ENVIRONMENTAL AND ECOLOGICAL FACTORS INFLUENCING INSECT-BORNE VIRUS EPIDEMIOLOGY

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Susan Bonner Claflin

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ENVIRONMENTAL AND ECOLOGICAL FACTORS INFLUENCING INSECT-BORNE VIRUS EPIDEMIOLOGY

Susan Bonner Claflin, PhD

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Vector ecology is essential to understanding the epidemiology of insect-borne pathogens. Yet, while much is known about vector population ecology, little work has been done on the impact of vector community ecology on disease spread. In my doctoral research, I studied environmental and ecological factors that influence the spread of a non-persistent plant pathogen, potato virus Y (PVY), focusing on the effect of landscape composition and the aphid vector assemblage on disease transmission. Chapter 1 shows that landscape composition within 1500m of a site strongly affects PVY prevalence; higher PVY prevalence was found in landscapes with greater amounts of agriculture. The work in this chapter also indicates that the effect of the landscape on PVY prevalence is most likely mediated by the direct effect of the landscape on the aphid vector community. Chapter 2 demonstrates that landscape composition and intra-annual variation interactively affect the aphid community in two cropping regions. Chapter 3 explores the effect of aphid density and community composition on aphid movement and PVY transmission. I found that aphid density positively affected aphid movement, and that conspecific density was more predictive than heterospecific density. Community composition affected both aphid movement and virus transmission, and these effects were driven by species identity, rather than species richness *per se*. Chapter 4 assessed the impact of predation and host plant abundance and spatial distribution on the movement of a common non-colonizing aphid species and PVY transmission. Predation, host plant

abundance, and plant spatial distribution interactively affected viral prevalence. Increasing the number of vector non-host plants increased the distance and frequency of aphid movement, and the effect was influenced by plant spatial distribution. However, aphid movement did not appear to mediate the effect of plant and predator treatments on PVY prevalence. Collectively, this work demonstrates the importance of vector community ecology and landscape epidemiology on insect-borne plant pathogens.

BIOGRAPHICAL SKETCH

Susan “Suzi” Claflin was raised on a farm in Bradford, Vermont. She received a BA in Biological Sciences and a BA in Peace and Justice Studies from Wellesley College in 2008, conducting ecological and biomechanical research along the way. In the two years between her undergraduate and graduate work, Suzi worked on various aspects of scientific inquiry and outreach, ranging from toxicology research to secondary science education. After developing an interest in both agricultural and disease ecology, she matriculated to Cornell University in 2011 in order to explore their intersection.

Dedicated to my family and friends, and to Andy.

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My thesis would have been logistically impossible without the participation of potato growers across the Finger Lakes region. I am so thankful for their generosity, curiosity, and kindness. I only hope that I didn't bother them too much.

Most importantly, I would not have made it through the unique gauntlet of graduate school without the squadron of loved ones that I am lucky enough to hold dear. My friends are brave, kind, and compassionate, and I am so grateful for them. I am particularly indebted to the Fist family, whose generous hospitality has made the last year one of the best of my life. Finally, none of this (or anything I have done or will do) would be possible without the support of my family. I am so fortunate to have been born a Claflin, and I am thankful for it every day.

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INTRODUCTION

Introduction

Diversity is essential to ecosystem functioning (Naeem et al. 2012), including the determination of pathogen prevalence. However, although there is potential for diversity at all levels to affect a pathosystem, research on the effect of diversity has largely been limited to the host level (e.g., Keesing et al. 2006). The influence of diversity at higher trophic levels, such as landscape structure and the vector assemblage, on pathogen prevalence remains understudied despite indications that their impact may be significant (Meenetemeyer et al. 2012; Roche et al. 2011). The aim of this quantitative review is to advance our knowledge of plant pathology by evaluating the direction and consistency of the impact of these levels of diversity on plant pathogens. It will summarize the work done to date; the review of landscape diversity will focus exclusively on plant pathogen prevalence, but, due to the limited number of studies in the area, the review of vector diversity will include both plant and animal pathosystems.

Landscape diversity is comprised of both landscape composition (the set of land uses or land cover types in the area) and connectivity (the proximity of similar land use or land cover types to each other), which in turn affect the permeability of the landscape to pests and pathogens (Margosian et al. 2009). Landscape diversity affects plant pathogen prevalence both directly and indirectly. The landscape could directly increase pathogen prevalence by increasing the concentration of the pathogen host plant species, building up pathogen inoculum over time, or including topographical features that enhance disease transmission (Root 1973; Meentemeyer et al. 2012). The landscape could also influence plant pathogen prevalence indirectly by affecting insect vector or predator assemblages.

Landscape diversity has been shown to significantly affect the local predatory insect community composition, which could have a cascading effect on insect vector abundance and behavior, and disease transmission (Chaplin-Kramer et al. 2011).

Vector diversity also mediates pathogen prevalence by both direct and indirect means. Pathogen prevalence is directly affected by vector diversity because vector species often vary in transmission efficiency and transmission-related behaviors (e.g., Boquel et al. 2011). Vector diversity could also indirectly impact pathogen prevalence through inter- and intra-species interactions. Vectors could affect the abundance of both hetero- and conspecifics through the release of pheromones or indirect competition, and alter transmission-related behaviors, such as movement (Kunert et al. 2005; Petersen and Sandström 2001; Mehrparvar et al. 2014; Smith et al. 2008).

This quantitative review will explore two questions about the effect of landscape and vector diversity on pathogen prevalence: 1) Do landscape and vector diversity consistently affect pathogen prevalence?, and 2) If so, what diversity metrics are the most common drivers and what is the direction of the effect? This review will explore the research done to date on plant landscape epidemiology and the effect of vector diversity on vector-borne pathogen prevalence. I will summarize general conclusions and overall findings in each field and will comment on areas for further investigation.

Methods

Landscape diversity

This review initially included the papers identified as empirical (as opposed to theoretical or conceptual) studies in the Meentemeyer et al. (2012) review of plant

landscape epidemiology (25/51 total), and replicated the search for 2012-2015 (search terms: Topic 1) epidem* OR disease OR pathogen, Topic 2) *spatial OR geograph* OR GIS OR "remote sens*" OR spread OR "landscape heterogen*" OR "landscape structure" OR risk, and Topic 3) landscape*; conducted in January 2016) to update to the present. The 2012-2015 search yielded 1597 studies, which were then refined by research area: ecology, environmental sciences, infectious diseases, evolutionary biology, entomology, geography, zoology, forestry, biology, plant sciences, agronomy, agricultural multidisciplinary, and remote sensing were included. The refinement resulted in 711 studies. These were sorted, and empirical studies of plant pathogens were selected, resulting in 23 studies.

Of the 48 total empirical studies, only those designed to test the relationship between landscape composition and/or connectivity and disease incidence or prevalence and included field-collected data were included in the final group, resulting in a total of 27 included studies.

Vector diversity

We conducted a Web of Science search for Topic 1) "vector diversity" Or "vector species richness" OR "vector abundance" and Topic 2) epidem* OR disease OR pathogen in January 2016, which yielded 170 papers. All papers were reviewed and those with data on the vector population as well as disease incidence or prevalence data were selected for inclusion in this review. Studies that analyzed climatic effects on vector populations, but had no infection data for the host population or vice versa, and those with no empirical data were excluded, leaving 27 included studies. Papers that mentioned

the potential importance of the vector community for the interpretation of their results, but did not quantify the vector community (a total of 10 studies), were assessed separately.

Results

Landscape diversity

The majority of studies investigated an oomycete (exclusively *Phytophora ramorum*, the cause of sudden oak death) or a fungal pathogen, accounting for 24 of the 27 studies reviewed (Table 1). Two studies were carried out in a virus pathosystem (Table 1) and in one, the identity of the pathogen was unresolved. Three studies evaluated effects on more than one pathogen in the same system. Nearly half (12/27) were multiyear studies, and a third (9/27) evaluated relationships at multiple scales. About 20% were conducted in an agricultural system (5/27).

More than half of the reviewed studies (16/27) evaluated the effect of abiotic environmental factors (such as rainfall) on the pathogen. Slightly less than half (10/27) studied the effect of different land uses or types, and/or explored the effect of host plant area or density (11/27). Only three studies quantified connectivity. Despite the wide variability in study systems and design, the results were very consistent; all but one of the studies reviewed found a significant relationship between the landscape metric (or at least one of the metrics, if multiple were used) and the pathogen. Of these, twenty-one studies demonstrated a positive effect and four studies showed a negative effect (Table 1).

Vector diversity

Ten papers mention the implications or potential importance of the vector community without measuring (and therefore testing) them. Eight papers mention the potential importance of vector abundance, two discuss the potential importance of vector diversity, and two suggest that the vector community composition might be important. About half of the studies that quantified the vector community addressed viruses (13/27), and slightly more than half worked with mosquito-transmitted pathogens (14/27). The majority of studies measured vector abundance (24/27), while only a little more than a third of the studies (11/27) measured some aspect of vector diversity (Table 2). Nearly half measured abiotic factors (13/27), about one fifth of the studies were agricultural (6/27) and more than one third included multiple years of data (10/27). The results of the reviewed studies were remarkably consistent; vector abundance was significantly related to the pathogen metric in 16 of the 24 studies in which it was measured. In fourteen of these studies, vector abundance had a positive effect on pathogen prevalence, and in two studies it had a negative effect. Vector abundance had no effect in four studies, and in remaining four it was not tested against the pathogen data (Table 2).

Of the 10 studies that measured vector diversity and analyzed its effect on the pathogen, 70% worked with a mosquito-borne pathogen (7/10), and 70% also measured vector abundance (Table 3). Most measured vector diversity as vector species richness (8/10), one calculated the Shannon diversity index, and one used genotypic diversity (Table 3). Only 3 of the 10 studies that measured vector diversity statistically compared it to the pathogen metric. Of those, two studies found that vector diversity was positively related to the pathogen metric, and one saw no effect (Table 3). Importantly, none of

Table 1: List of empirical plant landscape epidemiology studies (27 total). An I indicates an interactive effect.

	Pathogen				Landscape metric				Results			
Citation	Fungus	Oomycete	Virus	Multiple	Abiotic factors	Connectivity	Composition (land use)	Composition (host plant area/density)	Landscape had effect	Landscape had no effect	Positive effect	Negative effect
Anacker et al. 2008		X			X				X		X	
Avelino et al. 2012	X						X		X		X	
Busby et al. 2014	X			X	X				X		I	
Carrière et al. 2014			X					X	X		X	
Condeso et al. 2007		X					X		X		X	
Cushman et al.2008		X			X		X		X		X	
Dillon et al. 2014		X			X			X	X		X	
Ellis et al. 2010		X				X			X		X	
Garnas et al. 2013	X				X					X		
Gosme et al. 2012	X			X			X		X			X
Haas et al. 2011		X						X	X			X
Johnson et al. 2011	X				X	X			X		X	
Jules et al. 2002	X				X		X	X	X		X	
Kauffman et al. 2006							X		X		X	

	Pathogen				Landscape metric				Results			
Citation	Fungus	Oomycete	Virus	Multiple	Abiotic factors	Connectivity	Composition (land use)	Composition (host plant area/density)	Landscape had effect	Landscape had no effect	Positive effect	Negative effect
Kelly et al. 2002		X			X		X	X	X		X	
Laine et al. 2006	X						X	X	X		X	
LaManna et al. 2008	X				X				X		X	
Ma et al. 2015	X			X				X	X			X
Meetenmeyer et al. 2008a		X			X				X		X	
Müller et al. 2011	X				X				X		X	
Oguro et al. 2015	X					X		X	X		X	
Rodelo-Urrego et al. 2013			X		X			X	X		X	
Shearer et al. 2014	X				X			X	X		X	
Smith et al. 2011	X				X		X		X		X	
Vaclavik et al. 2010		X						X	X		X	
White et al. 2002	X				X		X		X		X	
Wilson et al. 2003	X				X				X		X	X
TOTAL	**15**	**9**	**2**	**3**	**16**	**3**	**10**	**11**	**26**	**1**	**22**	**4**

Table 2: List of studies that quantified the vector community (27 total).

	Vector community metric					Results						
Citation	Vector abundance	Vector community composition	Vector diversity (species richness)	Vector diversity (Shannon index)	Vector diversity (genetic diversity)	Vector abundance had effect	Vector abundance had no effect	Vector diversity had effect	Vector diversity had no effect	Relationship not analyzed	Positive effect	Negative effect
Bisanzio et al. 2015	X					X					X	
Chaves et al. 2014	X					X					X	
Cumming et al. 2006		X	X					X			X	
Daugherty et al. 2015	X						X					
Dusi et al. 2000	X					X					X	
Farias et al. 2012	X					X						X
Gajanana et al. 1997	X		X							X		
Godsey et al. 2012	X		X			X					X	
Gudex-Cross et al. 2015	X		X							X		
Gurney et al. 2014	X					X					X	
Kelly et al. 2014					X				X			

	Vector community metric					Results						
Citation	Vector abundance	Vector community composition	Vector diversity (species richness)	Vector diversity (Shannon index)	Vector diversity (genetic diversity)	Vector abundance had effect	Vector abundance had no effect	Vector diversity had effect	Vector diversity had no effect	Relationship not analyzed	Positive effect	Negative effect
Martínez-de la Puente et al. 2013	X					X					X	
Mayo et al. 2012	X					X					X	
Mori et al. 2012	X					X					X	
Nielsen et al. 2008	X						X					
Nouvellet et al. 2013	X					X					X	
Okanga et al. 2013	X		X				X					
Rahelinirina et al. 2010	X		X							X		
Reijniers et al. 2014	X					X					X	
Reynaud et al. 2009	X					X					X	
Roche et al. 2013				X				X			X	
Rúa et al. 2014	X									X		

	Vector community metric					Results						
Citation	Vector abundance	Vector community composition	Vector diversity (species richness)	Vector diversity (Shannon index)	Vector diversity (genetic diversity)	Vector abundance had effect	Vector abundance had no effect	Vector diversity had effect	Vector diversity had no effect	Relationship not analyzed	Positive effect	Negative effect
Rubio-Palis et al. 2011	X					X					X	
Rwegoshora et al. 2007	X		X				X					
Ryan et al. 1999	X		X			X					X	
Thammapalo et al. 2008	X					X					X	
Thomson et al. 1994	X						X					X
Total	**24**	**1**	**8**	**1**	**1**	**15**	**5**	**2**	**1**	**4**	**16**	**2**

Table 3: List of studies that quantified vector diversity (10 total).

