Received: 14 July 2022 | Accepted: 20 January 2023

DOI: 10.1111/afe.12564

ORIGINAL ARTICLE

Agricultural and Forest Entomology

Royal Entomological Society

Risk assessment for non-crop hosts of pea enation mosaic virus and the aphid vector *Acyrthosiphon pisum*

Robert E. Clark[1,2] | **Saumik Basu**[1] | **Sanford D. Eigenbrode**[3] | **Liesl C. Oeller**[1] | **David W. Crowder**[1]

[1]Department of Entomology, Washington State University, Pullman, Washington, USA

[2]EcoData Technology, Plantsville, Connecticut, USA

[3]Department of Entomology, Plant Pathology, and Nematology, University of Idaho, Moscow, Idaho, USA

Correspondence
Robert E. Clark, Department of Entomology, Washington State University, Pullman, WA, USA.
Email: robert.e.clark@wsu.edu

Funding information
USDA-NIFA, Grant/Award Numbers: 2022-67013-36422, 2017-67013-26537, 2017-07156

Abstract

1. Viral insect-borne plant pathogens have devastating impacts in agroecosystems. Vector-borne pathogens are often transmitted by generalist insects that move between non-crop and crop hosts. Insect vectors can have wide diet breadths, but it is often unknown which hosts serve as pathogen reservoirs and which non-crop host harbours the highest density of vectors.
2. In the Pacific Northwest USA, the pea aphid (Acyrthosiphon pisum) is a key virus vector in pulse crops. Despite pea aphid having a large number of potential non-crop plant hosts occuring in the region, no reservoir has yet been identified for the economically-costly pathogen Pea Enation Mosaic Virus (PEMV).
3. We addressed these issues by linking field surveys of an aphid vector and plant virus with statistical models to develop risk assessments for common non-crop legumes; in 2018, we completed a 65-site survey where aphids were surveyed in weedy legumes within and outside dry pea fields.
4. We quantified the abundance of pea aphids on 17 hosts, and plant tissue was tested for PEMV. Relatively high densities of A. pisum were found in habitats dominated by hairy vetch (Vicia villosa), which was the only legume other than cultivated dry pea where PEMV was detected.
5. Our results indicate that V. villosa is a key alternative host for PEMV, and that pest management practices in this region should consider the distribution and abundance of this weedy host in viral disease mitigation efforts for pulses.

KEYWORDS
aphids, legumes, non-crop hosts, plant viruses, pulse crops, reservoirs

INTRODUCTION

Plant viruses cause an average 10% reduction in global agricultural productivity, which translates to global economic losses of more than US $30 billion annually (Jones, 2021; Strange & Scott, 2005). Circulative-transmitted viruses require an insect vector, often phloem-feeding hemipterans like aphids (Hogenhout, Ammar, Whitfield, & Redinbaugh, 2008; Power, 2000). Despite the importance of vector-borne plant viruses in agriculture, our ability to predict virus occurrence across time and space remains poor for most pathosystems. Many vectors are generalists with a broad host range that includes food and cover crops, invasive agricultural weeds, and native plants (Bommarco, Wetterlind, & Sigvald, 2007; Mueller, Groves, & Gratton, 2012). Identifying host

reservoirs is key to determine the source(s) of vector-borne pathogens that can outbreak in a crop system (Gobatto et al., 2019; Peterson, 2018).

Like their vectors, many crop viruses occupy alternative hosts before infecting crop plants (Norris & Kogan, 2005). Non-crop hosts have been established as reservoirs for insect vectors and vector-borne pathogens that infect annual crops such as wheat, corn, and rice (Rashidi et al., 2020; Wu, Zhang, Ren, & Wang, 2020). The replication and spread of a plant virus across multiple hosts depend on the compatibility and coordinated interactions of virus- and host-encoded proteins, and the severity of infection often differs among hosts (Heinlein, 2015, Basu et al. 2018). Assessing whether certain hosts act as reservoirs of pathogens can be difficult, especially if alternative hosts do not show clear signs of infection (Lucas, 2006; Takahashi, Fukuhara, Kitazawa, & Kormelink, 2019). When infection symptoms are not easily observed in the field, management strategies for crop pathogens rely on detection through molecular diagnostic which test plant (Rageshwari, Renukadevi, Malathi, Amalabalu, & Nakkeeran, 2017). Understanding how pathogens and vectors move among distinct populations of hosts is also an important component of effective management of crop diseases.

When a non-crop host for pathogens or vectors is identified, integrated pest management (IPM) strategies suggest targeted removal to prevent crop infection (Catton, Lalonde, & De Clerck-Floate, 2015; Macharia, Backhouse, Wu, & Ateka, 2016). For example, management of wheat stem rust relies on control of the pathogen's alternative host American barberry (*Berberis canadensis*), a strategy that dates back almost a century (Peterson, 2018). Non-crop host removal can be difficult if these host plants are also weeds, however, particularly those that emerge early in seasons before crops are established (Norris & Kogan, 2005). Consequently, some agricultural weeds and cover crops allow pest insect populations to increase before moving into crops, exacerbating outbreaks (Wenninger, Dahan, Thornton, & Karasev, 2019). For example, Colorado potato beetle is observed feeding on horse nettle before moving into potato (Mena-Covarrubias, Drummond, & Haynes, 1996), and two spotted spider mites disperse from weeds to cotton (Norris & Kogan, 2005; Wilson, 1995).

