemp. Examples of waveform types representing the feeding behaviors of (b) non-probing (np), (c) pathway phase (C), (d) stylet difficulties (F), (e) potential drop (pd), (f) xylem ingestion (G), (g) phloem salivation (E1), and (h) phloem ingestion (E2). All waveforms pictured from an individual non-viruliferous *P. cannabis* feeding on hemp, displayed as voltage over time (seconds).

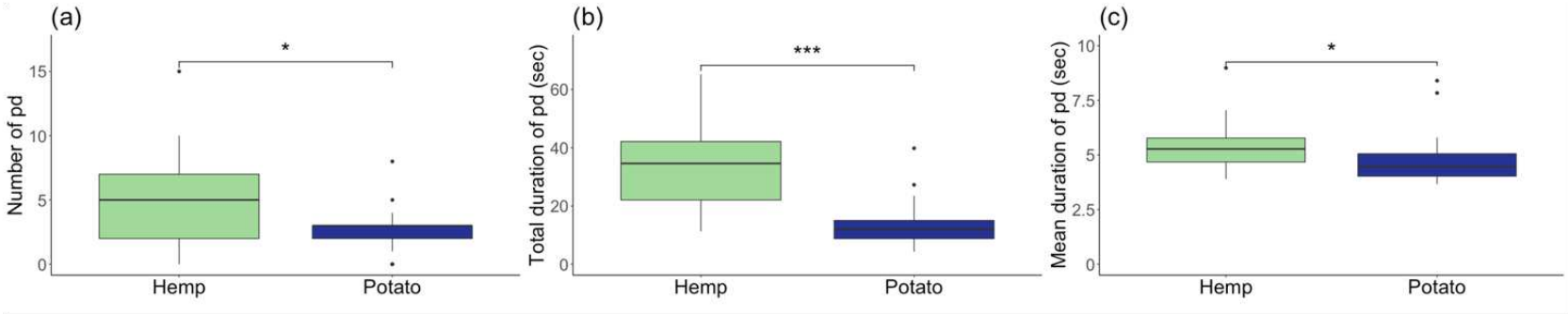

Figure 4.2. Feeding behavior parameters related to intracellular punctures (potential drops, pd) of non-viruliferous *Phorodon cannabis* feeding on hemp (host) and potato (non-host) plants during the initial 15 minutes of recording. Box plots display median (line), first and third quartiles (box), ± 1.5 x interquartile range (whiskers), and outliers (dots) of (a) number of pd, (b) total duration of pd (sec), and (c) mean duration of pd (sec). Asterisks indicate significant differences between groups according to Mann-Whitney U test (*P<0.05, ***P<0.001); n=21 hemp; n=20 potato.