	Metric					Results			
Citation	Vector abundance	Vector community composition	Vector diversity (species richness)	Vector diversity (Shannon index)	Vector diversity (genetic diversity)	Vector diversity had effect	Vector diversity had no effect	Positive effect	Negative effect
Cumming et al. 2006		X	X			X		X	
Gajanana et al. 1997	X		X						
Godsey et al. 2012	X		X						
Gudex-Cross et al. 2015	X		X						
Kelly et al. 2014					X		X		
Okanga et al. 2013	X		X						
Rahelinirina et al. 2010	X		X						
Roche et al. 2013				X		X		X	
Rwegoshora et al. 2007	X		X						
Ryan et al. 1999	X		X						
Total	**7**	**1**	**8**	**1**	**1**	**2**	**1**	**2**	**0**

these studies experimentally manipulated vector abundance or diversity, so their conclusions were limited to finding correlations.

Discussion

Landscape Diversity

Plant landscape epidemiology has demonstrated a consistent relationship between landscape composition and environmental factors, and plant pathogen dynamics at local scales. However, the generality of these conclusions is difficult to assess. To date, the field has focused on a narrow range of plant pathosystems; more than a third of all studies focused on a single pathogen, *Phytophora ramorum* (the cause of sudden oak death), greater than half worked with forest pathogens, and nearly all took place in temperate environments. As the field continues to grow, it is essential that future studies explore the complexity and nuances of these systems by testing the relative importance of landscape metrics, comparing pathogens within systems, and exploring both direct and indirect landscape effects.

1) Does landscape diversity consistently affect pathogen prevalence?, and 2) If so, what diversity metrics are the most common drivers and what is the direction of the effect?

There was a remarkable consistency in the results of the reviewed studies. All but one found a significant relationship between the pathogen and at least one landscape metric. Further, 21 of these found a positive effect, 3 found a negative effect, 1 found both a positive and negative effect, depending on the abiotic factor, and 1 found an interactive effect. The lone study that found no effect of the landscape by any metric,

Garnas et al. (2013), found that disease pressure was high across the study region. Because the pathogen was not dispersal-limited in any area, there was little variation for landscape factors to act upon, making it unsurprising that no pattern was observed. Landscape composition, measured as either land cover or host area, was the most common landscape driver of pathogen prevalence, accounting for 21 of the 26 studies that found a significant effect. However, while the findings of the reviewed studies offer a strong indication that the landscape can influence local plant pathogen dynamics, they address a relatively small range of pathogens, limiting the extent to which their conclusions can be extrapolated to other systems.

Gosme et al. (2012) and Busby et al. (2014) tested the effect of landscape composition, and environmental factors and host genotype, respectively, on multiple plant pathogens, and found that the relationship between the landscape metric and the disease varied among pathogens. While the mechanisms remain unclear, the differences between pathogens, such as variation in dispersal strategies and natural history, could drive the differential effect of landscape composition and connectivity.

Although more than 75% of studies focused on the direct effect of the landscape, landscape structure can also have indirect effects on pathogens, mediated by the vector assemblage. Eighty-nine percent of plant viruses are vectored (Power and Flecker 2008), yet only seven of the reviewed studies worked with a vector-borne pathogen. Carrière et al. (2014) conducted a multiyear, multiscale study that tested the effect of susceptible host abundance on the incidence of an insect-vectored virus. The authors found that host plant area had a positive effect on vector abundance, and that both host plant abundance and vector abundance had a positive relationship with disease prevalence, but vector

abundance explained the greatest amount of variation in infection. This work demonstrates that the importance of considering vector abundance in studies of pathogen prevalence.

Vector Diversity

1) Does vector diversity consistently affect pathogen prevalence?, and 2) If so, what diversity metrics are the most common drivers and what is the direction of the effect?

Vector abundance is the most common metric used to quantify the vector community; vector diversity is rarely measured. This is a puzzling oversight, given the strong theoretical prediction of an effect of vector species richness on pathogen prevalence (Roche et al. 2013). Of the reviewed studies, only ten measured vector diversity, and a mere three statistically tested the effect of vector diversity on the pathogen. Of these, two papers found a positive relationship between vector diversity and pathogen prevalence (Cumming et al. 2006; Roche et al. 2013), and the third explored the effect of vector genotypic diversity on pathogen diversity (Kelly et al. 2014). The authors found no significant genotypic variation within the vector community, meaning there was no variability to test against.

Cumming et al. (2006) and Roche et al. (2013) emphasize the importance of placing pathosystems within their community context. Cumming et al. (2006) found that while abiotic environmental factors weakly affected the pathogen community, the vector community had a strong effect. In addition, environmental factors strongly affected the vector community, indicating that the vector assemblage might be acting as the

mechanism for the effect of the environment on the pathogen. Roche et al. (2013) developed a theoretical model of a multi-host, multi-vector pathosystem, and validated it with empirical data collected during a West Nile virus outbreak. They demonstrated that increasing vector species richness amplified pathogen prevalence, even when the vector assemblage includes species with limited transmission efficiencies.

The data on the effect of vector diversity is too sparse to draw conclusions about general trends. However, the results of this review show that vector diversity, measured as species richness or Shannon diversity, can positively influence pathogen prevalence. It also shows that there are many more studies designed to collect the data relevant for addressing these questions than there are studies that actually analyze them. Future work should capitalize on the vector diversity data already being collected, harnessing it to assess the impact of the vector community on pathogen prevalence.

Conclusion

Plant landscape epidemiology and the study of vector diversity are relatively new fields, and have plenty of room to grow. Future work should seek to assess the complex interactions driving the relationship between landscape structure and local pathogen dynamics by exploring indirect effects, landscape connectivity, and a wider range of pathosystems. There is a significant absence of empirical studies testing the effect of vector diversity, leaving a substantial knowledge gap. Investigating the importance of the vector assemblage for disease dynamics is critical to understanding the community context of vector-borne pathogens.

The following chapters explore the effect of environmental and ecological variation on the prevalence of the plant pathogen, potato virus Y (PVY). **Chapter 1** assesses the impact of landscape complexity on predator and vector abundance and diversity, and PVY prevalence. **Chapter 2** contains an analysis of the effect of landscape composition and intra-annual variation on the vector community. **Chapter 3** is a study on the effect of vector density and community composition on vector movement and PVY transmission. **Chapter 4** explores the effect of host plant distribution and predation on a non-colonizing vector species (one that does not settle and reproduce on the virus host plant). Supplementary material for chapters 1, 2, and 3 can be found in the **Appendix**.

REFERENCES

Anacker, BL, NE Rank, D Hüberli, M Garbelotto, S Gordon, T Harnik, R Whitkus, and R Meentemeyer. (2008). Susceptibility to *Phytophthora ramorum* in a key infectious host: landscape variation in host genotype, host phenotype, and environmental factors. *New Phytologist*, 177:756-766.

Avelino, J, A Romero-Gurdián, HF Cruz-Cuellar, and FAJ Declerck. (2012). Landscape context and scale differentially impact coffee leaf rust, coffee berry borer, and coffee root-knot nematodes. *Ecological Applications*, 22(2):584-596.

Baleela, R, MS Llewellyn, S Fitzpatrick, K Kuhls, G Schönian, MA Miles, and IL Mauricio. (2014). *Leishmania donovani* populations in Eastern Sudan: temporal structuring and a link between human and canine transmission. *Parasites and Vectors*, 7:496.

Bisanzio, D, F Mutuku, AD LaBeaud, PL Mungai, J Muinde, H Busaidy, D Mukoko, CH King, and U Kitron. (2015). Use of prospective hospital surveillance data to define spatiotemporal heterogeneity of malaria risk in coastal Kenya. *Malaria Journal*, 14:482.

Blaustein, L, RS Ostfeld, and RD Holt. (2010). A community-ecology framework for understanding vector and vector-borne disease dynamics. *Israel Journal of Ecology and Evolution*, 56:251-262.

Boquel, S, A. Ameline, P. Giordanengo. (2011). Assessing aphids potato virus Y-transmission efficiency: a new approach. *Journal of Virological Methods*, 178: 63-67.

Busby, PE, G Newcombe, R Dirzo, and TG Whitham. (2014). Differentiating genetic and environmental drivers of plant-pathogen community interactions. *Journal of Ecology*, 102:1300-1309.

Carrière, Y, B Degain, KA Hartfield, KD Nolte, SE Marsh, C Ellers-Kirk, WJD Van Leeuwen, L Liesner, P Dutilleul, and JC Palumbo. (2014). Assessing transmission of crop diseases by insect vectors in a landscape context. *Journal of Economic Entomology*, 107(1):1-10.

Chaplin-Kramer, R., M.E. O'Rourke, E.J. Blitzer, and C. Kremen. 2011. A meta-analysis of crop pest and natural enemy response to landscape complexity. *Ecology Letters* 14:922-932.

Chaves, LF, JE Calzada, A Valderrama, and A Saldaña. (2014). Cutaneous leishmaniasis and sand fly fluctuations are associated with El Niño in Panamá. *PLOS Neglected Tropical Diseases*, 8(10):e3210.

Condeso, TE, and R Meentemeyer. (2007). Effects of landscape heterogeneity on the emerging forest disease sudden oak death. *Journal of Ecology*, 95:364-375.

Cumming, GS, and JF Guégan. (2006). Food webs and disease: is pathogen diversity limited by vector diversity. *EcoHealth*, 3:163-170.

Cushman, JH, and R Meetemeyer. (2008). Multi-scale patterns of human activity and the incidence of an exotic forest pathogen. *Journal of Ecology*, 96:766-776.

Daugherty, MP, S O'Neill, F Byrne, and A Zeilinger. (2015). Is vector control sufficient to limit pathogen spread in vineyards? *Environmental Entomology*, 44:789-797.

Dillon, WW, SE Haas, DM Rizzo, and RK Meetemeyer. (2014). Perspectives of spatial scale in a wildland forest epidemic. *European Journal of Plant Pathology*, 138:449-465.

Dusi, AN, D Peters, and W van der Werf. (2000). Measuring and modeling the effects of inoculation date and aphid flights on the secondary spread of Beet mosaic virus in sugar beet. *Annals of Applied Biology*, 136:131-146.

Ellis, AM, T Vclavk, and RK Meentemeyer. (2010). When is connectivity important? A case study of the spatial pattern of sudden oak death. *Oikos*, 119:485-493.

Enami, HR. (2009). Epidemiological survey on equine Filariasis in the Urmia area of Iran. *Journal of Animal and Veterinary Advances*, 8(2):295-296.

Farias, MEM, CT Atkinson, DA LaPointe, and S Jarvi. (2012). Analysis of the *trap* gene provides evidence for the role of elevation and vector abundance in the genetic diversity of *Plasmodium relictum* in Hawaii. *Malaria Journal*, 11:305.

Fourcade, Y, O Keis, DS Richardson, and J Secondi. (2014). Continental-scale patterns of pathogen prevalence: a case study on the corncrake. *Evolutionary Applications*, 1043-1055.

Gajanana, A, R Rajendran, PP Samuel, V Thenmozhi, TF Tsai, J Kimura-Kuroda, and R Reuben. (1997). Japenese encephalitis in South Arcot district, Tamil Nadu, India: a three-year longitudinal study of vector abundance and infection frequency. *Journal of Medical Entomology*, 34(6):651-659.

Garnas, JR, DR Houston, MJ Twery, MP Ayers, and C Evans. (2013). Inferring controls on the epidemiology of beech bark disease from spatial patterning of disease organisms. *Agricultural and Forest Entomology*, 15:146-156.

Geoghegan, J, PJ Walker, JB Duchemin, I Jeanne, and EC Holmes. (2014). Seasonal drivers of the epidemiology of arthropod-borne viruses in Australia. *PLOS Neglected Tropical Diseases*, 8(11):e3325.

Godsey, Jr., MS, K Burkhalter, G Young, M Delorey, K Smith, J Townsend, C Levy, and JP Mutebi. (2012). Entomologic investigations during an outbreak of West Nile virus disease in Maricopa county, Arizona 2010. *American Journal of Tropical Medicine and Hygiene*, 87(6):1125-1131.

Gosme, M, M de Villemandy, M Bazot, and MH Jeuffroy. (2012). Local and neighborhood effects of organic and conventional wheat management on aphids, weeds, and foliar diseases. *Agriculture, Ecosystems, and Environment*, 161:121-129.

Goswami, P, US Murty, SR Mutheneni, and ST Krishnan. (2014). Relative roles of weather variables and change in human population in malaria: comparison over different states of India. *PLOS One*, 9(6):e99867.

Gudex-Cross, D, RK Barraclough, DH Brunton, and JGB Derraik. (2015). Mosquito community and avian malaria prevalence in silvereyes (*Zosterops lateralis*) within forest edge and interior habitats in a New Zeland regional park. *EcoHealth*, 12:432-440.

Gurney, KEB, RG Clark, and PS Curry. (2014). Factors influencing mortality of Lesser Scaup (Aythya affinis) ducklings during a West Nile virus outbreak. *Canadian Journal of Zoology*, 92:365-370.

Haas, SE, MB Hooten, DM Rizzo, and RK Meetemeyer. (2011). Forest species diversity reduces disease risk in a generalist plant pathogen invasion. *Ecology Letters*, 14:1108-1116.

Haight, RG, FR Homans, T Horie, SV Mehta, DJ Smith, and RC Venette. (2011). Assessing the cost of an invasive forest pathogen: a case study with oak wilt. *Environmental Management*, 47:505-517.

Johnson, BL, and NM Haddad. (2011). Edge effects, not connectivity, determine the incidence and development of a foliar fungal plant disease. *Ecology*, 92:1551-1558.

Jules, ES, MJ Kauffman, WD Ritts, and AL Carroll. (2011). Spread of an invasive pathogen over a variable landscape: a nonnative root rot on Port Orford cedar. *Ecology*, 83:3167-3181.

Kauffman, MJ, and ES Jules. (2006). Heterogeneity shapes invasion: host size and environment influence susceptibility to a nonnative pathogen. *Ecological Applications*, 16:166-175.

Keesing, F, RD Holt, and RS Ostfeld. 2006. Effects of species diversity on disease risk. *Ecology Letters*, 9:485-498.

Kelly, RR, D Gaines, WF Gilliam, and RJ Brinkerhoff. (2014). Population genetic structure of the Lyme disease vector Ixodes scapularis at an apparent spatial expansion front. *Infection, Genetics and Evolution*, 27:543-550.

Kunert, G, S Otto, USR Rösse, J Gershenzon, and WW Weisser. (2005). Alarm pheromone mediates production of winged dispersal morphs in aphids. *Ecology Letters*, 8: 596-603.

Laine, A-L, and I Hanski. (2006). Large-scale spatial dynamics of a specialist plant pathogen in a fragmented landscape. *Journal of Ecology*, 94:217-226.