Movement of generalist vectors between non-crop and crop hosts can mediate the spread of pathogens (Davis, Wu, & Eigenbrode, 2015; Power, 1991; Srinivasan, Alvarez, Bosque-Pérez, Eigenbrode, & Novy, 2008). Aphids that migrate over long distances often establish population in crops rapidly in the spring months as non-crop hosts senesce, which is often accompanied by high prevalence of virus-infected plants (Clement, Husebye, & Eigenbrode, 2010; Reynolds, Chapman, & Harrington, 2006). Aphids with long-distance dispersal capability as alates can also complicate management efforts because outbreaks occur at regional scales (Mueller et al., 2012; Powell, Tosh, & Hardie, 2006). For these reasons, aphid-borne viruses are hard to track, and outbreaks are often unpredictable, hampering pest management (Damgaard, Bruus, & Axelsen, 2020). To address such challenges, identification of local sources of aphid-borne pathogens can be of great value in guiding optimal and cost-effective control strategies, such as removal of weedy reservoirs near crop fields, mitigating damage in outbreak years. To this end, the goal of our study was to track and quantify potential non-crop hosts for a problematic pathogen, PEMV or pea enation mosaic virus, which occurs in our study region of eastern Washington state and Northern Idaho, USA.

METHODS

Study system

The pea aphid *Acyrthosiphon pisum* is a frequent pest of pulse crops that acts as a vector for several economically important pathogens, including PEMV (Chatzivassiliou, 2021; Rashed et al., 2018). Plants infected with PEMV produce a range of species-specific symptoms, with malformed pods ultimately reducing yield (Clement et al., 2010). Extreme outbreaks can lead to up to 40% yield loss in pulses (Elbakidze, Lu, & Eigenbrode, 2011; Paudel, Bechinski, Stokes, Pappu, & Eigenbrode, 2018). In addition to dry pea (*Pisum sativum*), PEMV infects crops and weeds like alfalfa (*Medicago sativa* L.), yellow sweet clover (*Melilotus officinalis* L.), white sweet clover (*Melilotus albus* L.), wild white clover (*Trifolium repens* L.), common vetch (*Vicia sativa* L.), hairy vetch (*Vicia villosa* Roth), and broadbean (*Vicia faba* L.) (McEwen, Schroeder, & Davis, 1957). Pea aphids acquire PEMV from a few perennial legume hosts and agricultural weeds (Hull, 1981). However, *A. pisum* diet-breadth encompasses most of the Fabaceae (Peccoud, Ollivier, Plantegenest, & Simon, 2009), suggesting the diversity of PEMV-compatible hosts could be large.

Survey design

We conducted field surveys from May to July 2018 during an outbreak season of *A. pisum*. Pea aphids and virus have been historically monitored in eastern Washington and Idaho using a long-term trapping network for 17 growing seasons (https://www.legumevirusproject.org). In this trapping scheme, at least 10 locations have three pan traps placed at field edges starting after spring peas are planted (May). Pan traps contained propylene glycol for capturing alate aphids, and these were sampled biweekly. Sampling ceased when dry peas in the region complete pod development and are too desiccated to support aphid populations. All pan-trap collected, alate aphids were counted, and tested for viral pathogens, including PEMV. In this trapping network, the 2018 season had the second highest alate arrival counts on a per-trap basis over this entire period (Figure S1). This so-called 'outbreak year' thus provided an opportunity to discover the non-crop hosts for *A. pisum* and PEMV in a season when aphids are widespread, thus we targeted sampling at areas with patches of weedy legumes in 60 sites (30 locations >1 km apart, each with two repeated visits but samples taken 150 m apart). Plant and aphid communities were sampled in two climatic ecoregions: Palouse Prairie, a high-elevation grassland predominately converted to dryland wheat production (Looney & Eigenbrode, 2012) and shrub-steppe, a habitat found at lower elevations and warm slopes adjacent to the Palouse region (predominately along the Snake River in Washington and Idaho) (Knick & Rotenberry, 1997). Both habitat types harbour a diverse community