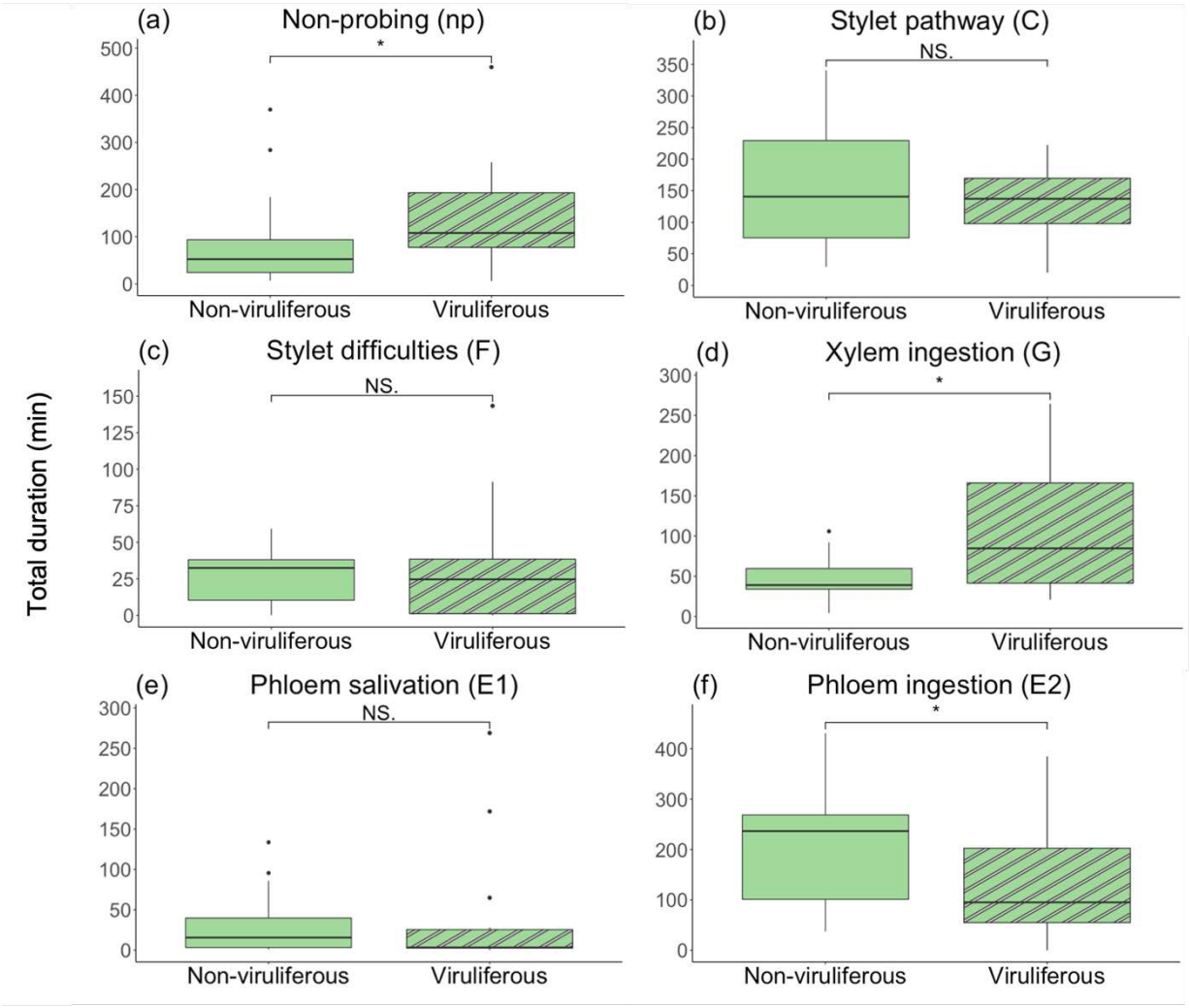

Figure 4.3. Durations of feeding behaviors of non-viruliferous and viruliferous *Phorodon cannabis* on hemp from the entire 8-hour recording. Box plots display median (line), first and third quartiles (box), ± 1.5 x interquartile range (whiskers), and outliers (dots) of durations in minutes spent in waveforms (a) np (non-probing), (b) C (pathway), (c) F (stylet difficulties), (d) G (xylem ingestion), (e) E1 (phloem salivation), and (f) E2 (phloem ingestion). Asterisks indicate significant differences and "NS." indicates non-significant differences between groups according to Mann-Whitney U test (*P<0.05); n=23 non-viruliferous aphids; n=22 viruliferous aphids.

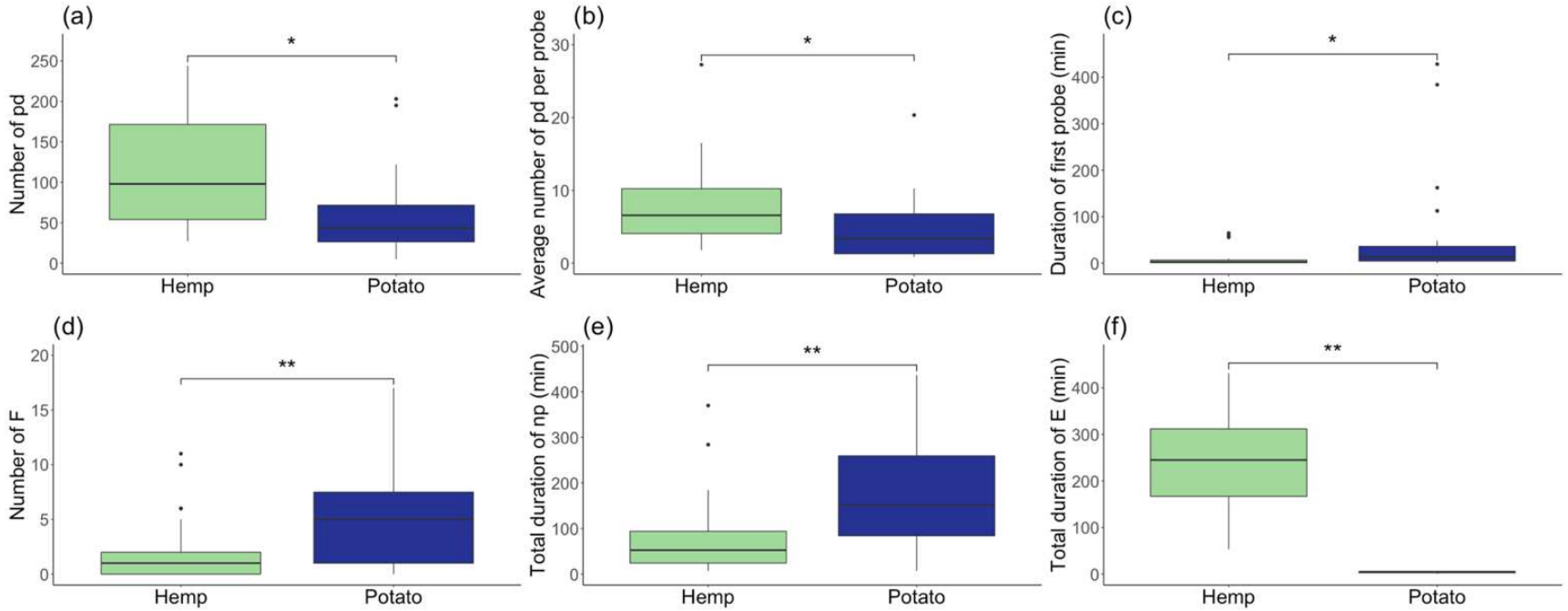

Figure 4.4. Feeding behavior parameters comparing non-viruliferous *Phorodon cannabis* feeding on hemp (host plant) and potato (non-host plant) from the entire 8-hour recording. Box plots display median (line), first and third quartiles (box), ± 1.5 x interquartile range (whiskers), and outliers (dots) of number of (a) intracellular punctures (pd), (b) average number of pd per probe, (c) duration of first probe (min), (d) number of F, (e) total duration of non-probing (np), (f) total duration of phloem feeding (E). Asterisks indicate significant differences between groups according to Mann-Whitney U test (*P<0.05, **P<0.01); n= 23 hemp; n=20 potato.

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CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

This research furthered understanding of 1) the effects of landscape composition on PVY prevalence and aphid vectors, 2) Aphid host use patterns in relation to PVY epidemiology, and 3) PVY transmission by a previously unknown vector species, the cannabis aphid. A better understanding of these interactions provides information that is useful to improve sustainable and effective management strategies.