La Manna, L, SD Matteucci, and T Kitzberger. (2008). Abiotic factors related to the incidence of the *Austrocedrus chilensis* disease syndrome at a landscape scale. *Forest Ecology and Management*, 256:1087-1095.

Ma, Z, J Zhu, Z Sun, J Liang, Z Zhang, L Zhang, L Sun, and W Li. (2015). The influence of biotic and abiotic factors on the occurrence and severity of poplar canker disease in Qingfeng County, China and the management implications. *Journal of Forest Research*, 26:1025-1034.

Margosian, M.L., K.A. Garrett, J.M.S. Hutchinson, and K.A. With. 2009. Connectivity of the American agricultural landscape: Assessing the national risk of crop pest and disease spread. *Bioscience* 59:141-151.

Martínez-de la Puente, J, J Martínez, J Rivero-de-Aguilar, S del Cerro, and S Merino. (2013). Vector abundance determines Trypanosoma prevalence in nestling blue tits. *Parasitology*, 140:1009-1015.

Mayo, CE, IA Gardner, BA Mullens, CM Barker, AC Gerry, AJ Guthrie, and NJ MacLachlan. (2012). Anthropogenic and meterological factors influence vector abundance and prevalence of bluetongue infection of dairy cattle in California. *Veterinary Microbiology*, 155:158-164.

Meentemeyer, RK, SE Haas, and T Václavík. (2012). Landscape epidemiology of emerging infectious disease in natural and human-altered ecosystems. *Annual Review of Phytopathology*, 50:379-402.

Meentemeyer, RK, NE Rank, BL Anacker, DM Rizzo, and JH Cushman. (2008). Influences of land-cover change on the spread of an invasive forest pathogen. *Ecological Applications*, 18:159-171.

Mehrparvar, M., S.M. Mansouri, and W.W. Weisser. (2014). Mechanisms of species sorting: effect of habitat occupancy on aphids' host plant selection. *Ecological Entomology*, 39:281-289.

Mobegi, VA, CW Duffy, A Amambua-Ngwa, KM Loua, E Laman, DC Nwakanma, B MacInnis, H Aspeling-Jones, L Murray, TG Clark, DP Kwiatkowski, and DJ Conway. (2014). Genome-wide analysis of selection on the malaria parasite Plasmodium falciparum in West African populations of differing infection endemicity. *Molecular Biology and Evolution*, 31(6):1490-1499.

Mori, N, F Pavan, N Reggiani, M Bacchiavini, L Mazzon, S Paltrinieri, and A Bertaccini. (2012). Correlation of bois noir disease with nettle and vector abundance in northern Italy vineyards. *Journal of Pest Science*, 85:23-28.

Müller, MEH, S Koszinski, A Brenning, G Verch, U Korn, M Sommer. (2011). Within field variation of mycotoxin contamination of winter wheat is related to indicators of soil moisture. *Plant Soil*, 342:289-300.

Naeem, S, JE Duffy, and E Zavaleta. 2012. The functions of biological diversity in an age of extinction. *Science*, 336:1401-1406.

Nielsen, CF, WK Reisen, MV Armijos, NJ MacLachlan, and TW Scott. (2008). High subclinical West Nile virus incidence among nonvaccinated horses in northern California associated with low vector abundance and infection. *American Journal of Tropical Medicine and Hygiene*, 78(1):45-52.

Nitatpattana, N, K Kanjanopas, S Yoksan, W Satimai, N Vongba, S Langdatsuwan, K Nakgoi, S Ratchakum, N Wauquier, M Souris, P Auewarakul, and JP Gonzalez. (2014). Long-term persistence of Chikungunya virus neutralizing antibodies in human populations of northeastern Thailand. *Virology Journal*, 11:183.

Nouvellet, P, E Dumonteil, S Gourbière. (2013). The improbable transmission of Trypansoma cruzi to human: the missing link in the dynamics and control of Chagas disease. *PLOS Neglected Tropical Diseases*, 7(11):e2505.

Oguro, M, S Imahiro, S Saito, and T Nakashizuka. (2015). Relative importance of multiple scale factors to oak tree mortality due to Japanese oak wilt disease. *Forest Ecology and Management*, 356:173-183.

Okanga, S, GS Cumming, and PAR Hockey. (2013). Avian malaria prevalence and mosquito abundance in the Western Cape, South Africa. *Malaria Journal*, 12:370.

Petersen, MK and JP Sandström. (2001). Outcome of indirect competition between two aphid species mediated by responses in their common host plant. *Functional Ecology*, 15: 525-534.

Pioz, M, H Guis, L Crespin, E Gay, D Calavas, B Durand, D Abrial, and C Ducrot. (2012). Why did bluetongue spread the way it did? Environmental factors influencing the velocity of bluetongue virus serotype 8 epizoonotic wave in France. *PLOS One*, 7(8):e43360.

Plantegenest, M, C Le May, and F Fabre. (2007). Landscape epidemiology of plant diseases. *Journal of the Royal Society Interface*, 4:963-972.

Power, AG and A Flecker. 2008. *In* Infectious Disease Ecology, ed. R.S. Ostfeld, F. Keesing, and V.T. Eviner. Princeton University Press.

Rahelinirina, S, JM Duplantier, J Ratovonjato, O Ramilijaona, M Ratsimba, and L Rahalison. (2010). Study on the movement of Rattus rattus and evaluation of the plague dispersion in Madegascar. *Vector-Borne and Zoonotic Diseases*, 10(1):77-84.

Real, LA, & R Biek. (2007). Spatial dynamics and genetics of infectious diseases on heterogeneous landscapes. *Journal of the Royal Society Interface*, 4: 935-948.

Reijniers, J, M Begon, VS Ageyev, and H Leirs. (2014). Plague epizootic cycles in central Asia. *Biology Letters*, 10.

Reynaud, B, H Delatte, M Peterschmitt, and D Fargette. (2009). Effects of temperature increase on the epidemiology of three major vector-borne viruses. *European Journal of Plant Pathology*, 123:269-280.

Roche, B, and JF Guégan. (2011). Ecosystem dynamics, biological diversity, and emerging infectious disease. *Comptes Rendes Biologies*, 334:385-392.

Roche, B, P Rohani, AP Dobson, and JF Gugan. (2013). The impact of community organization on vector-borne pathogens. *The American Naturalist*, 181(1).

Rodelo-Urrego, M, I Pagán, P González-Jara, M Betancourt, A Moreno-Letelier, MA Ayllón, A Fraile, D Piñero, and F. García-Arenal. (2013). Landscape heterogeneity shapes host-parasite interactions and results in apparent plant-virus codivergence. *Molecular Ecology*, 22:2325-2340.

Root, R.B. 1973. Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs* 43:95-124.

Rúa, MA, RL McCulley, and CE Mitchell. (2014). Climate drivers, host identity, and fungal endophyte infection determine virus prevalence in a grassland ecosystem. *Journal of Ecology*, 102:690-699.

Rubio-Palis, Y, LM Perez-Ybarra, M Infante-Ruiz, et al. (2011). Influence of climatic variables on dengue cases and abundance of Aedes aegyptii (Diptera: Culicidae) in Maracay, Venezuela. *Boletin de Malariologia y Salud Ambiental*, 51(2):145-157.

Rwegoshora, RT, PE Simonsen, DW Meyrowitsch, MN Malecela-Lazaro, E. Michael, and EM Pedersen. (2007). Bancroftian filariasis: house-to-house variation in the vectors and transmission- and the relationship to human infection- in an endemic community of coastal Tanzania. *Annals of Tropical Medicine & Parasitology*, 101(1):51-60.

Ryan, PA, KA Do, and BH Kay. (1999). Spatial and temporal analysis of Ross River virus disease patterns at Maroochy Shire, Australia: association between human morbidity and mosquito (Diptera: Culicidae) abundance. *Journal of Medical Entomology*, 36(4): 515-521.

Shearer, BL, CE Crane, RG Fairman, MJ Dillon, and RM Buehrig. (2014). Spatio temporal variation in invasion of woodlands and forest by *Phtophthora cinnamomi*. *Australian Plant Pathology*, 43:327-337.

Smith, R.A., K.A. Mooney, and A.A. Agrawal. (2008). Coexistence of three specialist aphids on common milkweed, Asclepias syriaca. *Ecology*, 89(8):2187-2196.

Stoddard, ST, AC Morrison, GM Vazquez-Prokopec, VP Soldan, TJ Kochel, U Kitron, JP Elder, and TW Scott. (2009). The role of human movement in the transmission of vector-borne pathogens. *PLOS Neglected Tropical Diseases*, 3(7):e481.

Thammapalo, S, Y Nagao, W Sakamoto, S Saengtharatip, M Tsujitani, Y Nakamura, PG Coleman, C Davies. (2008). Relationship between transmission intensity and incidence of dengue hemorrhagic fever in Thailand. *PLOS Neglected Tropical Diseases*, 2:e263.

Thomson, MC, U Dalessandro, S Bennett, et al. (1994). Malaria prevalence is inversely related to vector density in the Gambia, West-Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 6:638-643.

Václavík, T, A Kanaskie, EM Hansen, JL Ohmann, and RK Meentemeyer. (2010). Predicting potential and actual distribution of sudden oak death in Oregon: prioritizing landscape contexts for early detection and eradication of disease outbreaks. *Forest Ecology and Management*, 260:1026-1035.

White, MA, TN Brown, and GE Host. (2002). Landscape analysis of risk factors for white pine blister rust in the Mixed Forest Province of Minnesota, U.S.A. *Canadian Journal of Forest Research*, 32:1639-1650.

Wilson, BA, A Lewis, and J Aberton. (2003). Spatial model for predicting the presence of cinnamon fungus (*Phytophthora cinnamomi*) in sclerophyll vegetation communities in south-eastern Australia. *Austral Ecology*, 28:108-115.

CHAPTER ONE
SIMPLE LANDSCAPE HAVE HIGHER VECTOR-BORNE PLANT VIRUS PREVALENCE

Summary

1. Landscape composition affects local arthropod biodiversity, including herbivorous insects and their predators, yet landscape effects on insect-vectored plant diseases have to date received little attention. In this study, we examine how landscape complexity affects the prevalence of an insect-vectored viral pathogen in host-plants, and the role the arthropod vector assemblage plays in mediating landscape effects.
2. We measured the effect of landscape composition on the plant virus *Potato virus Y (PVY)*, its aphid vectors, and their coccinellid predators during the 2012 and 2013 field seasons at 19 to 21 farms in upstate New York.
3. In both years, we found a positive relationship between final virus prevalence and percent cropland within 500, 1000, and 1500 meters surrounding study sites. Percent cropland also had a significant negative effect on aphid species richness, and the aphid community composition in turn affected PVY prevalence. By contrast, landscape composition had no measurable effect on coccinellid abundance or species richness in this study.
4. *Synthesis and applications*. Our work demonstrates that landscape composition plays an important role in vector-borne pathogen spread, and that pathogen spread appears to be mediated by the effect of the landscape on the vector community. The close proximity of the effect seen in our study indicates that virus prevalence may be manageable on small-scale farms.

Introduction

Agricultural intensification and uniformity of land use results in homogeneous, highly connected landscapes that are vulnerable to the spread of pests and pathogens (Margosian *et al.* 2009). Landscape structure also drives local community composition. Landscape effects have been clearly demonstrated in predatory insects; predator abundance and diversity are known to decrease with decreased complexity of the surrounding landscape (Bianchi, Booij & Tscharntke 2006; Chaplin-Kramer *et al.* 2011). However, the effect of landscape complexity on herbivorous insect populations is inconsistent, with conflicting responses in both herbivore abundance and diversity (Chaplin-Kramer *et al.* 2011). While increasing attention is being paid to landscape effects on plant pathogens (e.g., Plantegenest, Le May & Fabre 2007; Metz *et al.* 2012), there are to date relatively few landscape studies of plant viruses (but see Carrière *et al.* 2014). Yet as the majority of plant viruses are insect-transmitted (Power & Flecker 2008), the effect of landscape complexity on virus spread is likely to be strong.

Because the incidence of an insect-vectored pathogen depends on transmission, the landscape complexity of a region could affect local disease prevalence through both direct and indirect effects on the insect vector population. As many aphid species are specialist pests on agricultural crops, simplifying the landscape by increasing the amount of agricultural land might directly increase aphid abundance and species richness through resource concentration (Root 1973). Vector community composition could in turn affect disease transmission in two ways. First, because vector species have different transmission efficiencies, the species composition of the vector assemblage could dramatically change the rate of disease spread (Mello *et al.* 2011). Second, interactions

between co-occurring vector species, including competition, may influence vector behavior and distribution, altering disease prevalence.

Landscape composition could also affect viral prevalence by indirectly influencing the vector community via their natural enemies. The aforementioned inverse relationship between landscape complexity and natural enemy abundance and diversity could be caused by a reduction in species spillover (Tscharntke *et al.* 2012); increasing the amount of agricultural land in a region often demands a reduction in natural habitats. Natural enemy populations can have a cascading impact on disease, by affecting prey abundance, behavior, and movement (Gardiner *et al.* 2009; Finke 2012). Natural enemies have the potential to act as a control for vector-borne diseases by suppressing vector movement and abundance (Moore, Borer & Hosseini 2009). However, predators could also encourage greater vector movement, which may increase transmission and disease prevalence (Finke 2012).

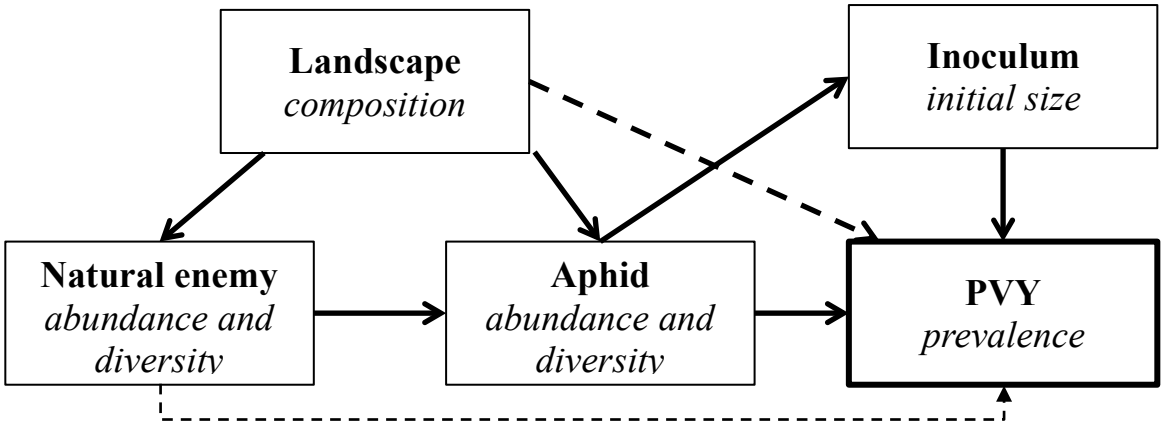


Figure 1: Diagram of interactions in the PVY system. Solid arrows represent direct relationships and dashed arrows represent indirect relationships.