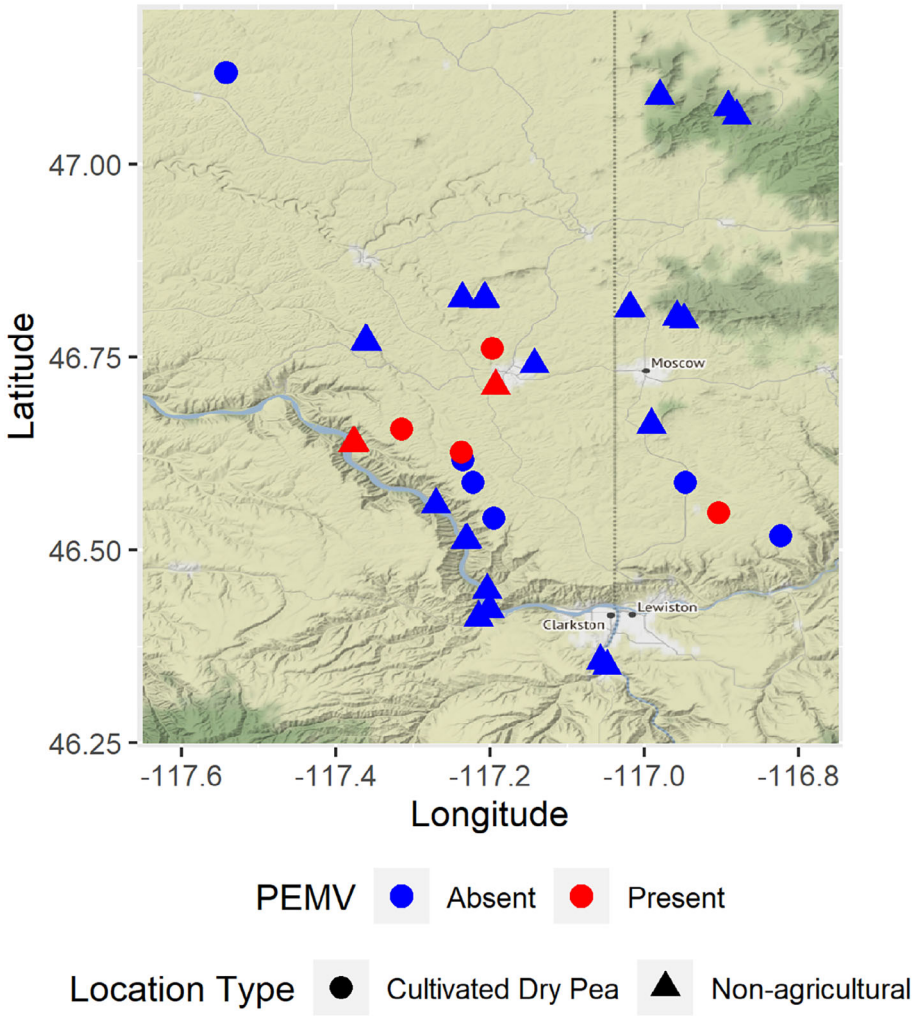

FIGURE 1 Sampling locations for each unique crop and non-crop site. All cultivated dry pea fields were spring-planted fields in rotation with cereals. Non-agricultural sites included open public lands or lands that gave permission to sample. Colour indicates presence or absence of PEMV at a given transect. Repeated sampling locations 150–250 m in proximity not shown to prevent overlapping points on the map

of herbaceous legumes and are purported sources of pea aphid outbreaks (Clement, 2006). All non-agricultural sites were in either roadside edges, native prairie, or shrub-steppe. Agricultural sites were spring-planted pea fields on the lower Palouse in Whitman Co. Washington and Latah Co. Idaho between 47.46° N and 46.33° N (Figure 1).

Aphid, plant, and virus surveys were conducted using a line-transect (Figure S2). At each of sites sampled, we ran 20 m line transects and quantified plant diversity (species identity) of all forbs touching the line transect; forb percent cover was calculated by measuring the length of the line transect (in cm) covered by plant material. At each transect, we collected canopy arthropods using two 180° sweeps through the foliage; insects collected were stored in 95% ethanol until identification to species. Samples of aboveground terminal leaf tissue of legume species overlapping the meter-line transect were harvested, wrapped in aluminium foil, frozen in liquid N_2, and held on dry ice before storing at −80°C. These tissue samples were used to determine the presence of PEMV.

PEMV-1 detection in plants by RT-PCR

To test all crop and non-crop legumes for PEMV, we used a two-stage protocol (Sint et al., 2016). First, we tested for PEMV by using reverse transcription-polymerase chain reaction (RT-PCR) from pooled samples of all tissue collected from each transect ($n = 60$). Subsamples of tissue from each plant, regardless of species, were pooled and ground into fine powder under liquid N_2 by mortar and pestle (Chisholm, Sertsuvalkul, Casteel, & Crowder, 2018) and combined into a transect-wide mix. Second, if PEMV was detected in the pooled sample, the remaining tissue from all host plants was tested individually. This method allows efficient scoring of each of plant in a sample for the presence PEMV while avoiding unnecessary and costly sampling of individual plants if the entire population is free of the virus.

For detection of PEMV from plant tissue samples, 100 mg of homogenized tissue was run through Promega SV total RNA isolation kits (Promega), producing cDNA from 1 µg of total RNA using Bio-Rad iScript cDNA synthesis kits (Lee, Clark, Basu, & Crowder, 2021). Then