Consistent with theoretical evidence suggesting that higher host diversity leads to higher pathogen prevalence when the pathogen has a wide host range (Keesing et al. 2006), I showed that higher crop diversity within landscapes led to higher PVY prevalence. Additionally, the virus host/non-host status of crop species likely mediates this effect. Increased potato within landscapes led to increased PVY prevalence. This suggests that potato is an important source of inoculum within a landscape, and that growers should plant certified seed potato to limit spread. Despite being a host plant for several aphid vector species, barley, which is not a host of PVY, led to decreased PVY incidence. This suggests that the influence of barley on PVY inoculum was more important than its influence on vector communities for determining PVY prevalence, but additional studies should be conducted to assess this conclusion. Further, alfalfa, which is a non-host, did not show a detectable association with PVY incidence. However, alfalfa is a host of several important vector species of PVY, so future research should assess the impact of alfalfa on PVY transmission within landscapes. Knowledge of what crops are in the surrounding landscape can assist potato growers in determining their risk of PVY infection early in the season. Further, my finding that aphid species richness was positively associated with PVY

incidence underscores the importance of monitoring non-colonizing aphid vectors (i.e., vectors that probe but cannot colonize potato) for the prevention of PVY spread.

Future research should aim to describe mechanisms that are involved in the association of crop diversity with PVY prevalence. While differences in management between crop types may have influenced the amplification effect of crop diversity on PVY prevalence, another possible mechanism is increased vector movement resulting from non-consumptive effects of natural enemies. Indeed, crop diversity leads to reduced aphid densities via increased predation/parasitism (Bosem Baillod et al. 2017; Redlich et al. 2018), and there is evidence that predator/parasitoid-avoidance behaviors lead to increased virus transmission (Hodge et al. 2011; Finke et al. 2012; Crowder et al. 2019). Further research is needed to assess the influence of natural enemies on PVY transmission by aphid vectors within landscapes. From an applied perspective, my findings of crop species effects on PVY prevalence can assist the development of spatial risk-assessment models for PVY transmission by aphids during the growing season.

For the first time, I utilized high throughput molecular gut content analysis (GCA) to characterize host use patterns of aphid vector species of potato virus Y and found that aphids probed upon plants that are outside of their reported host ranges, including many plants that are presumed hosts of PVY. These findings provide novel evidence for the importance of non-colonizing vectors for PVY spread. Further, these findings provide additional evidence that aphids passively disperse within a landscape and probe many non-host plants while searching for a suitable host. Specific vector species that may be important for PVY spread in the San Luis Valley include *Diuraphis noxia* and *Capitophorus elaeagni*. Indeed, *D. noxia* probed upon *Solanum* during both years of study, and *C. elaeagni* probed upon many weed genera that are presumed hosts of PVY. This suggests that weed host plants of these aphid species, in addition to

weeds that are PVY host plants, should be considered when developing strategies for PVY management.

Future studies should analyze virus status of the aphids when conducting GCA. This could be achieved through simultaneously extracting DNA and RNA from aphid vectors. Alternatively, stylets could be dissected from aphids and utilized for RNA extraction and PVY testing, while aphid bodies could be utilized for DNA extraction and GCA. This type of study would provide more precise information on which plants within a landscape are important for PVY spread. And as *Brassica,* and *Erodium* were detected within vectors in my study, future studies should assess the importance of these plants on PVY prevalence within landscapes. Further, GCA has enormous potential to gain insights into plant – aphid vector interactions, and additional studies of GCA of aphid vectors should be conducted, particularly with focus on other pathosystems. Specifically, the risk of emerging aphid-vectored viruses could be estimated by predicting patterns of virus movement based on long-term datasets of host use patterns of aphid vector species. Another important knowledge gap that remains is how aphids acquire DNA from plants. Specifically, it is not known during what feeding behaviors aphids acquire plant DNA, although it seems likely that plant DNA acquisition occurs during intra- or intercellular probes (Tjallingii 1985; Cooper et al. 2016) without feeding from the phloem. Further, it is not known how long aphids retain plant DNA after acquisition, but this information would allow for more precise inference of plant – plant movement of aphids detected using GCA. Experiments combining electrical penetration graph (EPG) analysis and GCA could answer these questions.

I assessed the ability of the cannabis aphid (*Phorodon cannabis*) to transmit PVY and found that the cannabis aphid is a competent vector. And consistent with previous research (Blua and Perring 1992; Boquel et al. 2012; Eigenbrode et al. 2018), I found that PVY, a non-

persistently transmitted virus, altered cannabis aphid feeding behaviors in ways that would discourage host acceptance and encourage vector dispersal, which could favor virus spread. These findings show that PVY infection in hemp has the potential to pose a risk for potato production via transmission by cannabis aphids. Further, these findings provide additional support for the negative effect of non-persistent viruses on aphid performance (Mauck et al. 2012; Eigenbrode et al. 2018).

From an applied perspective, future research should identify the effect of PVY infection on yield and cannabinoid levels in hemp. This information is necessary to determine the risk that PVY poses for hemp cultivation. Further, the extent of viruses and viroids that the cannabis aphid is able to transmit should be assessed, as this aphid species is a common pest of hemp in the United States (Cranshaw et al 2019).

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APPENDIX

A.1 CHAPTER 2 Supplemental Information

Supplemental Table A1.1: Details of potato fields sampled for PVY from 2020 and 2021.