This study tests the effects of the landscape, predator and vector assemblages on the prevalence of an aphid-vectored plant virus, *Potato virus Y* (PVY), a widespread and

economically important pathogen introduced into the system every spring by newly planted seed tubers. We hypothesize that landscape simplicity, here measured as the percentage of agricultural land in a given area, 1) increases virus prevalence, and 2) this effect is mediated by the effects of landscape composition on the vector assemblage (Figure 1). To test these hypotheses, and to determine if the effects are direct or indirect (via landscape influence on the predator assemblage), we explored the following three questions:

1) What is the effect of landscape simplicity on PVY prevalence, and the aphid vector and coccinellid predator communities?

2) What is the effect of aphid vector abundance and species richness on PVY prevalence and the predator community? And

3) What is the effect of coccinellid predator abundance and species richness on PVY prevalence and the vector community?

We investigated these questions by measuring PVY prevalence, and aphid and coccinellid abundance and species richness in potato fields across landscapes spanning a gradient of simplicity during the 2012 and 2013 growing seasons.

Materials and Methods

Study system.

PVY is a potyvirus of significant concern for potato growers, as it can severely reduce potato quality and yield (Nolte et al. 2004; Scholthof *et al.* 2011). There are no known environmental reservoirs of PVY in the northeast, but because the virus is transmitted in seed tubers, it is often introduced into potato fields at planting (Gray *et al.*

2010; Karasev & Gray 2013). The median PVY infection in sample seed lots was 2.4% for US states (Gray *et al.* 2010). The symptoms of PVY vary in presentation and severity between potato cultivars and among its multiple strains, making infected plants difficult to identify in the field for removal. The rapidity of transmission (less than a minute during aphid probing or feeding) and the large number of vector species (over 50 aphid species transmit PVY) also limit effective mitigation strategies (Gray *et al.* 2010). Aphid vector species vary widely in transmission efficiency and life history (Gray *et al.* 2010), and may be differentially affected by landscape composition, especially as many aphid vector species are non-colonists (do not settle and reproduce) on potato. Coccinellids are important, naturally occurring aphid predators in potato fields (Alyokhin & Sewell 2004), and they were the only aphid predators in significant numbers at our study sites. While the relationship between coccinellid predators and aphids has been extensively studied in the context of biological control, the effect of predators on vector populations is variable and their impact on disease transmission is unresolved (Finke 2012).

Farm sites

Monitoring took place at 19 farms in 2012 and 21 farms in 2013, including 17 of the farms sampled in 2012. The farms were located throughout the Finger Lakes region of New York State, all grew multiple cultivars of potatoes, and there was a wide range of cultivars grown among farms. The potato fields varied dramatically in size, from 21m^2 to 12,140,000m^2. Field management also varied, though all but three farms used little to no pesticides. Nearby agriculture consisted of similar, highly diverse farms. To our knowledge, there were no significant concentrations of PVY host plants in the study areas.

The farms were selected to represent a gradient of landscape simplicity, ranging from 3.6% to 92.3% cropland within 500m, 5% to 77.8% cropland within 1000m, and 7.1% to 73.4% cropland within 1500m. Land use within 500m 1000m, and 1500m of the site was calculated using the 2012 and 2013 Crop Data Layers (USDA National Agricultural Statistics Service Cropland Data Layer), respectively, and ArcGIS software (ESRI, ArcMap 10.2), and was divided into three categories: cropland, natural habitat, and non-agricultural land. All managed land, including orchards and pasture, was included as cropland. Fallow or idle cropland and forested land categories were included as natural habitat. Developed land, barren land, and open water were categorized as non-agricultural land. The sites were at least 1 km apart. While some sites were close enough to allow for long distance movement between farms, transmission of PVY from one potato field to another is not supported by our data. Preliminary analyses using the USDA Cropland Data Layer demonstrated that the percentage of potato in the landscape had no effect on PVY prevalence. Mantel tests were used to test for spatial autocorrelation of landscape simplification. The results for the Mantel tests were not significant (Supplemental Table 1) indicating that our measure of landscape simplification was not spatially autocorrelated.

Sampling schedule

Sites were divided into two groups, based on their distance from Ithaca: those sampled weekly (11 farms in 2012 and 12 in 2013), and those sampled periodically (2-3 times) during the growing season (8 farms in 2012 and 9 in 2013). For the sites sampled each week, the insect traps (see below) were collected and replaced at each visit. The traps were collected and removed after a week at the sites sampled periodically. Virus

sampling was biweekly at the weekly visited sites, and done at each visit for the sites visited 2-3 times a season.

Monitoring viral prevalence

To monitor viral prevalence, twenty potato plants grown as part of the regular field crop at each farm were randomly selected for sampling for PVY using a random number table, and the sampled stem was marked with a survey flag. Each subsequent sample was taken from new apical leaves, and when possible, from the same stem.

In addition to the representative field plants sampled, twenty sentinel plants were planted at 11 farms in 2012 and 10 farms in 2013. The sentinel plants ensured that some of the plants sampled would have uniformity of cultivar (and therefore uniform susceptibility to PVY), uniform soil, and be disease free at planting. Each plant (cv. Yukon Gold) was grown from a disease-free seed tuber (Uhlein Farm, Cornell University) in a two-gallon plastic pot full of commercially available potting media (SunGro Sunshine Organic Potting Soil) and 45mg fertilizer (Jobe's Organics Vegetable & Tomato, 2:5 (N:P)). The pots were distributed haphazardly throughout the plot. The sentinel plants were sampled in concert with the representative field plants, and in the same fashion. The number of plants sampled was sufficient to detect infection.

Sampling occurred every two weeks on the weekly sampled farms, and every visit on those sampled periodically. One to three of the newest fully expanded leaflets were taken at each virus sample, and immediately placed in a BioReba extraction bag (BioReba AG, Switzerland) and stored in a cooler. Upon return to the lab, each sample was weighed and stored in a -20°C freezer for later enzyme-linked immunosorbent assay (ELISA; Ellis *et al.* 1996) analysis.

Final PVY prevalence (i.e. cumulative incidence) was calculated as the proportion of all plants sampled (field and sentinel plants) that resulted in a positive ELISA reading throughout the season. Sentinel and field plants were not evaluated separately, because the rate of infection in the sentinel plants was too low to permit a comparison (average sentinel plant final prevalence: 0.04; average field plant final prevalence: 0.11, in the total dataset). Because PVY is not uniformly distributed throughout the potato plant (Kogovšek *et al.* 2011), it is difficult to collect an accurate plant tissue sample. We address this difficulty by using final prevalence, a cumulative measure, for greater accuracy in our results.

Monitoring insect abundance and community composition

To monitor the aphid and coccinellid communities, 18 traps (9 water traps and 9 sticky traps) were placed at each farm approximately 3-4 weeks after planting, when plants were emerging, with 6 traps (3 of each type) in each of three rows. The traps were placed in the two peripheral rows and the center row of each site. The water traps were made in the style of green tile traps; a hard 17.5x16x9cm plastic box with green plastic that mimics the reflectance of leaves (#4430 filter, Rosco, Markam, Ontario, Canada) glued to the bottom was filled with water and about two drops of dish soap and were suspended approximately 1m off the ground on a rebar stake (Boiteau 1990). The yellow sticky traps were tied approximately 1m off the ground on bamboo stakes using commercial twist ties. The traps were placed approximately midway down the length of a potato row, alternating between trap types, with 1m between traps.

Each trap was collected after one week; the sticky traps were stored at 4°C for later analysis. The water traps were sieved using a $1mm^2$ mesh screen, and the collected

specimens were stored at room temperature in 70% ethanol for later identification. Following collection, aphids and the coccinellid predators were identified to species using morphological characters (D. Voegtlin and D. Lagos, and J. Losey, respectively). Seventy-two percent (1240) of aphid specimens were identified to species, 21% (366) were identified to genus, and the remaining 7% (113) could not be resolved. Ninety-five percent (135) of coccinellid predator specimens were identified to species, and the remaining 5% (7) were unidentified. A more detailed analysis of the relationship between landscape composition and aphid community composition is underway (S. Claflin, unpublished data).

Aphid abundance was calculated as the mean number of aphids per water trap. Aphid species richness was calculated as the number of species collected over the season at a site. As most genera only included one or a few aphids, each genera was treated as a unique species in these analyses. Unidentified specimens were excluded from analyses. Coccinellid predator abundance and species richness were calculated in the same fashion.

Statistical Analysis

Preliminary analysis showed that year had a significant effect on pathogen prevalence. Consequently, final models analyze study years separately. Conversely, preliminary analysis demonstrated that field size had no effect, and field size was omitted from final models.

Sequential models for over-dispersed, zero-inflated data

Because the data from our study is zero-inflated (characterized by excess zeros), we used a two-step modeling process for our statistical analysis. Theory suggests that

excess zeros are generated by a separate process from that responsible for nonzero count values, and that the excess zeros can and should be modeled independently. Because PVY is primarily introduced via infected seed tubers (tuber pieces used to seed fields), and not through environmental reservoirs, the initial presence of PVY in the landscape is unrelated to the environment or to landscape parameters. Therefore farms without infected seed or observed plant infection cannot be used to test the effect of landscape composition on PVY prevalence.

We assume that Bernoulli probability governs the binary outcome of whether a count variate has a zero (no observed infection = 0) or a positive (observed infection = 1) outcome. In two-step models it is typically then assumed that the positive count data is governed either by a Poisson process or by a Binomial (success/failure) process. However, as our data is both zero-inflated and over-dispersed, we cannot assume a Poisson process for the count data, and we model it as binomial success/failure.

The two-step modeling therefore employs an initial model for the binary "presence" versus "absence" of disease, and includes information on presence and absence of disease from both infected and non-infected farms, thus the entire dataset. This step shows that, as expected, there was no relationship between landscape parameters and the presence or absence of infection (Supplementary Table 2). The second step utilizes a reduced dataset including only positive count data from infected farms in a binomial model with disease "successes" (number of infected samples) versus "failures" (number of uninfected samples) regressed against landscape parameters. In both 2012 and 2013, 8 farms with no observed PVY were excluded from the reduced dataset. Because year was a significant factor in preliminary analyses, and because the

number of sites varies between years, each year was analyzed separately. We also performed success/failure analysis on the full dataset (including zero values from uninfected farms) and get consistent, though less significant results (see the Supplementary Information).

PVY prevalence versus landscape parameters.

Analysis of the effects of landscape composition on PVY prevalence examined effects of percent cropland at three distance scales (500m, 1000m, and 1500m). The effect of percent cropland was significant at all three scales, but AIC scores were computed to determine the most predictive model and distance. For both 2012 and 2013, the distance with the most explanatory power is 1000 meters. The effect of natural habitat on final virus prevalence was also evaluated, using percent natural habitat at scales of 500, 1000, and 1500 meters from the sampling site. Again, the model with the lowest AIC value was at the 1000 m scale for both years, and was selected as the best model.

PVY prevalence versus insect parameters.

Analysis of the effects of aphid and coccinellid species richness and abundance on PVY prevalence was performed using a binomial (success/failure) model for positive count data on the reduced and full datasets.

Effects of Landscape on Insect Communities.

In models analyzing the effect of landscape simplicity on coccinellid and aphid communities, percent cropland at three distance scales (500m, 1000m, and 1500m) was included as the explanatory variable and the abundance and species richness of each insect community as the response variable, using the reduced dataset and assuming a linear Poisson or quasi-Poisson family model. To determine the effect of the natural

habitat area on the insect community, these analyses were repeated with the percent natural habitat replacing the percent cropland. AIC scores were computed for both scenarios so that critical distances could be determined.

Because percent agriculture and percent natural habitat were significantly negatively correlated at all three scales, in both the reduced and full datasets, only the results of the models including percent agriculture are reported below (Supplementary Table 2). The results of models including percent natural habitat are in the supplemental materials (Supplementary Table 3). All analyses were performed in ***R*** (R Statistical Software, version 3.0.1).

Results

Virus prevalence

The effect of landscape composition on virus prevalence was consistent and strong. There was a significant positive relationship between final virus prevalence and percent cropland in both years (Figure 2, Table 1). As expected, given the negative correlation between the percent agriculture and the percent natural habitat, the effect of percent natural habitat on the final PVY prevalence was inverse to effects of percent cropland. The amount of natural habitat had a significant negative effect on final PVY prevalence in both years (Figure 2, Table 1). In both years, the most predictive scale was 1000m (Table 1, Supplementary Table 4). These relationships were also evident in the full dataset that included farms with no infection (Supplementary Figure 1, Supplementary Table 5 and 6).

Aphid community parameters also consistently affected final viral prevalence. Aphid abundance (Figure 3a) significantly affected viral prevalence in both years, but in opposing directions. In 2012, aphid abundance had a significant positive effect on final disease prevalence ($p<0.001$), while in 2013 it had a significant negative effect ($p<0.001$). Removal of potential outliers did not affect the results. Aphid species richness had a negative effect on final virus prevalence, but the effect was only significant in 2013 ($p<0.001$; Figure 3b). For results from the full dataset see Supplementary Figure 3a, 3b.