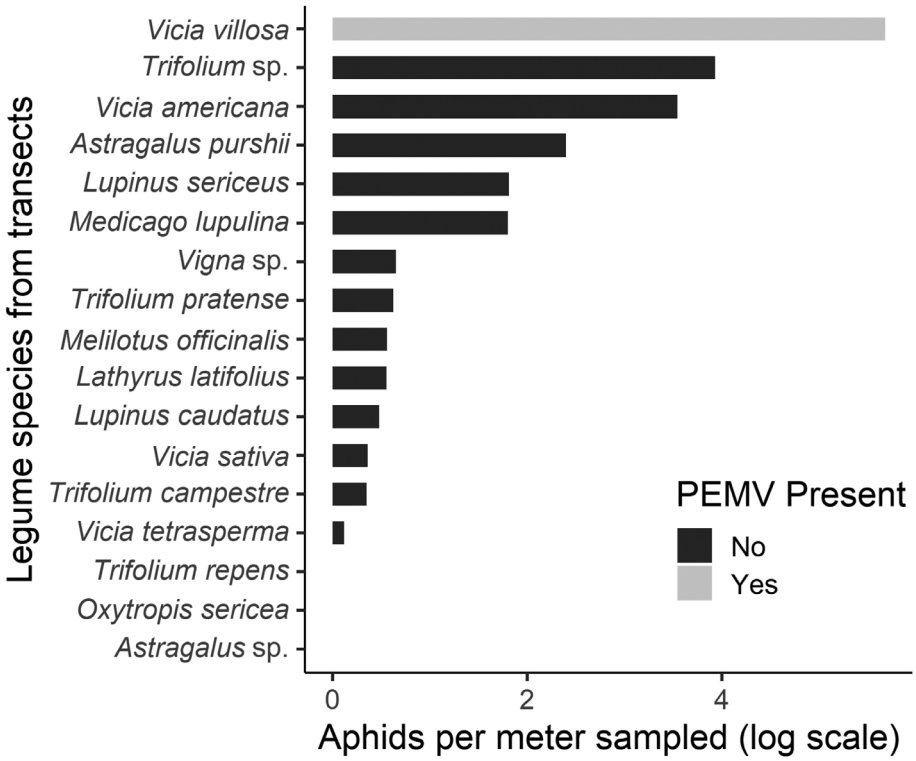

FIGURE 2 Cumulative aphid counts per meter of sampled plants (log transformed). Bar length equals the total abundance of aphids divided by the total metres covered by each individual host plant. Bar colours indicate whether a host plant was discovered with PEMV through RT-PCR. Six host plant species are not shown as they occurred only incidentally in a single transect and did not have aphids or PEMV

RT-PCR was performed using PEMV-1 coat protein specific primers (PEMV CP FP: 5′ GTGGTGGCACCCTCTATG 3′; PEMV CP RP: 5′ GTGTCCACATGGTAGGCTATG 3′). Primers were designed using the IDT Primer Quest Tool for RT-PCR reaction (10 μL) containing 3 μL of ddH2O, 5 μL of dream Taq mastermix (Thermo Scientific, Waltham, MA, usa), 1 μL of diluted primer mix (forward and reverse [concentration 10 μM]), and 1 μL of cDNA template. The RT-PCR program included an initial denaturation for 5 min at 95°C followed by 21 cycles of denaturation at 95°C for 30 s, annealing for 30 s at 56°C, and extension for 45 s at 72°C and final extension of 10 min at 72°C. After PCR was complete, agarose gels (1%) were run at 90 v for 45 min, after which gel pictures were taken in a documentation system (Bio-Rad, Hercules, CA). For one large population of hairy vetch that contained PEMV (Wawawei Park Road, 46.630, −117.378), we revisited the site later in the season and sampled living, adjacent hairy vetch population, validating that PEMV was indeed persistent in this location via collecting. These five additional *V. villosa* samples were processed to rule out contamination as the cause of PEMV detection at this site.

Statistical analyses

All data analyses were completed using R version 4.1.2 (R Development Core Team, 2021) using base functions unless otherwise specified. For analyses of plant and aphid data, we used generalized linear mixed models (GLMM) applying the 'lme4' package (Bates, Maechler, Bolker, & Walker, 2015); model estimates and P-values were extracted using the 'car' package (Fox & Weisberg, 2011). For plotting results and post hoc tests, we used the 'emmeans' package (Lenth, 2016). Aphid counts, or cumulative abundance models used a negative-binomial link function appropriate for zero-inflated count data. These abundance data were then transformed for plotting by dividing abundance estimates by total host plant area (Figure 2). Probability of aphid presence in transects was modelled as the ratio of presence and absence among sites (Figure 4). Statistical analyses for line transects used 'site' as a random effect. Analyses of pooled long-term monitoring data from https://www.legumevirusproject.org/ were completed using a GLMM with a negative binomial link function. These source data were comprised of samples from a minimum of 30 pan traps monitored weekly over a 17-year survey period (Figure S1).

RESULTS

Among all transects, we collected 15,289 *A. pisum* aphids in total and assayed 1076 candidate plant tissue samples for PEMV. In our transects, we recorded 145 species of annual plants, of which 23 were in the family Fabaceae. We observed a range of abundances of aphids on non-crop hosts (Figure 2) and abundance of non-crop legumes (Figure 3).

Hairy vetch had the highest abundance of pea aphids and was the most abundant non-crop, weedy legume (Figure 3). At the community level, increasing coverage of vetch in transects was related to a greater likelihood of pea aphid presence (GLMMs, $\chi^2 = 15.02$, $p < 0.0001$, Figure 4). Notably, adjacent habitats also had high