2020				
Field	**Potato Variety**	**Field Size (hectares)**	**Seed or Commercial[a]**	**End-of-season PVY Incidence (%)**
C-9	Norkotah 3	24.3	Seed	5
C-13	Norkotah 296	24.3	Commercial	15
RS-1	Rocky Mountain Russet	50.6	Commercial	0
8--15	Reveille Russet	24.3	Commercial	5
MV-4	?[b]	46.8	Commercial	5
MV-12	Norkotah 278	51.8	Seed	0
MV-16	Norkotah 3	16.3	Commercial	15
6N6E	Norkotah 296	49.7	?[b]	0
2021				
Field	**Potato Variety**	**Field Size (hectares)**	**Seed or Commercial**	**End-of-season PVY Incidence (%)**
C-9	Mesa Russet	25.0	Seed	5
C-13	Norkotah 296	24.3	Commercial	55
RS-1	Canela Russet	24.5	Commercial	5
8--5	Norkotah 278	49.4	Commercial	90
8--7	Norkotah 296	54.4	Commercial	80
8--15	Norkotah 296	24.4	Commercial	35
MV-4	Norkotah 296	51.7	Seed	5
MV-12	Norkotah 296	50.7	Commercial	25
MV-16	Norkotah 90	24.3	?[b]	10

[a]"Commercial" indicates that potatoes in that field were grown for commercial sale and "Seed" indicates that potatoes in that field were grown to be tested for re-certification as seed potatoes
[b]Question marks indicate an absence of information

Supplemental Table A1.2: Landcover types from RGWCD map within the study region, including crop species which were considered for analyses.

Landcover Types Within Study Region				
Alfalfa	Fallowed	Meadow/Pasture	Peas	Sudan Grass Hay
Alfalfa Grass	Grain	Native Grass Hay	Potatoes	Trees
Barley	Grass Cover Crop	Native Hay	Quinoa	Triticale Hay
Brush Pasture	Grass Hay	Native Hay Pasture	Reservoir	Vegetables
Canola	Grass Hay/Pasture	No Longer Farmed	Rye	Weeds
Carrots	Green Manure	Non-crop	Sainfoin	Wheat
Clover	Hemp	Oat Hay	Sorghum	Wildflowers
Corn	Lettuce	Oats	Spinach	Willows
CREP	Meadow	Pasture	Stubble	Willows/Other Trees
Landcover Types (Crop Species) Considered for Analyses[a]				
Alfalfa	Corn	Peas	Sainfoin	Triticale Hay
Barley	Hemp	Potatoes	Sorghum	Wheat
Canola	Lettuce	Quinoa	Spinach	
Carrots	Oats	Rye	Sudan Grass Hay	
Crop Species Found Within 3 km Buffers of Study Sites[b]				
Alfalfa	Carrots[*]	Peas	Rye	Sudan Grass Hay
Barley	Hemp	Potatoes	Sorghum[**]	Triticale Hay[**]
Canola	Lettuce	Quinoa	Spinach	Wheat[*]

[a]Landcover categorizations which allowed identification of individual crop species and which were considered for analyses

[b]Crop species within 3 km buffer of study sites from 2020 and 2021 used for landscape analyses

[*]Denotes presence of that crop species only in 2020

[**]Denotes presence of that crop species only in 2021

Supplementary Table A1.3: Daily weather parameters from May 1st – August 31st, 2020, and 2021 at the San Luis Valley Research Center weather station in Colorado.

Year	Month	Min. temp. (C)	Mean temp. (C)	Max. temp. (C)	Total Precipitation (mm)	Total Wind Run (Km)
2020	May	-2.0	13.5	27.4	10.2	8,286
	June	1.3	16.7	30.2	1.6	7,269
	July	2.9	17.4	31.1	51.6	5,179
	August	5.1	18.3	31.7	14.5	6,052
	May – August	-2.0	16.5	31.7	77.9	26,785
2021	May	-2.6	11.2	25.1	24.9	7,472
	June	2.0	16.6	33.3	17.6	5,253
	July	7.1	18.3	32.6	28.7	4,217
	August	4.9	17.8	30.2	5.9	5,161
	May – August	-2.6	16.0	33.3	77.1	22,103

Data were obtained from CoAgMET archives (https://coagmet.colostate.edu/table/daily/ctr01?units=m&from=2021-05-01&to=2021-08-31).

Supplemental Table A1.4: Logistic regression models used to test association between PVY incidence and landscape composition, and linear regression models used to test association between aphid abundance, species richness, and species evenness and landscape composition.

Logistic Regression Models[a]
Buffer Radius = 1 km
PVY Incidence ~ Shannon Diversity Index of Crops
PVY Incidence ~ % Potato + % Barley + % Alfalfa
Buffer Radius = 2 km
PVY Incidence ~ Shannon Diversity Index of Crops
PVY Incidence ~ % Potato + % Barley + % Alfalfa
Buffer Radius = 3 km
PVY Incidence ~ Shannon Diversity Index of Crops
PVY Incidence ~ % Potato + % Barley + % Alfalfa
Linear Regression Models[a]
Buffer Radius = 1 km
Aphid Abundance ~ Shannon Diversity Index of Crops
Aphid Abundance ~ % Potato + % Barley + % Alfalfa
Buffer Radius = 2 km
Aphid Abundance ~ Shannon Diversity Index of Crops
Aphid Abundance ~ % Potato + % Barley + % Alfalfa
Buffer Radius = 3 km
Aphid Abundance ~ Shannon Diversity Index of Crops
Aphid Abundance ~ % Potato + % Barley + % Alfalfa

[a]If significant correlations between landscape predictors were detected, variables were removed from the model

Supplemental Table A1.5: Results from spatial autocorrelation analysis using Moran's I.