Table 1: Results from binomial positive counts models (step 2 in the sequential modeling process) evaluating the effect of landscape composition, measured as percent agriculture, on final PVY prevalence, and results from a linear poisson-family models evaluating the effect of landscape composition, measured as percent agriculture, on aphid species richness (number of different species captured at each site). Analysis is of the reduced dataset, only the most predictive scale is shown, and bold type shows significant p-values. For results from the less predictive scales and full dataset, please see *Supplementary Table 4, 5, and 6.*

Response variable	Explanatory scale (m)	df	estimate	p	AIC
2012 PVY prevalence	1000	2	0.064	**3.41E-06**	44.65
2013 PVY prevalence	1000	2	0.031	**5.26E-05**	98.8
2012 Aphid species richness	1000	2	-0.01316	**0.00545**	92.15
2013 Aphid species richness	500	2	-0.006566	**0.0136**	111.7

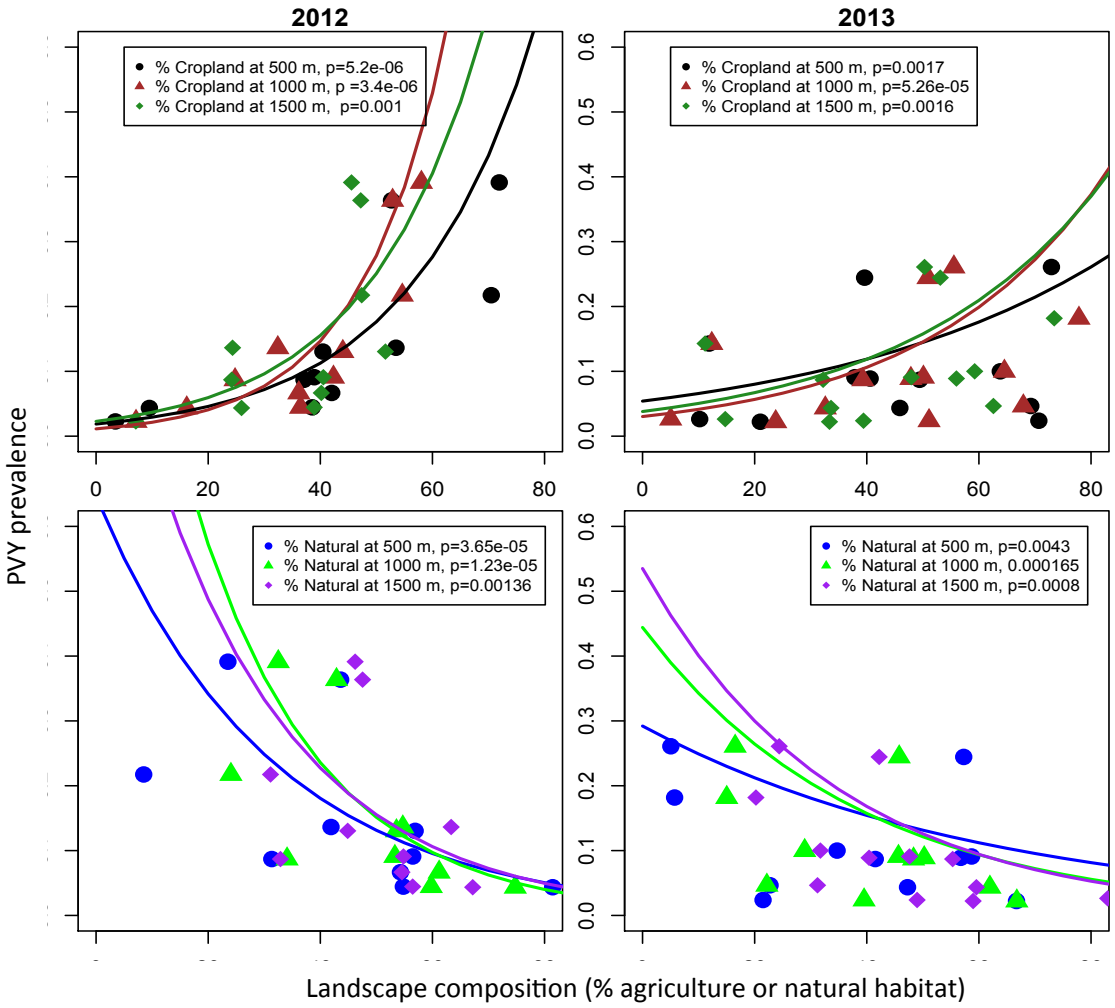
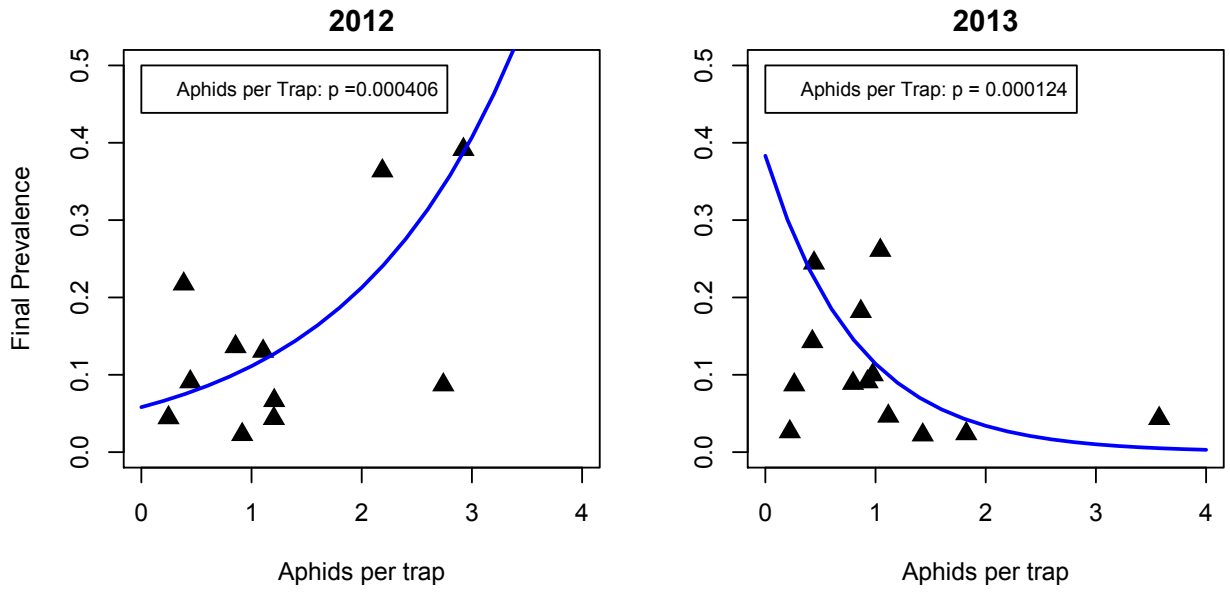


Figure 2: Top two panels: PVY prevalence (y-axis) increases with increasing percentage of agricultural land (x-axis), years 2012 in left panel and 2013 in right panel. Percent cropland at 500 m, black symbol and line, at 1000 m (the most predictive scale in 2012 and 2013), red symbol and line, and at 1500 m, green symbol and line. *Bottom two panels:* PVY prevalence decreases with increasing percentage of natural habitat in the landscape, years 2012 in left panel and 2013 in right panel. Percent natural at 500, blue symbol and line, percent natural at 1000 m (the most predictive scale in 2012 and 2013), green symbol and line and percent natural at 1500 m, purple symbol and line.

a.



b.

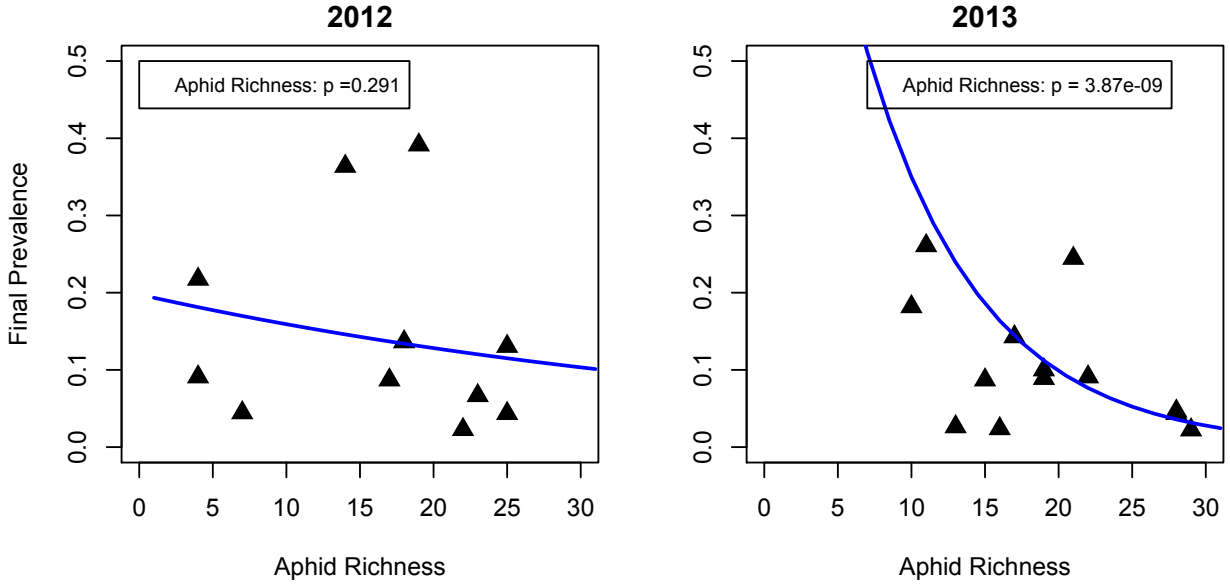


Figure 3: *3a:* Aphid abundance (aphids per trap) and final PVY prevalence in 2012 and 2013. Both analyses are performed on the reduced dataset. *3b*: Aphid species richness and final PVY prevalence in 2012 and 2013. For equivalent results from the full dataset, see Supplementary Figure 3ab.

Coccinellid species richness had a significant negative effect on final virus prevalence in 2012 ($p=0.005$), but no effect in 2013 ($p=0.37$). Coccinellid abundance had no effect in either year. In the full dataset, the effect of predators was similar to that using the reduced dataset, except that coccinellid abundance had a significant negative effect ($p=0.026$) in 2012 (Supplementary Figure 3a, 3b).

Insect community composition

Aphid species richness had a significant negative relationship with percent cropland in both years (Figure 4, Table 1). The most predictive scale for this study was 1000 meters in 2012 and 500 meters in 2013. Analysis of the full dataset yields consistent results (Supplementary Figure 4, Supplementary Table 6). Again, similar analysis of the effects of percent natural landscape on aphid species richness shows the inverse relationship. In this case, a significant positive relationship (Supplementary Table 6). Percent cropland had no effect on aphid abundance, coccinellid abundance, or coccinellid species richness in either year. There was no effect of the coccinellid community on the aphid community in either year. Summary statistics for the insect communities on infected farms are provided in Table 2.

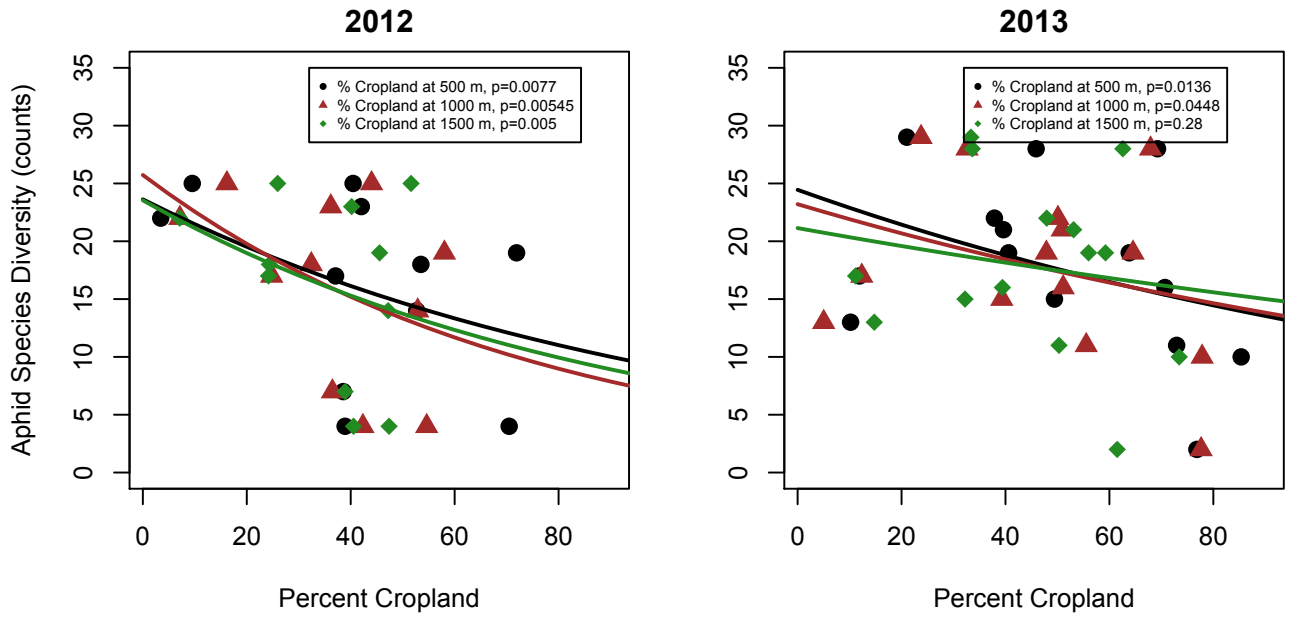


Figure 4: Aphid species richness decreases with increasing agriculture in a landscape. Percent cropland at 500 m (the most predictive scale in 2013), black symbol and line, at 1000 m (the most predictive scale in 2012), red symbol and line, and at 1500 m, green symbol and line. See Table 3 for parameter estimates, p-values, and AIC values, and for results from analysis of the effects of natural landscape on aphid species richness. Analysis was completed on the reduced dataset. For equivalent results from the full dataset, see Supplementary Table 2.

Table 2: Summary statistics for both the aphid and coccinellid communities on infected farms in 2012 and 2013.

Year	Insect community	Number caught	Mean abundance (#/trap)	Abundance range (#/trap)	Species richness range	Mean species richness
2012	Aphid	587	0.31-3	1.32	4-25	16.18
2012	Coccinellid	36	0-0.19	0.084	0-3	1.9
2013	Aphid	704	0.31-3.76	1.08	2-29	18
2013	Coccinellid	44	0-0.4	0.086	0-5	1.92

Discussion

Increasing landscape simplicity had a consistent, positive effect on PVY prevalence, with farms in the most complex landscapes near zero prevalence and those in highly simplified areas exhibiting prevalences over 30%. Although much work has been done on the relationship between landscape composition and arthropod communities, few studies have examined the effects of landscape composition on plant pathogens. To our knowledge, only two previous empirical landscape studies have tested the effect of landscape composition on a plant virus (Carrière *et al.* 2014; Rodelo-Urrego *et al.* 2013). Our findings are some of the first to demonstrate that landscape simplicity can have a dramatic effect on local plant virus prevalence.

We hypothesized that due to the greater concentration of resources, increasing landscape simplicity would result in a greater abundance and diversity of aphid vector species, and that the inverse would be true for the coccinellid predator assemblage. We further assumed that as a consequence, increased landscape simplicity would result in greater PVY prevalence. We tested these hypotheses by addressing the questions discussed below.

1) What is the effect of landscape simplicity on PVY prevalence, and the vector and predator communities?