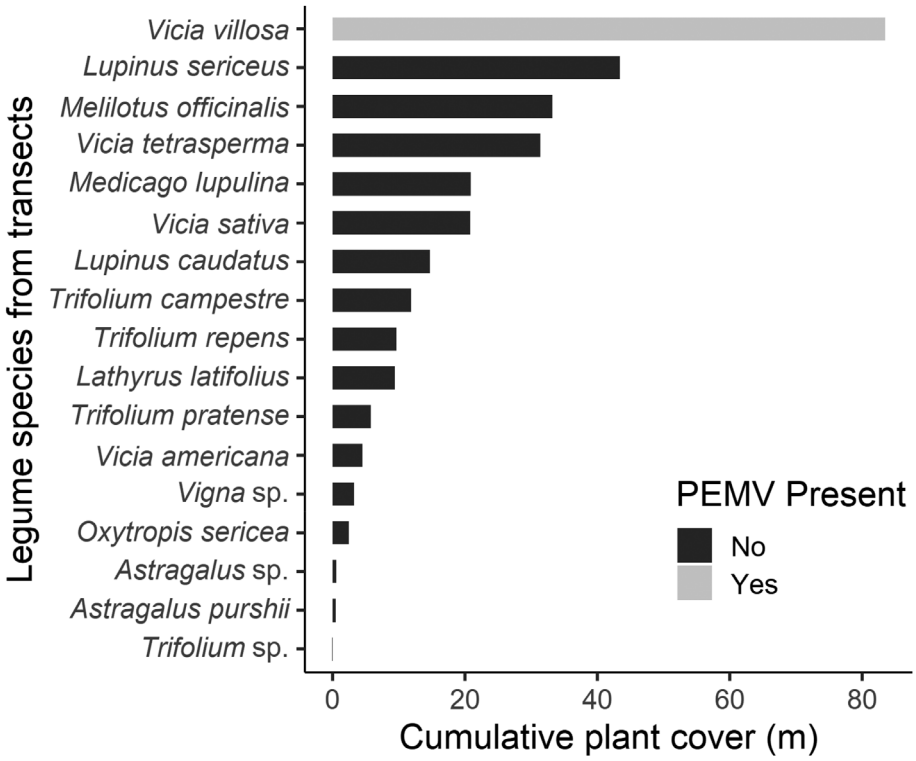

FIGURE 3 Cumulative plant coverage for non-crop legumes found among all surveys; hairy vetch was the most common. Bar length indicates the cumulative coverage among our sites

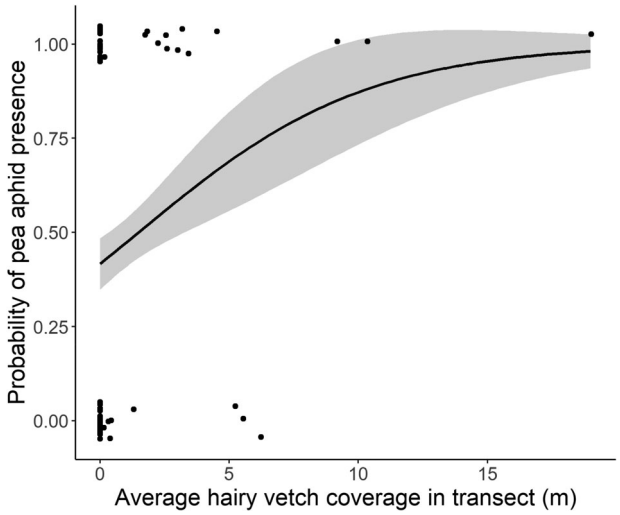

FIGURE 4 Probability predictions from GLMM (binomial fit) for pea aphid presence or absence in transects fitted to the abundance of non-crop host hairy vetch. The line indicates estimates means from GLMM, and the shaded area indicates the standard error of those model predictions. As hairy vetch coverage increased, aphids were more likely to be present in plant communities

populations of hairy vetch colonized by pea aphids upon subsequent revisit dates (Figure S3). Finally, PEMV was only detected in hairy vetch (Figure S4) and crop (dry pea) sites colonized by pea aphids, but not in other hosts.

DISCUSSION

Effective prediction of viral plant pathogen outbreaks requires a detailed understanding of vector and pathogen movement from crop to non-crop hosts at the landscape scale (Srinivasan et al., 2008). While our results are limited to a single field season, the first step in risk assessment is evaluating potential alternative hosts during an outbreak (Holt, Colvin, & Muniyappa, 1999). During an outbreak year for pea aphids, hairy vetch plants were suitable and heavily occupied alternative hosts for pea aphids and is a competent host for PEMV. While other alternative hosts may be found with additional surveys, we found that hairy vetch has high densities in non-agricultural environments, and it is conventionally used in the western U.S. as a cover crop (Luna, Mitchell, & Shrestha, 2012). Our surveys of plant communities in habitats adjacent to pea fields suggest that there are at least 23 potential hosts that can be resampled in future years, and the absence of aphids or PEMV does not rule them out as compatible hosts for either.

Our understanding of pea aphid and PEMV outbreaks in the Palouse considers that pea aphids likely colonize Palouse agroecosystems following wind currents from the Columbia Basin and Willamette Valley, where milder winters allow aphids to overwinter on alfalfa and clover (Clement et al., 2010; Hampton, 1983). Genetic data show that the pea aphid biotype found on dry pea in the Palouse has shared markers with biotypes collected in these areas (Eigenbrode et al., 2016). Our study suggests two possibilities that align with this information. First, hairy vetch, which emerges early and in low

elevation areas that warm up early in the growing season, is an effective 'stopover' host for aphid alates dispersing from warmer western regions to the Palouse. Second, hairy vetch occurs in relatively warm microhabitats along the edge of the Palouse and in the lower elevations in the Columbia Basin, and aphids may overwinter in these areas. Vetch is a facultative biennial with an above-ground rosette during ideal climatic conditions (Mischler, Duiker, Curran, & Wilson, 2010; Pokorny, Filbey, Kilian, Scianna, & Jacobs, 2020), and may have a small second generation in mesic habitats in the fall and winter (Clark personal observations). In either case, vetch may act as a short- (months) or long-term (years) reservoir for aphids and PEMV. In some years, PEMV-infected hairy vetch may provide inoculum for arriving aphids, contributing to more injurious infections associated with early transmission to pulse crops (Paudel et al., 2018). Furthermore, in years when infectious aphids arrive later in the season, if they colonize vetch, the pathogen can gain an overwintering foothold for possible infection of legume crops in the following growing season.

Once non-crop hosts for specific plant pathogens are discovered, management implications arise. Removing non-crop host plants could reduce the incidence of that pathogen in crops (Peterson, 2018; Strickland, Carroll, & Cox, 2020). In other systems, management of weeds may reduce pest populations in crops (Norris & Kogan, 2005). However, in many cases, removal of non-crop hosts may not be viable if they occur over large geographic regions or when movement of pests between hosts occurs over long distances, so local control would not prevent outbreaks. In Palouse agroecosystems, it is unclear if a weed removal strategy is tenable. Hairy vetch seeds are spread to produce cattle forage (Golden, Hogge, Hines, Packham, & Falen, 2016), possibly explaining how it persists at high abundance in the region. Hairy vetch also improves soil nitrogen, prevents erosion, but it is not listed as a noxious weed in this climatic zone (Mischler et al., 2010, Pokorny et al., 2020). Our results suggest that in this region, cover cropping of hairy vetch may increase PEMV outbreak risk in dry peas if in the same fields, but further work would be needed to verify if region-wide control would reduce likelihood of economic impacts of PEMV.