	1 km		2 km		3 km	
	Moran's I	P-Value	Moran's I	P-Value	Moran's I	P-Value
	Shannon Diversity Index of Crops					
2020 Aphid Abundance	-0.19	0.60	-0.15	0.55	-0.13	0.52
2021 Aphid Abundance	-0.18	0.59	-0.06	0.43	-0.12	0.51
2020 Aphid Richness	0.04	0.34	-0.267	0.67	-0.70	0.96
2021 Aphid Richness	-0.03	0.40	-0.23	0.65	-0.60	0.93
2020 Aphid Evenness	-0.59	0.93	-0.68	0.96	-0.60	0.93
2021 Aphid Evenness	-0.07	0.45	0.12	0.24	-0.05	0.43
2020 PVY Incidence	-0.01	0.36	0.08	0.23	0.22	0.19
2021 PVY Incidence	-0.39	0.76	-0.54	0.87	-0.61	0.90
	Crop Species Models					
2020 Aphid Abundance	-0.31	0.72	-0.10	0.48	-0.08	0.46
2021 Aphid Abundance	0.26	0.10	-0.27	0.70	-0.41	0.85
2020 Aphid Richness	0.17	0.18	-0.242	0.65	-0.27	0.68
2021 Aphid Richness	0.03	0.32	-0.05	0.43	-0.05	0.43
2020 Aphid Evenness	-0.61	0.94	-0.65	0.96	-0.68	0.96
2021 Aphid Evenness	0.03	0.33	-0.27	0.69	-0.24	0.66
2020 PVY Incidence	0.14	0.24	-0.08	0.39	-0.08	0.39
2021 PVY Incidence	-0.41	0.77	0.02	0.13	0.04	0.28

Supplemental Table A1.6: Aphid community composition from 2020 and 2021.

2020		
Aphid Species/Genus Group	**Number of Individuals**	**Percent of Total Aphid Community**
Acyrthosiphon spp.	8055	47.3
Phorodon cannabis	3362	19.7
Myzus persicae	1768	10.4
Protaphis middletonii	466	2.7
Aphis spp.	331	1.9
Rhopalosiphum spp.	296	1.7
Metopolophium dirhodum	216	1.3
Aphis craccivora	161	0.9
Capitophorus elaeagni	160	0.9
Pemphigus sp.	61	0.4
Hayhurstia atriplicis	57	0.3
Lipaphis pseudobrassicae	46	0.3
Nearctaphis spp.	45	0.3
Diuraphis noxia	27	0.2
Therioaphis trifolii	19	0.1
Tinocallis saltans	10	0.1
Myzocallis sp.	8	0.05
Sitobion avenae	6	0.04
Uroleucon spp.	4	0.02
Capitophorus hippophaes	3	0.02
Sipha elegans	3	0.02
Illinoia spp.	2	0.01
Periphyllus spp.	2	0.01
Aphis fabae	1	0.01
Hyperomyzus sp.	1	0.01
Iziphya sp.	1	0.01
Macrosiphum sp.	1	0.01
Mindarus sp.	1	0.01
Pachypappa sp.	1	0.01
Damaged[a]	1826	10.7
?[b]	81	0.5
Apterous[c]	8	0.05
2021		
Aphid Species/Genus Group	**Number of Individuals**	**Percent of Total Aphid Community**
Myzus persicae	805	25.4
Protaphis middletonii	601	19.0
Capitophorus elaeagni	400	12.6
Pemphigus sp.	237	7.5

Aphis spp.	208	6.6
Rhopalosiphum spp.	176	5.6
Hayhurstia atriplicis	138	4.4
Nearctaphis spp.	79	2.5
Acyrthosiphon spp.	67	2.1
Lipaphis pseudobrassicae	61	1.9
Metopolophium dirhodum	44	1.4
Hyperomyzus sp.	27	0.9
Capitophorus hippophaes	23	0.7
Uroleucon spp.	22	0.7
Aphis craccivora	16	0.5
Tinocallis saltans	12	0.4
Therioaphis trifolii	10	0.3
Myzocallis sp.	6	0.2
Mindarus sp.	5	0.2
Chaitophorus sp.	4	0.1
Cavariella sp.	3	0.1
Illinoia spp.	3	0.1
Pleotrichophorus sp.	3	0.1
Sitobion avenae	3	0.1
Tuberculatus sp.	2	0.1
Brevicoryne brassicae	1	0.03
Durocapillata utahensis	1	0.03
Eriosoma sp.	1	0.03
Iziphya sp.	1	0.03
Macrosiphum sp.	1	0.03
Obtusicauda sp.	1	0.03
Pachypappa sp.	1	0.03
Damaged[a]	175	5.5
?[b]	30	0.9
Apterous[c]	2	0.1

[a]"Damaged" indicates specimens that were too damaged for morphological identification
[b]Question marks indicate specimens that were not able to be identified
[c]"Apterous" indicates apterous aphid specimens; these specimens were not identified

Supplemental Table A1.7: Results from linear regression of aphid species richness and percent cover of potato, barley, and alfalfa for 2020 (n=10) and 2021 (n=10).