Landscape simplicity within scales ranging from 500m to 1500m around a site was positively associated with final PVY prevalence on farms in this study. While plant pathology has lagged behind its zoological counterpart in incorporating spatial data into research (Plantegenest, Le May & Fabre 2007), and nearly all landscape-level plant

pathogen studies explore fungal and oomycete pathogens in non-agricultural landscapes (Meentemeyer, Haas & Václavík 2012), our results agree with the findings of three broadly related studies. First, working with West Nile virus (WNV), Crowder *et al.* (2013) found that land use, specifically orchards, positively affected the abundance and prevalence of WNV in mosquito vectors at the local level, and significantly increased WNV prevalence in animal reservoirs and hosts at the landscape level. Second, researching two whitefly-transmitted viral pathogens in wild pepper, Rodelo-Urrego *et al.* (2013) demonstrated that greater amounts of human management had a positive relationship with pathogen prevalence at the landscape scale. Finally, Carrière *et al.* (2014) showed that the amount of the landscape containing reservoir host plants positively affected the prevalence of a whitefly-transmitted virus in melons, and the authors found 1500m to be the most predictive scale in this system. As in these studies, the local nature of the effect seen in our study indicates that local landscape and on-farm management can influence PVY prevalence, making the virus potentially manageable by farmers. Larger landscape scales may also play a role, and should be explored in future studies. While field size had no effect in this experiment, other management practices may affect disease prevalence.

Landscape composition did not have a significant effect on aphid abundance in this study. However, the significant relationship between landscape composition and aphid species richness along with the significant relationship between aphid abundance and species richness, and disease prevalence (discussed below) indicates that, although other possibilities cannot be ruled out, the vector community is the most likely mechanism for the landscape effect on disease. This is in agreement with previous work.

Landscape complexity within similar scales (<1500m) has previously been demonstrated to influence aphid abundance (Hassan *et al.* 2013), population dynamics (Vialatte *et al.* 2006), and reproductive mode (Gilabert *et al.* 2009), as well as the species richness of an array of herbivore and predator groups, including leafhoppers (Rosch *et al.* 2013), wasps, bees, and their antagonists (Steckel *et al.* 2014). Local and aerial aphid populations colonize cultivated wheat fields, though the aerial populations may have a greater impact (Vialatte *et al.* 2007, Vialatte *et al.* 2005); the scale of effect demonstrated in this study (500-1500m) could influence both local and long-distance migrants. Landscape composition could shape the local reservoir population by determining its source species pool and attract the aerial population through the concentration of resources. Accordingly, Carrière *et al.* (2014) found that increasing the amount of host plant in the landscape increased the abundance of the insect vector, and that vector abundance had a strong effect on virus prevalence. There are several viable hypotheses explaining the effect of the landscape on the vector community in this system, discussed below.

The amount of natural area in the landscape at the most predictive scale had a strong negative relationship with virus prevalence. While our study was not designed to test Tscharntke *et al.*'s hypotheses (2012), these results do not support the *cross-habitat spillover hypothesis* as a possible mechanism for the landscape effect. This hypothesis posits that by moderating population dynamics via species spillover between habitats, including agriculturally managed and unmanaged land, landscape affects local vector communities. If vector species spillover were occurring, increasing the amount of natural habitat (the source of overwintering aphids) should positively affect virus prevalence. Although not directly tested by this study, aphid species functional traits may be

important, as suggested by the *landscape-moderated functional trait selection hypothesis*. This hypothesis posits that species functional roles and local vector community assembly may be impacted by the selection of functional traits, such as colonization and vector ability, by the landscape. The effect of landscape composition may be mediated by the landscape species pool; different species occupying different habitats may affect the overall transmission efficiency of the vector community (*landscape species pool hypothesis*; Tscharntke *et al.* 2012). In this case, it could mean that the cumulative effect of the landscape on vector community composition and species identity, rather than abundance or species richness, drives virus prevalence. It is also possible that the landscape effect shown here is influenced by virus amplification in potatoes. Because many farmers in this study save potato seed from one growing season to replant in the next, increased within-season disease spread in areas with greater amounts of agricultural land could be amplified, and result in greater virus prevalence over time. These pockets of high disease prevalence could also serve as a source for within-season virus spread between fields or farms, mediated by the aphid community. The dominant mechanism behind the landscape effect will depend on the relative importance of aphid species identity, function, and diversity on PVY transmission.

2) What is the effect of aphid vector abundance and species richness on PVY prevalence and the predator community?

In this study, we found a significant relationship between aphid abundance and virus prevalence. This finding is consistent with previous work demonstrating the importance of vector abundance to disease spread in agroecosystems (e.g. Carrière *et al.*

2014). However, we found that the direction of the relationship between aphid abundance and final PVY prevalence varied by year: positive in 2012 and negative in 2013. This shift might be explained by changes in aphid community composition between years, with a greater abundance of inefficient or non-vector species resulting in less disease spread (S. Claflin, unpublished data). This may reflect annual variation in aphid community composition, which is supported by the relationship between aphid species richness and PVY prevalence, discussed below.

The negative relationship between aphid species richness and final PVY prevalence found in this study could be driven by several factors. First, the assembly of the aphid community may be nonrandom. Working with another aphid-vectored virus, *Barley yellow dwarf virus*, Lacroix *et al.* (2014) found that the dilution effect (decreased disease prevalence with increased biodiversity) evident in western grasslands was caused by nonrandom host species loss, indicating that host species identity and functional traits, and thereby species loss order, affected virus prevalence. In the PVY system, the same mechanism could be at work, with nonrandom vector community assembly creating a progressively less efficient group of transmitters and decreasing virus prevalence. Second, greater species richness may modify vector behavior through inter-specific competition (Gianoli 2000); this mechanism would likely have a stronger effect on colonizing species, which remain on the host plant for extended periods, but may also apply to the broader community. Finally, the variance of the relationship between aphid species richness and abundance and viral prevalence among the two years may reflect annual shifts in community composition caused by differences in temperature, precipitation or population cycles.

3) What is the effect of coccinellid predator abundance and species richness on PVY prevalence and the vector community?

While there is abundant evidence demonstrating the ability of coccinellids to suppress aphid populations (Obrycki *et al.* 2009), as well as a well-developed theoretical basis for expecting predators to influence vector-borne disease prevalence (Moore *et al.* 2009, Finke 2012), in this study the coccinellid community did not have a significant effect on the aphid community. Despite this lack of relationship, predator species richness had a negative effect on disease prevalence in 2012, though not in 2013. However, given the relative scarcity of coccinellids observed in the field and the fact that the majority of aphids were non-colonists, the impact of landscape simplicity on PVY is more likely mediated by its direct effect on the vector assemblage, rather than by its indirect effect on vectors through its influence on the predator community.

By focusing on a vectored agricultural plant virus, this study makes a novel contribution to the growing field of landscape epidemiology, and offers a starting point for the investigation of landscape effects on PVY prevalence. The landscape effect on PVY prevalence is clearly demonstrated, and appears to be mediated by the direct effect of the landscape on the vector community. To advance understanding of plant virus systems and aid in development of disease mitigation strategies, it is critical that further research be conducted on the effects of landscape composition and connectivity on local arthropod communities. Given the strong effects of landscape composition at local and on-farm scales (500m to 1500m) demonstrated in this study, our work suggests that PVY

management approaches should be explored for small-scale farmers in diverse landscapes.

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REFERENCES

Alyokhin, A, and G Sewell. (2004). Changes in a lady beetle community following the establishment of an alien species. *Biological Invasions*, 6: 463-471.

Bianchi, FJJA, CJH Booij, and T Tscharntke. (2006). Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society B*, 273. 1715-1727.

Boiteau, G. (1990). Effect of trap color and size on relative efficiency of water-pan traps for sampling alate aphids (Homoptera: Aphididae) on potato. *Journal of Economic Entomology*, 83: 937-942.

Carrière, Y, B Degain, KA Hartfield, KD Nolte, SE Marsh, C Ellers-Kirk, WJD Van Leeuwen, L Liesner, P Dutilleul, and JC Palumbo. (2014). Assessing transmission of crop diseases by insect vectors in a landscape context. *Journal of Economic Entomology*, 107: 1-10.

Chaplin-Kramer, R, ME O'Rourke, EJ Blitzer, and C Kremen. (2011). A meta-analysis of crop pest and natural enemy response to landscape complexity. *Ecology Letters*, 14: 922-932.

Crowder, DW, EA Dykstra, JM Brauner, A Duffy, C Reed, E Martin, W Peterson, Y Carrière, P Dutilleul, and JP Owen. (2013). West Nile virus prevalence across landscapes is mediated by local effects of agriculture on vector and host communities. *Plos One*, 8: e55006.

Ellis P, R Stace-Smith, G Bowler, and DJ Mackenzie. (1996). Production of monoclonal antibodies for detection and identification of strains of potato virus y. *Canadian Journal of Plant Pathology*, 18: 64-70.

Finke, D. (2012). Contrasting the consumptive and non-consumptive cascading effects of

predators on vector-borne pathogens. *Entomologia Experimentalis et Applicata*, 144: 45-55.

Gardiner, MM, DA Landis, C Gratton, CD DiFonzo, M O'Neal, JM Chacon, MT Wayo, NP Schmidt, EE Mueller, and GE Heimpel. (2009). Landscape diversity enhances biological control of an introduced crop pest in the north-central USA. *Ecological Applications*, 19: 143-154.

Gilabert, A, JC Simon, L Mieuzet, F Halkett, S Stoeckel, M Plantegenest, and CA Dedryver. (2009). Climate and agricultural context shape reproductive mode variation in aphid crop pest. *Molecular Ecology*, 18: 3050-3061.

Gianoli, E. (2000). Competition in cereal aphids (Homoptera: Aphididae) on wheat plants. *Environmental Entomology*, 29: 213-219.

Gray, S, S De Boer, J Lorenzen, A Karasev, J Whitworth, P Nolte, R Singh, A Boucher, and H Xu. (2010). Potato virus y: an evolving concern for potato crops in the United States and Canada. *Plant Disease*, 94: 1384-1397.

Hassan, DA, E Georgelin, T Delattre, F Burel, M Plantegenest, P Kindlmann, and A Butet. (2013). Does the presence of grassy strips and landscape grain affect the spatial distribution of aphids and their carabid predators? *Agricultural and Forest Entomology*, 15: 24-33.

Karasev, AV, and SM Gray. (2013). Continuous and emerging challenges of Potato virus Y in potato. *Annual Review of Phytopathology*, 51: 571-586.

Kogovšek, P, A Kladnik, J Mlakar, M Tušek Žnidarič, M Dermastia, M Ravnikar, and M Pompe-Novak. (2011). Distribution of Potato virus Y in potato plant organs, tissues, and cells. *Phytopathology*, 101: 1292-1300.

Lacroix, C, A Jolles, EW Seabloom, AG Power, CE Mitchell, and ET Borer. (2014).

Nonrandom biodiversity loss underlies predictable increases in viral disease prevalence. *Journal of the Royal Society Interface*, 11: 20130947.

Margosian, ML, KA Garrett, JMS Hutchinson, and KA With. (2009). Connectivity of the American agricultural landscape: Assessing the national risk of crop pest and disease spread. *Bioscience*, 59: 141-151.

Meetenemeyer, RK, SE Haas, and T Václavík. (2012). Landscape epidemiology of emerging infectious diseases in natural and human-altered ecosystems. *Annual Review of Phytopathology*, 50: 379-402.

Mello, AFS, RA Olarte, SM Gray, and KL Perry. (2011). Transmission efficiency of Potato virus Y strains PVY(o) and PVY(N-Wi) by five aphid species. *Plant Disease*, 95: 1279-1283.

Metz, MR, KM Frangioso, AC Wickland, RK Meentemeyer, and DM Rizzo. (2012). An emergent disease causes directional changes in forest species composition in coastal California. *Ecosphere*, 3: 1-23.

Moore, SM, ET Borer, and PR Hosseini. (2009). Predators indirectly control vector-borne disease: linking predator-prey and host-pathogen models. *Journal of the Royal Society Interface*, 7: 161-176.

Nolte, P, JL Whitworth, MK Thornton, and CS McIntosh. (2004). Effect of seed-borne potato virus y on performance of Russet Burbank, Russet Norkotah, and Shepody potato. *Plant Disease*, 88: 248-252.

Obrycki, JJ, JD Harwood, TJ Kring, and RJ O'Neil. (2009). Aphidophagy by Coccinellideae: application of biological control in agroecosystems. *Biological Control*, 51: 244-254.

Plantegenest, M, C Le May, and F Fabre. (2007). Landscape epidemiology of plant diseases. *Journal of the Royal Society Interface*, 4: 963-972.

Power, AG, and AS Flecker. (2008). The role of vector diversity in disease dynamics. In: RS Ostfeld, et al. (eds.), *Infectious disease ecology: The effects of ecosystems on disease and of disease on ecosystems*. Princeton University Press, Princeton, NJ, pp.30-47.

Rodelo-Urrego, M, I Pagán, P González-Jara, M Betancourt, A Moreno-Letelier, MA Ayllón, A Fraile, D Piñero, and F García-Arenal. (2013). Landscape heterogeneity shapes host-parasite interactions and results in apparent plant-virus codivergence. *Molecular Ecology*, 22: 2325-2340.

Root, RB. (1973). Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*, 43: 95-124.

Rösch, V, T Tscharntke, C Scherber, and P Batáry. (2013). Landscape composition, connectivity, and fragment size drive effects of grassland fragmentation on insect communities. *Journal of Applied Ecology*, 50: 387-391.

Scholthof, KB, S Adkins, H Czosnek, P Palukaitis, E Jacquot, T Hohn, B Hohn, K Saunders, T Candresse, P Ahlquist, C Hemenway, and GD Foster. (2011). Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology*, 12: 938-954.

Steckel, J, C Westphal, MK Peters, M Bellach, C Rothenwoehrer, S Erasmi, C Scherber, T Tscharntke, and I Steffan-Dewenter. (2014). Landscape composition and configuration differently attract bees, wasps and their atagnoists. *Biological Control*, 172: 56-64.

Tscharntke, T, JM Tylianakis, TA Rand, RK Didham, L Fahrig, P Batáry, J Bengtsson, Y

Clough, TO Crist, CF Dormann, RM Ewers, J Fründ, RD Holt, A Holzschuh, AM Klein, D Kleijn, C Kremen, DA Landis, W Laurance, D Lindenmayer, C Scherber, N Sodhi, I Steffan-Dewenter, C Thies, WH van der Putten, and C Westphal. (2012). Landscape moderation of biodiversity patterns and processes – eight hypotheses. *Biological Reviews*, 87: 661-682.

Vialatte, A, CA Dedryver, JC Simon, M Galman, and M Plantegenest, M. (2005). Limited genetic exchanges between populations of an insect pest living on uncultivated and related cultivated host plants. *Proceedings of the Royal Society B*, 272: 1075-1082.