The optimization of pathogen detection from field samples also depends on precision and specificity of the procedure to ensure efficient and accurate detection of true positive samples (Yazdkhasti, Hopkins, & Kvarnheden, 2021). The use of more advanced molecular detection techniques, such as real time-PCR with much lower detection threshold can be used to detect pathogens with low titre (Rubio et al. 2020). Another key step towards improved pathogen detection is to maintain the quality and integrity of field samples by following proper collection technique in order to enhance detection of pathogens from field samples. Based on our observations, Hairy vetch did not exhibit any outward signs of infection by PEMV (e.g. Takahashi et al., 2019), meaning molecular diagnostics would remain necessary to track the spread of these pathogens in this reservoir.

Hairy vetch emerges earlier and hosts pea aphids earlier in the season compared to cultivated legumes. The phenological difference between weeds and crops suggest that a survey of PEMV in vetch may be available to predict seasonal prevalence of PEMV prior to crop emergence. Greenhouse work has demonstrated that pea aphid adults feeding on vetches with PEMV can then transmit these viral pathogens to dry pea (Clark, Basu, Lee, & Crowder, 2019). Sampling hairy vetch for aphids and PEMV may be a way to indicate if there are risks of large-scale, catastrophic outbreaks of PEMV likely to occur each year. While we only have one season of data reported here, PEMV and aphid populations go through large and difficult to predict population cycles, thus more data is necessary to predict population cycles (e.g. Northfield, Paini, Funderburk, & Reitz, 2008; Pernek et al., 2008). Consequently, it appears likely that if PEMV is found in April or early May in many hairy vetch populations along the lower Palouse, it would portend an areawide impact of PEMV in the growing season for pulse farmers. Similar strategies could be employed in other non-crop and crop source-sink dynamics systems where the non-crop host is a perennial plant that emerges earlier in the growing season.

AUTHOR CONTRIBUTIONS

REC, DWC, and SDE and conceived project design. REC and ECO completed surveys and data analysis. REC and SB completed molecular assays. SDE provided long-term aphid trap data. All authors wrote, edited, and approved the final manuscript.

ACKNOWLEDGEMENTS

We thank multiple undergraduate research technicians for their contributions to field surveys and molecular diagnostic work. These include D. Roach, L. Dean, L. Hope, S. McGranaghan, and R. McNannay. M. Brousil and I. Cranston aided in data manipulation. This work was supported by USDA-NIFA awards 2017-07156 & 2017-67013-26537 and completion of statistical analysis was supported for R. Clark by USDA-NIFA award 2022-67013-36422. We also thank public land managers and Washington & Idaho farmers for permission to collect plant samples on their property.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data and R code is available through a publicly available GitHub repository curated by REC at https://github.com/robclark19/vetch2018.

ORCID

Robert E. Clark https://orcid.org/0000-0001-5819-6251
Saumik Basu https://orcid.org/0000-0002-3904-6984
Sanford D. Eigenbrode https://orcid.org/0000-0003-0054-8511
Liesl C. Oeller https://orcid.org/0000-0003-3894-2880
David W. Crowder https://orcid.org/0000-0002-3720-1581

REFERENCES

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.

Basu, S., Kumar Kushwaha, N., Kumar Singh, A., Pankaj Sahu, P., Vinoth Kumar, R. & Chakraborty, S. (2018) Dynamics of a geminivirus-encoded pre-coat protein and host RNA-dependent RNA polymerase