			Potato		**Barley**[a]		**Alfalfa**	
	df	AICc	Estimate	P-value	Estimate	P-value	Estimate	P-value
2020 Aphid Richness								
1 km	4	55.2	0.14	0.06	-	-	*0.20*	*0.02*
2 km	4	60.5	0.07	0.56	-	-	0.16	0.18
3 km	4	60.3	0.09	0.50	-	-	0.20	0.18
2021 Aphid Richness								
1 km	5	70.2	0.01	0.94	-0.03	0.80	0.08	0.39
2 km	5	70.5	-0.10	0.49	0.11	0.47	0.02	0.86
3 km	5	64.9	-0.23	0.06	0.17	0.10	-0.1	0.56

[a]Percent cover of barley in 2020 was significantly correlated with other landscape predictors, so those data were not included in models

Italicized values indicate a significant association ($P < 0.05$)

Supplemental Table A1.8: Results from linear regression of log-transformed aphid abundance and Shannon Diversity Index of crop species (top) and percent cover of crops (potato, barley, alfalfa) (bottom) for 2020 (n=10) and 2021 (n=10).

	df	AICc	Estimate	P-value
2020 Aphid Abundance				
1 km	3	17.2	0.02	0.96
2 km	3	17.1	-0.10	0.77
3 km	3	15.2	-0.51	0.22
2021 Aphid Abundance				
1 km	3	25.2	-0.25	0.75
2 km	3	25.1	0.27	0.69
3 km	3	25.4	0.03	0.95

	Potato				**Barley**[a]		**Alfalfa**	
	df	AICc	Estimate	P-value	Estimate	P-value	Estimate	P-value
2020 Aphid Abundance								
1 km	4	21.8	0.01	0.39	-	-	0.01	0.34
2 km	4	22.3	-0.01	0.61	-	-	0.001	0.93
3 km	4	22.4	-0.01	0.69	-	-	0.002	0.94
2021 Aphid Abundance								
1 km	5	28.0	0.02	0.28	0.01	0.46	*0.030*	*0.025*
2 km	5	33.2	0.01	0.51	-0.01	0.77	0.04	0.09
3 km	5	35.5	0.01	0.65	-0.01	0.66	0.04	0.15

[a]Percent cover of barley in 2020 was significantly correlated with other landscape predictors, so those data were not included in models

Italicized values indicate a significant association ($P < 0.05$)

Supplemental Table A1.9: Results from linear regression of aphid species evenness and Shannon Diversity Index of crop species (top) and percent cover of crops (potato, barley, alfalfa) (bottom) for 2020 (n=10) and 2021 (n=10).

	df	AICc	Estimate	P-value
2020 Aphid Evenness				
1 km	3	-18.8	-0.09	0.13
2 km	3	-16.3	-0.04	0.50
3 km	3	-15.7	-0.01	0.92
2021 Aphid Evenness				
1 km	3	-8.0	0.01	0.94
2 km	3	-9.2	-0.11	0.35
3 km	3	-8.0	0.00	0.97

		Potato			**Barley**[a]		**Alfalfa**	
	df	AICc	Estimate	P-value	Estimate	P-value	Estimate	P-value
2020 Aphid Evenness								
1 km	4	-10.8	-0.001	0.80	-	-	0.001	0.74
2 km	4	-10.6	-0.001	0.70	-	-	0.001	0.83
3 km	4	-10.4	-0.001	0.71	-	-	0.0004	0.93
2021 Aphid Evenness								
1 km	5	-0.1	0.001	0.89	0.00	0.88	-0.003	0.27
2 km	5	1.2	-0.001	0.76	0.00	0.68	-0.01	0.15
3 km	5	1.6	-0.003	0.57	0.004	0.32	-0.01	0.13

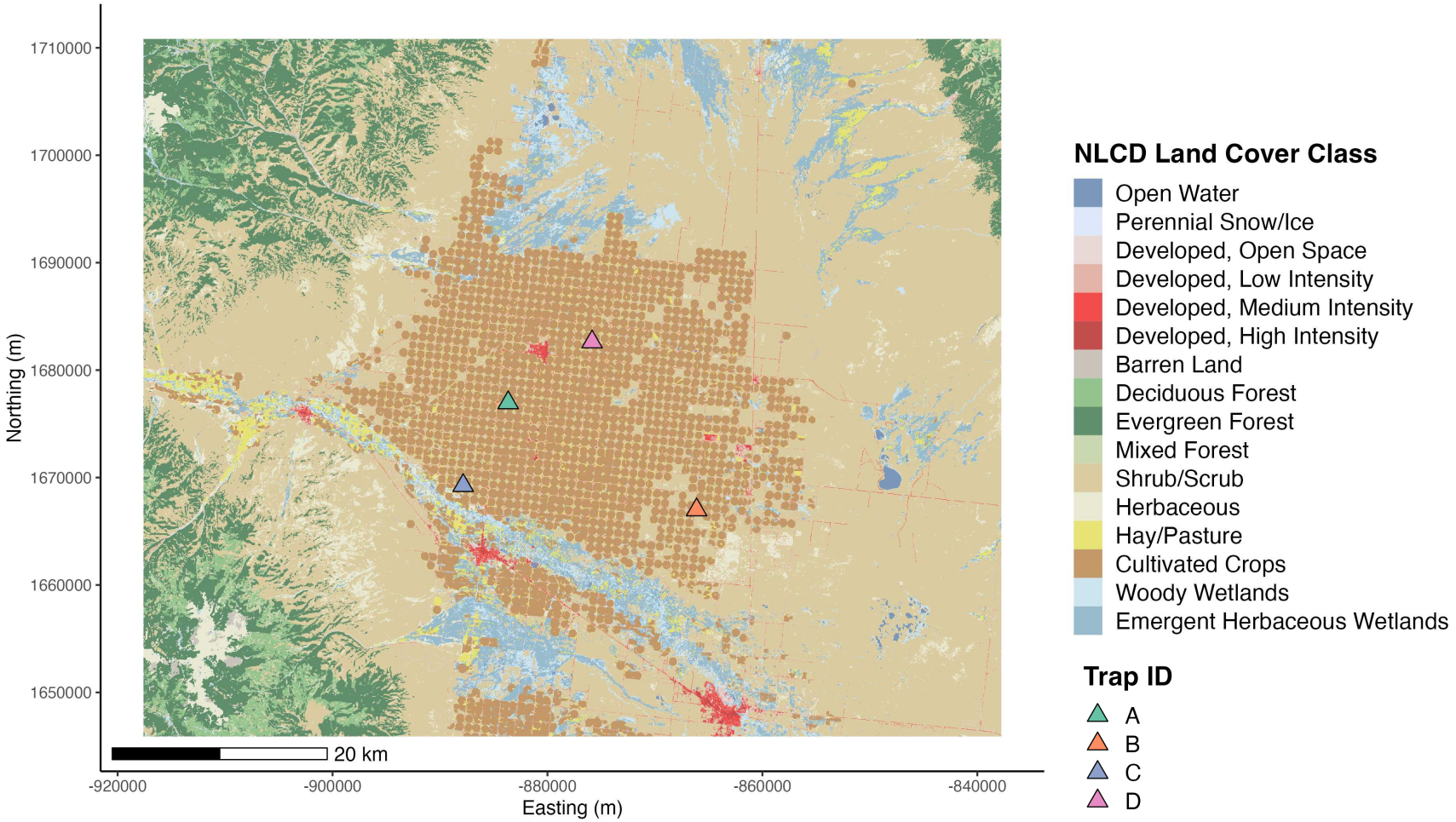
[a]Percent cover of barley in 2020 was significantly correlated with other landscape predictors, so those data were not included in models