Vialatte, A, JC Simon, CA Dedryver, F Fabre, and M Plantegenest. (2006). Tracing individual movement of aphids reveals preferential routes of population transfers in agroecosystems. *Ecological Applications*, 16: 839-844.

Vialatte, A, M Plantegenest, JC Simon, and CA Dedryver. (2007). Farm-scale assessment of movement patterns and colonization dynamics of the grain aphid in arable crops and hedgerows. *Agricultural and Forest Entomology*, 9: 337-346.

CHAPTER TWO
INTRA-ANNUAL VARIATION AND LANDSCAPE COMPOSITION INTERACTIVELY AFFECT APHID ABUNDANCE, SPECIES RICHNESS, AND FUNCTIONAL COMMUNITY COMPOSITION IN TWO AGRICULTURAL REGIONS

Abstract

Agricultural intensification impacts local arthropod communities. The temporal and spatial variation of agricultural environments can have a significant impact on insect pest populations. In this study, we explored the effect of landscape composition, intra-annual variation, and their interaction on aphid abundance, aphid species richness, and functional community composition (in this case, crop virus transmission− or vectoring− ability, and colonization status) in two agricultural regions. We compared a diversified agricultural region in NY and an agriculturally intense potato-growing region in WI. We found that the interactive effect of landscape composition and intra-annual variation significantly affected aphid abundance, species richness, and functional community composition in both study regions. Our results also demonstrate that intra-annual variation explained a substantial amount of the observed variation, and differentially affected vector functional groups in NY. These results indicate that intra-annual variation is an important driver of local aphid communities, and that spatiotemporal shifts in agroecosystems have significant implications for aphid functional community composition.

Introduction

The effect of landscape composition on local communities often varies over time, with temporal shifts and landscape structure interactively shaping local communities. Tscharntke et al. (2012) describe this phenomenon in their *landscape-moderated concentration and dilution hypothesis*, which posits, "spatial and temporal changes in landscape composition can cause transient concentration or dilution of populations with functional consequences". However, most research in this area has explored the effect of inter-annual variation in landscape composition (e.g. habitat fragmentation) on local species richness or the abundance of particular species (Debinski and Holt, 2000). These studies show a wide range of species specific responses to landscape modification, and demonstrate that transient effects dominate in many systems (Debinski and Holt, 2000; Grez et al. 2004). Inter-annual habitat modification not only results in in the transient concentration and dilution of species (Thies et al. 2008), it can also impact species interactions (Tylianakis et al. 2007). Both effects could impact the functional composition of the community. Yet to date, little work has been done on intra-annual (within season) shifts or their impact on the functional composition of local communities (Tscharntke et al. 2012). In this study, we compare data from two potato-producing areas to explore the interactive effects of landscape composition and intra-annual variation on local insect pest abundance, species richness, and functional community composition.

Agricultural systems are fragmented both spatially and temporally, and are subject to high levels of disturbance and variation. Because landscape composition in the surrounding area affects the connectivity and permeability of the landscape for agricultural pests (Margosian et al. 2009), these shifts can affect the abundance and

composition of the local arthropod assemblage. Shifts in landscape composition over time, such as habitat loss and fragmentation, increased chemical inputs, and increased crop cover, associated with agricultural intensification can affect insect pest abundance, and these effects are often species-specific (Grez et al. 2008; Braschler et al. 2003; Banks 1998; Zhao et al. 2015; Weibull and Östman 2003; O'Rourke et al. 2011). Land use, vegetation type, and management style have also been found to affect the composition of arthropod assemblages in groups ranging from bees to spiders (Woodcock et al. 2014; Rusch et al. 2014; Torma et al. 2014). For example, Woodcock et al. (2014) found that the amount of arable crop production and semi-natural habitat cover had opposing effects on the functional diversity of ground beetles and bees. The arable crop production was negatively correlated with functional diversity, while the semi-natural habitat cover was positively correlated with functional redundancy.

Temporal shifts, such as seasonal variation in weather, precipitation, and environmental disturbance, such as raking or other management practices, can affect insect pest abundance and movement (Davis 2014; Cocu et al. 2005; Narayandas and Alyokhin 2006; Krauss et al. 2011). Cardinale et al. (2006) found that the asynchronous mowing of alfalfa fields caused a difference of several orders of magnitude in pea aphid (*Acyrthosiphon pisum* Harris) density among alfalfa fields, resulting in significant spatial and temporal variation. Seasonal insect pest movement is not random; Vialatte et al. (2006) found that aphids have preferred routes, and that most of the aphids colonizing wheat came from another crop, maize. By changing the proportion of each species in the insect pest assemblage, seasonal shifts in species abundances could alter the species composition, and possibly the functional composition of the community.

Aphids are an excellent study system for these questions. Although they are a small insect group, with only approximately 4,000 species globally (Dixon et al. 1987), aphids are a major crop pest and significant plant disease vectors. Aphids vector nearly 50% of the 600 known invertebrate-transmitted plant viruses (Hull 2002). Potatoes are a particularly susceptible crop: 13 of the 30 viral diseases that infect potatoes (Salazar 1996) are transmitted by aphids (Brunt 2001). The majority of the 275 known aphid-transmitted viruses are non-persistent (stylet-borne; Hull 2002), including the focus of this study, potato virus Y (PVY), a potyvirus of significant concern for potato growers, as it can severely reduce potato quality and yield (Nolte et al. 2004; Scholthof et al. 2011; Karasev and Gray 2013).

A large suite of aphid species vector PVY. Aphid vector species vary widely in transmission efficiency (the probability of virus transmission from an infected to an uninfected host plant) and life history (Gray et al. 2010). Many aphid vector species are non-colonists (do not settle and reproduce) on potato. Although the most efficient PVY vector is a potato colonizing species (i.e. settles and reproduces on potato), the large vector assemblage is mostly comprised of non-colonists (Gray et al. 2010). DiFonzo et al. (1997) found that non-colonizing alates (winged morphs) are much more common in potato fields than those of colonizing species, an observation that led them to hypothesize that the non-colonizing vectors are the most significant driver of PVY spread.

In this study, we explored three important functional attributes in the aphid community: 1) the proportion of aphids that were colonists and non-colonists, 2) the proportion of aphids that were PVY vectors and non-vectors, and 3) the proportion of aphids that were unestimated (with no published transmission efficiency), low-

transmitting, and high-transmitting PVY vectors. In this context, altering the aphid functional community composition could have significant implications for predicting pest pressure and PVY risk. Furthermore, assessing the relative impact of intra-annual variation and landscape composition may also help explain previous findings from this data, which showed a strong negative relationship between the percent cropland within 1500m and end-of-season PVY prevalence on small-scale farms in New York State, which appeared to be mediated by the direct effect of the landscape on the aphid community (Claflin et al., unpublished data).

The aim of this study was to evaluate the effects of intra-annual variation and landscape composition on the aphid community. To explore this, we investigated the following research question: How do intra-annual variation and landscape composition (measured as percent agriculture and percent natural habitat) affect aphid abundance, species richness and functional community composition? To address this question, we evaluated two study regions, in order to assess the consistency of our results. We analyzed data collected in two, two-year surveys of the aphid community in potato fields: one in an agriculturally intense region in Wisconsin (WI) in 2010 and 2011 and the other in the diversified Finger Lakes region of New York State (NY) in 2012 and 2013.

Methods

Study system

Aphids are a major crop pest and cause significant crop damage, both directly through phloem feeding and indirectly by vectoring crop diseases (Dedryver et al. 2010). Because plants infected with PVY are difficult to identify in the field for removal and

transmission is so rapid (it can happen in less than a minute during aphid probing or feeding), effective mitigation strategies are limited (Gray et al. 2010). There is also an unusually large (>40 species) suite of vectors species. These characteristics make the aphid community tractable for exploring questions about functional community composition and abundance.

Farm sites

In NY, sampling took place at 19 farms in 2012 and 21 farms in 2013. Seventeen of the farms from 2012 were sampled again in 2013. The farms were located throughout the Finger Lakes region, all grew multiple cultivars of potatoes, and there was a wide diversity of cultivars grown among farms. Field management varied, though all but three farms used little to no pesticides. The farms were selected to constitute a gradient of landscape composition, ranging from 3.6% cropland within 500m to 92.3%, 5% to 77.8% cropland within 1000m, and 7.1% to 73.4% cropland within 1500m (Supplemental Table 1).

In WI sampling took place at 9 sites in 2010 and 9 sites in 2011. One 2010 site was excluded from analyses because there was significant overlap in the surrounding landscape (i.e. it was too near) with other sites. Two sites from each year were also excluded from the 1000m scale analyses because of their proximity to other sites, leaving 14 total sites. Five sites from 2010 and three sites from 2011 were excluded from the 1500m scale analyses because of their proximity to other sites, leaving 10 total sites. The sites constituted a gradient of landscape composition, ranging from 41.5% agricultural

land within 500m to 95.6%, 22.7% to 94.6% agricultural land within 1000m, and 37.2% to 91.7% within 1500m (Supplemental Table 1).

Land use within 500m 1000m, and 1500m of the site was calculated using the 2010, 2011, 2012, and 2013 Crop Data Layers (USDA National Agricultural Statistics Service Cropland Data Layer), respectively, and ArcGIS software (ESRI, ArcMap 10.2), and was divided into three categories: cropland, natural habitat, and other. All managed land, including pasture, was included as cropland. Fallow or idle cropland and forested land categories were included as natural habitat. Developed land, barren land, and open water were categorized as other. The sites were at least 1km apart. Mantel tests were used to test for spatial autocorrelation of landscape simplification. The results for the Mantel tests were not significant (Supplemental Tables 2 and 3) indicating that our measure of landscape simplification was not spatially autocorrelated.

Sampling schedule

In NY, the sites were divided into two groups, based on their distance from Ithaca: those sampled weekly (11 farms in 2012 and 12 in 2013), and those sampled periodically (2-3 times) during the growing season (8 farms in 2012 and 9 in 2013). For the sites sampled each week, the insect traps (see below) were collected and replaced at each visit. The traps were collected and removed after a week at the sites sampled periodically. In WI, all sites were sampled weekly.

Sampling insect abundance and community composition

To sample the aphid and coccinellid communities in NY, 9 pan traps were placed at each farm approximately 3-4 weeks after planting, when plants were emerging, with 3 traps in each of three rows. The traps were placed in the two peripheral rows and the center row of each site. The pan traps were made in the style of green tile traps; a hard 17.5x16x9cm plastic box with green plastic that mimics the reflectance of leaves (#4430 filter, Rosco, Markam, Ontario, Canada) glued to the bottom was filled with water and about two drops of dish soap and were suspended approximately 1m off the ground on a rebar stake (Boiteau 1990). The traps were placed approximately midway down the length of a potato row, with 2m between traps.

In WI, sampling was conducted in a similar manner, except that the pan traps contained a green tile− instead of plastic material− to mimick the surrounding plants. They also contained a mixture of 50:50 propylene glycol and water, instead of a water and soap mixture. Twenty-one traps were set at each site along four transects of five traps. One transect was arranged in each cardinal direction with traps positioned at 3.05m outside the potato field, in the weedy margin, at the field edge (0m), between the weedy margin and potatoes, 3.05m into the potato field, 7.62m into the potato field, and 15.24m into the potato field. The final trap was positioned as close to the center of the field as possible.

Each trap was collected after one week; the sticky traps were stored at 4°C for later analysis. The water traps were sieved using a $1mm^2$ mesh screen, and the collected specimens were stored at room temperature in 70% ethanol for later identification. Following collection, aphids were identified to species using morphological characters (Pike et al. 2003, key; D. Voegtlin and D. Lagos, personal communication). In NY, 72%

(1240) of aphid specimens were identified to species, 21% (366) were identified to genus, and the remaining 7% (113) could not be resolved. Aphid abundance was calculated by dividing the total number of aphids collected at a site by the number of water traps. In WI, 79% (3604) of the aphid specimens were identified to species, 18% (809) were identified to genus, and the remaining 3% (130) could not be resolved. Aphid species richness was calculated as the number of species collected over the season at a site. As most genera only included one or a few aphids, each genera was treated as a unique species in these analyses. Unidentified specimens were excluded from analyses.

Analysis

For analyses, specimens were categorized in three ways, according to their species or genus functional traits (Pelletier et al. 2012; Boquel et al. 2011; Verbeek et al. 2010; Mello et al. 2011; Halbert et al. 2003):

1) colonization status (binary distinction between colonizer or non-colonizer),

2) vector status (binary distinction between PVY vector or non-vector),

3) transmission efficiency (non-binary distinction between non-vector, unestimated vector (no accepted transmission efficiency), low-transmitting vector (transmission efficiency <0.1 probability), or high-transmitting vector (transmission efficiency >0.1 probability))

The NY results were analyzed as follows. The colonization status (colonist vs. non-colonist) data was analyzed with generalized mixed models that included colonization status (as an average count) as a response variable, the sampling week (the week of the first sample = 1), a single landscape metric (i.e. the percent natural habitat or

agricultural land within one of three scales), and their interaction as predictor variables, and the farm and year as random effects. The vector status data (vector or non-vector) was analyzed in a similar model, but with vector status as the response variable. These models used a binomial distribution.

The vector ability status data (unestimated vs. low-transmitting vs. high-transmitting vectors) was analyzed with linear mixed models that included vector ability status (as a proportion of the total number of aphids collected) as a response variable, the sampling week (the week of the first sample = 1), a single landscape metric (i.e. the percent natural habitat or agricultural land within one of three scales), and their interaction as predictor variables, and the farm and year as random effects. Because the response variable has >2 categories, these models used a poisson distribution. To compensate for uneven sampling, these models included the log of the number of traps as an offset variable.

The WI results were analyzed in a similar fashion, but because farms were not sampled in both years, the farm was not included as a random effect. And because there was even sampling, no offset variable was included to adjust for trap number. For both sampling regions, the results of the models with the best explanatory value for each response variable, as determined by AIC values, are reported and discussed. Because the percent agriculture and the percent natural habitat were significantly negatively correlated at the most all scales except the 500m scale in WI (where the relationship was marginal), only the models including percent agriculture are reported (Supplemental Table 4). The average percent agriculture and natural habitat and the average number of aphids per trap

of the two study regions were compared using Wilcox t-tests. All analyses were conducted in R (version 3.2.1).

Most predictive models

In NY, the 500m scale models had the greatest explanatory value for aphid abundance, species richness, and the proportion of aphids that were high-transmitting vectors. Models at the 1500m scale had the most explanatory power for the proportion of aphids that were PVY vectors, the proportion that were unestimated vectors and the proportion that were low-transmitting vectors in NY. In WI, the 1500m scale models were the most predictive for all models, except the model exploring the proportion of aphids that were unestimated vectors, where the 1000m scale was most predictive.