1 in regulating symptom recovery in tobacco. *Journal of Experimental Botany*, 69, 2085–2102.
Bommarco, R., Wetterlind, S. & Sigvald, R. (2007) Cereal aphid populations in non-crop habitats show strong density dependence. *Journal of Applied Ecology*, 44, 1013–1022.
Catton, H.A., Lalonde, R.G. & De Clerck-Floate, R.A. (2015) Nontarget herbivory by a weed biocontrol insect is limited to spillover, reducing the chance of population-level impacts. *Ecological Applications*, 25, 517–530.
Chatzivassiliou, E.K. (2021) An annotated list of legume-infecting viruses in the light of metagenomics. *Plants*, 10, 1413.
Chisholm, P.J., Sertsuvalkul, N., Casteel, C.L. & Crowder, D.W. (2018) Reciprocal plant-mediated interactions between a virus and a non-vector herbivore. *Ecology*, 99, 2139–2144.
Clark, R.E., Basu, S., Lee, B.W. & Crowder, D.W. (2019) Tri-trophic interactions mediate the spread of a vector-borne plant pathogen. *Ecology*, 100, 1–8.
Clement, S.L. (2006) Pea aphid outbreaks and virus epidemics on peas in the US Pacific northwest: histories, mysteries, and challenges. *Plant Health Progress*, 7, 34.
Clement, S.L., Husebye, D.S. & Eigenbrode, S.D. (2010) *Ecological Factors Influencing Pea Aphid Outbreaks in the US Pacific Northwest. In Aphid Biodiversity under Environmental Change: Patterns and Processes (ed. by Kindlmann, P., Dixon, A.F.G. & Michaud, J.P.).* Netherlands, Dordrecht: Springer, pp. 107–128.
Damgaard, C., Bruus, M. & Axelsen, J.A. (2020) The effect of spatial variation for predicting aphid outbreaks. *Journal of Applied Entomology*, 144, 263–269.
Davis, T.S., Wu, Y. & Eigenbrode, S.D. (2015) Chickpea variety and phenology affect acquisition of pea enation mosaic virus, subsequent plant injury and aphid vector performance. *Annals of Applied Biology*, 167, 420–425.
Eigenbrode, S.D., Davis, T.S., Adams, J.R., Husebye, D.S., Waits, L.P. & Hawthorne, D. (2016) Host-adapted aphid populations differ in their migratory patterns and capacity to colonize crops. *Journal of Applied Ecology*, 53, 1382–1390.
Elbakidze, L., Lu, L. & Eigenbrode, S. (2011) Evaluating vector-virus-yield interactions for peas and lentils under climatic variability: a limited dependent variable analysis. *Journal of Agricultural and Resource Economics*, 36, 504–520.
Fox, J. & Weisberg, S. (2011) *An R companion to applied regression*. Thousand Oaks, CA: Sage Publications.
Gobatto, D., de Oliveira, L.A., de Siqueira Franco, D.A., Velásquez, N., Daròs, J.-A. & Eiras, M. (2019) Surveys in the chrysanthemum production areas of Brazil and Colombia reveal that weeds are potential reservoirs of chrysanthemum stunt viroid. *Viruses*, 11, 355.
Golden, L., Hogge, J., Hines, S., Packham, J. & Falen, C. (2016) Cover crops for grazing use in Idaho. University of Idaho Extension, December, 0–14.
Hampton, R.O. (1983) Pea leaf roll in northwestern US pea seed production areas. *Plant Disease*, 67, 1306–1310.
Heinlein, M. (2015) Plant virus replication and movement. *Virology*, 479–480, 657–671.
Hogenhout, S.A., Ammar, E.-D., Whitfield, A.E. & Redinbaugh, M.G. (2008) Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology*, 46, 327–359.
Holt, J., Colvin, J. & Muniyappa, V. (1999) Identifying control strategies for tomato leaf curl virus disease using an epidemiological model. *Journal of Applied Ecology*, 36, 625–633.
Hull, R. (1981) Pea enation mosaic virus. In: Kurstak, E. (Ed.) *Handbook of plant virus infections and comparative diagnosis*. Amsterdam, Netherlands: Elsevier/North-Holland Biomedical Press, pp. 239–256.
Jones, R.A.C. (2021) Global plant virus disease pandemics and epidemics. *Plants*, 10, 1–41.
Knick, S.T. & Rotenberry, J.T. (1997) Landscape characteristics of disturbed shrub steppe habitats in southwestern Idaho (U.S.A.). *Landscape Ecology*, 12, 287–297.
Lee, B.W., Clark, R.E., Basu, S. & Crowder, D.W. (2021) Predators affect a plant virus through direct and trait-mediated indirect effects on vectors. *BioRxiv*, 2021.02.17.431666.
Lenth, R.V. (2016) Least-squares means: the R package lsmeans. *Journal of Statistical Software*, 69, 1–33.
Looney, C. & Eigenbrode, S.D. (2012) Characteristics and distribution of Palouse prairie remnants: implications for conservation planning. *Natural Areas Journal*, 32, 75–85.
Lucas, W.J. (2006) Plant viral movement proteins: agents for cell-to-cell trafficking of viral genomes. *Virology*, 344, 169–184.
Luna, J.M., Mitchell, J.P. & Shrestha, A. (2012) Conservation tillage for organic agriculture: evolution toward hybrid systems in the western USA. *Renewable Agriculture and Food Systems*, 27, 21–30.
Macharia, I., Backhouse, D., Wu, S.-B. & Ateka, E.M. (2016) Weed species in tomato production and their role as alternate hosts of tomato spotted wilt virus and its vector Frankliniella occidentalis. *Annals of Applied Biology*, 169, 224–235.
McEwen, F.L., Schroeder, W.T. & Davis, A.C. (1957) Host range and transmission of the pea enation mosaic virus. *Journal of Economic Entomology*, 50, 770–775.
Mena-Covarrubias, J., Drummond, F.A. & Haynes, D.L. (1996) Population dynamics of the Colorado potato beetle (coleoptera: Chrysomelidae) on horsenettle in Michigan. *Environmental Entomology*, 25, 68–77.
Mischler, R., Duiker, S.W., Curran, W.S. & Wilson, D. (2010) Hairy vetch management for no-till organic corn production. *Agronomy Journal*, 102, 355–362.
Mueller, E.E., Groves, R.L. & Gratton, C. (2012) Crop and non-crop plants as potential reservoir hosts of alfalfa mosaic virus and cucumber mosaic virus for spread to commercial snap bean. *Plant Disease*, 96, 506–514.
Norris, R.F. & Kogan, M. (2005) Ecology of interactions between weeds and arthropods. *Annual Review of Entomology*, 50, 479–503.
Northfield, T.D., Paini, D.R., Funderburk, J.E. & Reitz, S.R. (2008) Annual cycles of Frankliniella spp. (Thysanoptera: Thripidae) thrips abundance on North Florida uncultivated reproductive hosts: predicting possible sources of pest outbreaks. *Annals of the Entomological Society of America*, 101, 769–778.
Paudel, S., Bechinski, E.J., Stokes, B.S., Pappu, H.R. & Eigenbrode, S.D. (2018) Deriving economic models for pea aphid (Hemiptera: Aphididae) as a direct-pest and a virus-vector on commercial lentils. *Journal of Economic Entomology*, 111, 2225–2232.
Peccoud, J., Ollivier, A., Plantegenest, M. & Simon, J.C. (2009) A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. *Proceedings of the National Academy of Science*, 106, 7495–7500.
Pernek, M., Pilas, I., Vrbek, B., Benko, M., Hrasovec, B. & Milkovic, J. (2008) Forecasting the impact of the gypsy moth on lowland hardwood forests by analyzing the cyclical pattern of population and climate data series. *Forest Ecology and Management*, 255, 1740–1748.
Peterson, P.D. (2018) The barberry eradication program in Minnesota for stem rust control: a case study. *Annual Review of Phytopathology*, 56, 203–223.
Pokorny, M., Filbey, S., Kilian, R., Scianna, J. & Jacobs, J. (2020) Evaluation of Cool Season Cover Crops in Southern Montana. Plant Materials Technical Note No. MT-123. USDA Natural Resources Conservation Service.
Powell, G., Tosh, C.R. & Hardie, J. (2006) Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology*, 51, 309–330.
Power, A.G. (1991) Virus spread and vector dynamics in genetically diverse plant populations. *Ecology*, 72, 232–241.