A.2 CHAPTER 3 Supplemental Information

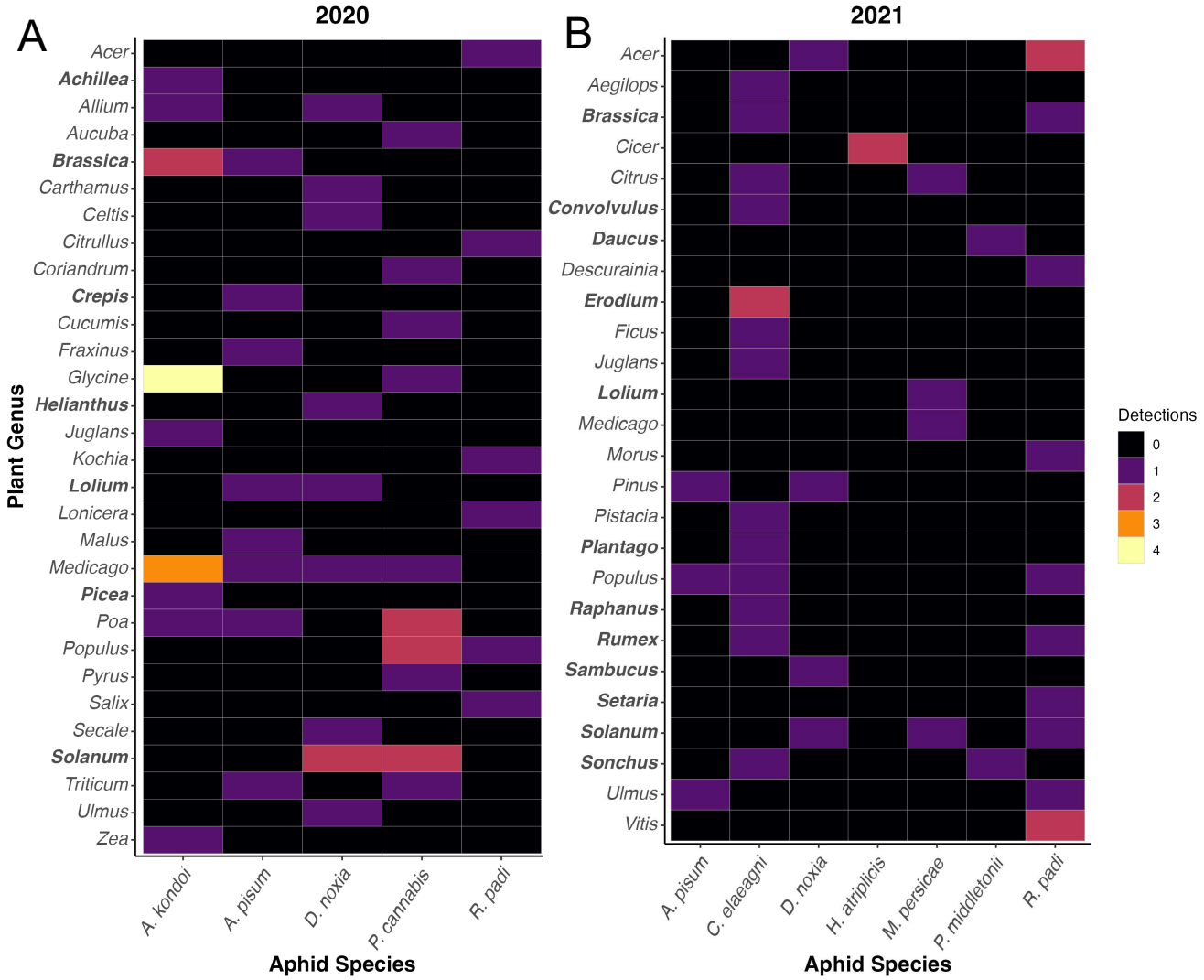
Supplemental Table A2.1: Summary of the number of individual aphids which were sequenced and which probed.