Power, A.G. (2000) Insect transmission of plant viruses: a constraint on virus variability. *Current Opinion in Plant Biology*, 3, 336–340.

R Development Core Team. (2021) *R version 4.1.2. R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Available from: https://www.R-project.org/

Rageshwari, S., Renukadevi, P., Malathi, V.G., Amalabalu, P. & Nakkeeran, S. (2017) Dac-elisa and RT-PCR based confirmation of systemic and latent infection by tobacco streak virus in cotton and parthenium. *Journal of Plant Pathology*, 99, 469–475.

Rashed, A., Feng, X., Prager, S.M., Porter, L.D., Knodel, J., Karasev, A. et al. (2018) Vector-borne viruses of pulse crops, with a particular emphasis on north American cropping systems. *Annals of the Entomological Society of America*, 111, 205–227.

Rashidi, M., Cruzado, R.K., Hutchinson, P.J.S., Bosque-Pérez, N.A., Marshall, J.M. & Rashed, A. (2020) Grassy weeds and corn as potential sources of barley yellow dwarf virus (BYDV-PAV) spread into winter wheat. *Plant Disease*, 105, 1–39.

Reynolds, D.R., Chapman, J.W. & Harrington, R. (2006) The migration of insect vectors of plant and animal viruses. *Advances in Virus Research*, 67, 453–517.

Rubio, L., Galipienso, L. & Ferriol, I. (2020) Detection of Plant Viruses and Disease Management: Relevance of Genetic Diversity and Evolution. *Frontiers in Plant Science*, 11.

Sint, D., Sporleder, M., Wallinger, C., Zegarra, O., Oehm, J., Dangi, N. et al. (2016) A two-dimensional pooling approach towards efficient detection of parasitoid and pathogen DNA at low infestation rates. *Methods in Ecology and Evolution*, 7, 1548–1557.

Srinivasan, R., Alvarez, J.M., Bosque-Pérez, N.A., Eigenbrode, S.D. & Novy, R.G. (2008) Effect of an alternate weed host, hairy nightshade, Sofonum sarrachoides, on the biology of the two most important potato leafroll virus (Luteoviridae: Polerovirus) vectors, Myzus persicae and Macrosiphum euphorbiae (Aphididae: Homoptera). *Environmental Entomology*, 37, 592–600.

Strange, R.N. & Scott, P.R. (2005) Plant disease: a threat to global food security. *Annual Review of Phytopathology*, 43, 83–116.

Strickland, D., Carroll, J. & Cox, K. (2020) Cedar apple rust. *New York State Integrated Pest Management Program*. Available from: https://ecommons.cornell.edu/handle/1813/41246

Takahashi, H., Fukuhara, T., Kitazawa, H. & Kormelink, R. (2019) Virus latency and the impact on plants. *Frontiers in Microbiology*, 10, 2764.

Wenninger, E.J., Dahan, J., Thornton, M. & Karasev, A.V. (2019) Associations of the potato psyllid and "Candidatus Liberibacter solanacearum" in Idaho with the noncrop host plants bittersweet nightshade and field bindweed. *Environmental Entomology*, 48, 747–754.

Wilson, L.J. (1995) Habitats of twospotted spider mites (Acari: Tetranychidae) during winter and spring in a cotton-producing region of Australia. *Environmental Entomology*, 24, 332–340.

Wu, N., Zhang, L., Ren, Y. & Wang, X. (2020) Rice black-streaked dwarf virus: from multiparty interactions among plant–virus–vector to intermittent epidemics. *Molecular Plant Pathology*, 21, 1007–1019.

Yazdkhasti, E., Hopkins, R.J. & Kvarnheden, A. (2021) Reservoirs of plant virus disease: occurrence of wheat dwarf virus and barley/cereal yellow dwarf viruses in Sweden. *Plant Pathology*, 70, 1552–1561.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting Information.

How to cite this article: Clark, R.E., Basu, S., Eigenbrode, S.D., Oeller, L.C. & Crowder, D.W. (2023) Risk assessment for non-crop hosts of pea enation mosaic virus and the aphid vector *Acyrthosiphon pisum*. *Agricultural and Forest Entomology*, 25(3), 427–434. Available from: https://doi.org/10.1111/afe.12564