Vector Species	Number of individuals sequenced	Number of individuals that probed[a]	Number of individuals that probed more than one plant	Proportion of individuals that probed more than one plant
Acyrthosiphon kondoi	20	10	4	0.40
Acyrthosiphon pisum	32	11	0	0.00
Capitophorus elaeagni	20	9	2	0.22
Diuraphis noxia	31	11	2	0.18
Hayhurstia atriplicis	10	2	0	0.00
Myzus persicae	10	3	1	0.33
Phorodon cannabis	30	10	2	0.20
Protaphis middletonii	7	2	0	0.00
Rhopalosiphum padi	40	14	3	0.21
Total	200	72	14	0.19

[a]Excluding *Cannabis, Beta,* and plant genera with < 6 reads



Supplemental Figure A2.1: Map of study area in the San Luis Valley, CO. Land cover data (30 x 30 m) represent land cover types present in 2019 and were obtained from the USGS National Land Cover Database (Dewitz and USGS 2021). Trap A was present during 2020 and 2021 but traps B, C, and D were present only during 2021. Figure was constructed in R Studio (R Core team 2022) using the packages 'sp', 'tidyverse', 'ggspatial', 'terra', 'raster', 'FedData', 'ggnewscale', and 'ggpubr' (Bivand et al. 2013; Wickham et al. 2019; Dunnington 2023; Hijmans 2023a; Hijmans 2023b; Bocinsky 2023; Campitelli 2023; Kassambara 2023).



Supplemental Figure A2.2: Heatmap displaying sum of binary detections of each plant genus for each aphid vector species during 2020 (**A**) and 2021 (**B**). Sample size refers to the number of individual aphids that returned sequences. Plant genera that are bolded are presumed host plants of PVY (**Table 3.2**).

A.3 CHAPTER 4 Supplemental Information

Supplemental Table A3.1: Number of individual aphids, excluding the mother aphid, on each potato and control (hemp) plant from population growth experiments.

	Number of Individuals			
	Day 1	**Day 2**	**Day 3**	**Day 4**
Biological Rep 1				
Potato plant 1	-	-	3	0
Potato plant 2	-	-	0	0
Potato plant 3	-	-	1	0
Potato plant 4	-	-	0	0
Potato plant 5	-	-	2	0
Potato plant 6	-	-	1	0
Control plant (Hemp)	-	-	10	9
Biological Rep 2				
Potato plant 1	0	0	-	-
Potato plant 2	0	0	-	-
Potato plant 3	0	0	-	-
Potato plant 4	0	0	-	-
Control plant (Hemp)	5	5	-	-

Dashes indicate an absence of observations for the specified time period. Mother aphids on potato from biological rep 2 did not produce any offspring and did not survive longer than two days

Supplemental Table A3.2: Feeding behaviors (potential drops or pd and probing behaviors) (mean ± SE) pertaining to virus transmission by *Phorodon cannabis* on hemp and potato in the initial 30 minutes of recording.

Parameters	Hemp				Potato	
	Non-viruliferous (n=21)	Viruliferous (n=21)	*U*	p-value	Non-viruliferous (n=20)	Viruliferous (n=2)[a]
Probing behaviors						
Time to first probe from start of EPG (sec)	**209.20 ± 28.34**	**474.49 ± 88.12**	***140***	**0.04**	230.06 ± 30.54	205.25 ± 77.84
Duration of first probe (sec)	375.73 ± 115.34	502.90 ± 134.46	*198*	0.58	814.66 ± 135.19	1007.96 ± 664.63
Number of probes	3.10 ± 0.50	3.05 ± 0.65	*240.5*	0.62	2.20 ± 0.28	2.00 ± 1.00
Intracellular punctures						
Mean duration of pd (sec)	5.38 ± 0.24	4.95 ± 0.15	*233*	0.38	5.14 ± 0.23	4.43 ± 0.09
Average number of pd per probe	5.17 ± 0.82	4.77 ± 0.80	*217*	0.64	3.89 ± 0.78	8.17 ± 6.83
Number of pd	10.33 ± 1.49	9.10 ± 1.39	*243.5*	0.57	6.15 ± 1.08	9.50 ± 5.50
Total duration of pd (sec)	59.98 ± 7.12	45.83 ± 7.22	*253*	0.15	33.78 ± 5.87	42.52 ± 25.16
Time from the beginning of the 1st probe to first pd (sec)	192.11 ± 56.20	117.30 ± 42.18	*236*	0.33	210.69 ± 79.09	27.42 ± 10.18
Time from the end of the last pd to the end of the probe (sec)	271.27 ± 89.36	276.57 ± 92.66	*220*	0.59	368.41 ± 95.27	92.57 ± 88.26
Total duration of subphase II1 for the pd (sec)	21.15 ± 2.54	19.11 ± 3.07	*195*	0.69	11.96 ± 2.09	17.12 ± 9.94
Total duration of subphase II2 for the pd (sec)	13.34 ± 1.74	10.33 ± 1.81	*223.5*	0.21	5.97 ± 1.12	9.00 ± 4.04
Total duration of subphase II3 for the pd (sec)	25.01 ± 3.06	19.26 ± 2.90	*227.5*	0.17	15.46 ± 2.88	16.40 ± 11.18

[a]Due to the low sample size of viruliferous aphids on potato, statistical comparisons were not made between these aphids and non-viruliferous aphids on potato

P-values calculated according to the Mann-Whitney *U* Test, with bolded values indicating significant differences ($P<0.05$)