Results

Study region and aphid community comparison

The WI study region had significantly lower average percent natural habitat, higher percent agriculture, higher aphid abundance, and higher aphid abundance per trap, compared to the NY study region. The WI study region had more than 25% greater average percent agriculture than the NY study region at the 500m scale, the 1000m scale, and 1500m scale (Table 1). Conversely, the WI study region had more than 36% less average percent natural habitat than the NY study region at both the 500m scale and 1000m scale, and 25% less natural habitat at the 1500m scale (Table 1). The WI study region also had more than six times higher average aphid abundance and 1.14 times higher aphid abundance per trap compared to the NY study region (Table 1).

Table 1: Results of Wilcox t-tests comparing descriptive statistics between the NY and WI sampling regions.

Category	Scale	NY	WI	W	p-value
Average percent agriculture	500	47.9	77.9	100	8.91E-06
	1000	45.0	70.4	91	2.62E-04
	1500	43.7	69.0	71	1.13E-03
Average percent natural habitat	500	45.6	9.4	627	5.81E-07
	1000	47.4	19.0	446	4.39E-05
	1500	48.0	23.0	340	3.38E-03
Average aphid abundance per trap		1.0	1.1	221.5	3.96E-02

In NY, the 1606 identified aphids collected over the two seasons included 87 species and genus groups (when the species could not be determined). In WI, the 4413 identified aphids collected included 110 species and genus groups. There was a wide range in the abundance of different species, from 1 to 241 specimen collected in NY and 1 to 686 in WI. In both study regions *Acyrthosiphon pisum*, a low-transmitting PVY vector, was the most abundant species, with 75 more specimens than the second most abundant species in NY, *Capitopherous elaeagni*, and 368 more specimen than the second most abundant species in WI, *Aphis craccivora*. The most abundant colonizing species was *Macrosiphum euphorbiae* in both the NY and WI study regions, with 62 and 60 specimens collected, respectively.

The two study regions had similar functional aphid community compositions. Of the 1606 identified specimen in NY, approximately 66% were PVY vectors. Of the identified specimen, about 22% of the total were unestimated vectors, about 33% were low-transmitting vectors, and about 11% were high-transmitting vectors. In WI,

approximately 70% of the identified specimen were PVY vectors. Of these, about 33% of the total were unestimated vectors, about 31% were low-transmitting vectors, and about 6% were high-transmitting vectors. The vast majority of specimen (about 94% in NY and 98% in WI) were non-colonizing species. Only approximately 6% and 2% were potato colonizing species in NY and WI, respectively, all of which were PVY vector species (Table 2).

Table 2: Counts and percent of total number of identified aphids for each aphid species category in both study regions.

Category	**Total**	**Percent**	**Total**	**Percent**
State	**NY**		**WI**	
Total	1606	100	4413	100
Vector	1056	65.75	3088	69.98
Non-Vector	550	34.25	1219	27.62
Colonizing	97	6.04	106	2.40
Non-colonizing	1509	93.96	4307	97.60
Unestimated vector	347	21.61	1147	32.89
Low transmitting vector	537	33.44	1358	30.77
High transmitting vector	172	10.71	283	6.41

Interactive effects of intra-annual variation and landscape composition on aphid abundance, species richness and functional community composition.

In this study, the interactive effect of landscape composition and intra-annual variation significantly affected aphid abundance, species richness, and functional community composition in both study regions (Tables 3 and 4; Supplemental Tables 5, 7, 8, and 9). In both regions, the relationship between aphid abundance and landscape composition varied throughout the growing season, with trends in the data showing

similar shifts in the relationship over time (Figure 1). The interaction of the landscape composition and intra-annual variation also had a significant effect on aphid species richness in both study regions (Table 4; Figure 2). Data trends indicate that the shifts in the relationship between landscape composition and aphid species richness were similar between the two regions, and similar to those for aphid abundance (Figure 2).

Table 3: Results of a generalized linear mixed effects model with the total number of aphids per trap collected in NY as a response variable and the number of traps as an offset variable, and of a generalized linear mixed effects model with number of aphids collected in WI as a response variable. Significant p values are in bold. For results from the percent natural habitat models, see Supplemental Table 5.

Response Variable	Explanatory Scale (m)	Predictor Variable	Estimate	Std. Error	z value	p value	R^2
NY							
Number of aphids per trap	500	Week	-0.41	0.026	-15.57	**<2E-16**	0.52
Number of aphids per trap	500	Percent agriculture	-0.12	0.0051	-2.38	**1.71E-02**	0.0034
Number of aphids per trap	500	Week:Percent agriculture	0.0037	0.00049	7.49	**6.74E-14**	
WI							
Number of aphids	1500	Week	-4.30E-02	1.17E-02	-3.68	**0.000235**	
Number of aphids	1500	Percent agriculture	3.05E-02	2.72E-03	11.2	**<2E-16**	0.11
Number of aphids	1500	Week:Percent agriculture	-5.49E-03	4.88E-04	-11.2	**<2E-16**	

Table 4: Results of a generalized linear mixed effects model with the aphid species richness as a response variable. Significant p values are in bold. For results from the percent natural habitat models, see Supplemental Table 5.

Response Variable	Explanatory Scale (m)	Predictor Variable	Estimate	Std. Error	z value	p value	R^2
NY							
Richness	500	Week	-0.194	0.032	-6.045	**1.50E-09**	0.46
Richness	500	Percent agriculture	-0.0059	0.0039	-1.521	0.12835	
Richness	500	Week:Percent agriculture	0.0015	0.0006023	2.503	**0.01232**	
WI							
Richness	1500	Week	-0.0812	0.0174	-4.67	**3.22E-06**	0.51
Richness	1500	Percent agriculture	0.00973	0.00428	2.27	**0.023**	0.034
Richness	1500	Week:Percent agriculture	-0.00142	0.000669	-2.12	**0.0339**	

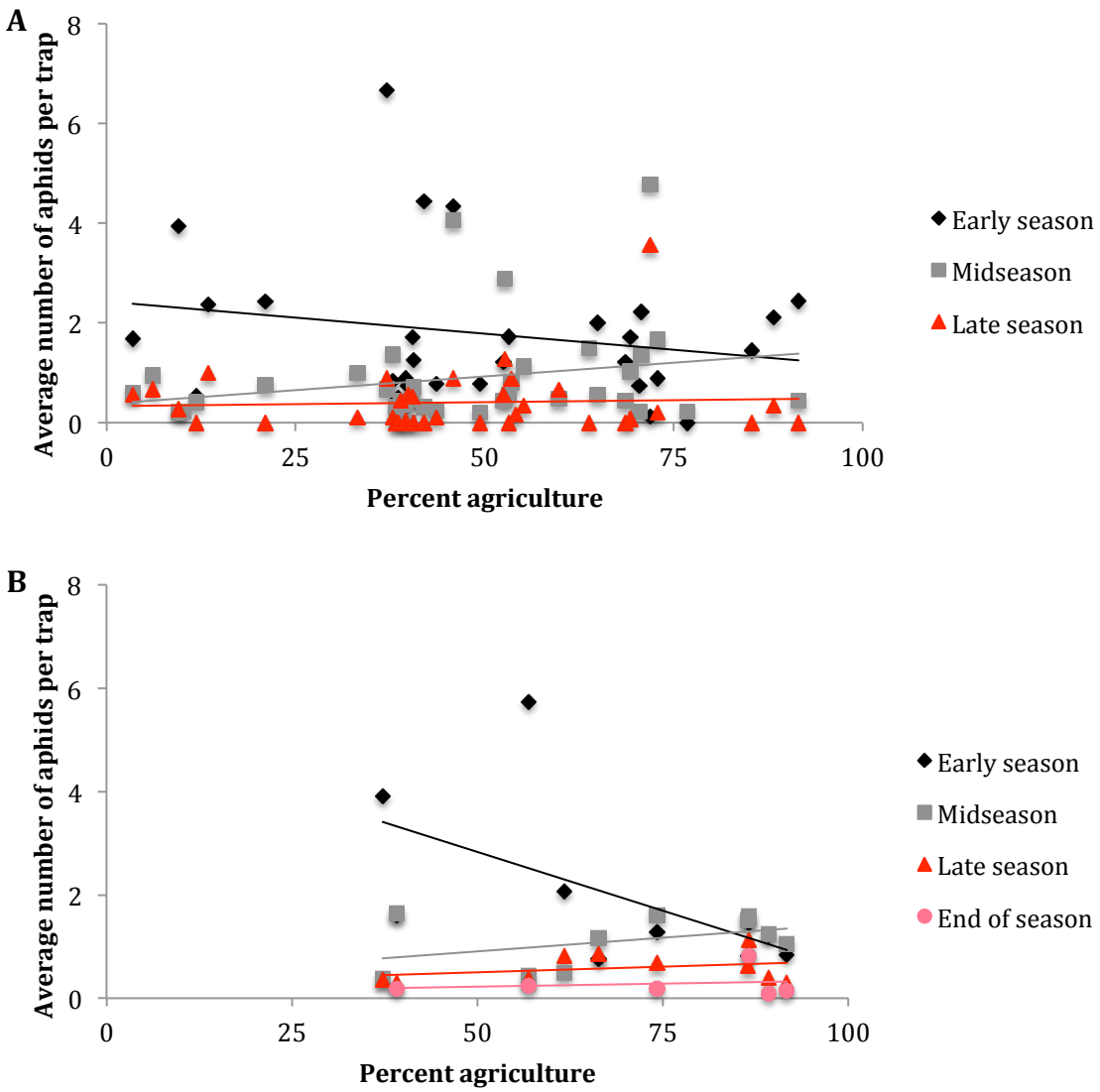

Figure 1: The average total number of aphids across a landscape composition gradient, as measured by the percent agriculture surrounding a site A) within 500m in NY and B) within 1500m in WI. Sites have been grouped by sampling week: early season (week 1-4), midseason (week 5-8), and late season (week 9-12), and end of season (week 13-14). Lines show trends in the data, not statistical significance.

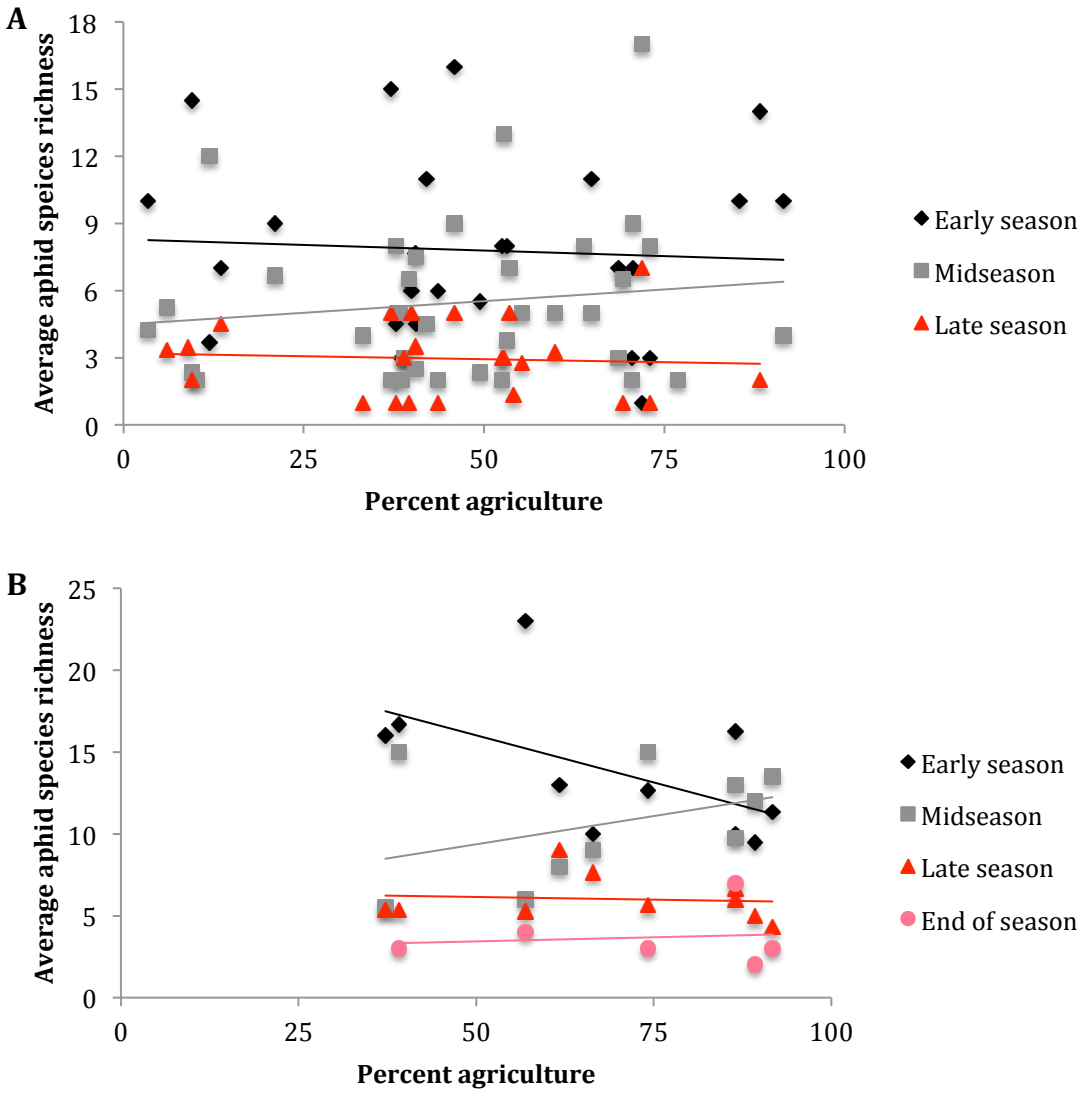

Figure 2: The average aphid species richness across a landscape composition gradient, as measured by the percent agriculture surrounding a site A) within 500m in NY and B) within 1500m in WI. Sites have been grouped by sampling week: early season (week 1-4), midseason (week 5-8), and late season (week 9-12), and end of season (week 13-14). Lines show trends in the data, not statistical significance.

In both study regions, the interaction between landscape composition and intra-annual variation significantly affected aphid functional community composition. In NY, the landscape composition and intra-annual variation interactively affected the proportion of aphids that were PVY vectors (Supplemental Table 8; Figure 3), with the early season differing from the mid and late season. In WI, the interaction of landscape composition

and intra-annual variation significantly affected the proportion of aphids that were colonizers and low-transmitting PVY vectors (Supplemental Table 7 and 9; Figure 4), and the proportion of aphids that were low-transmitting PVY vectors (Supplemental Table 9; Figure 5).

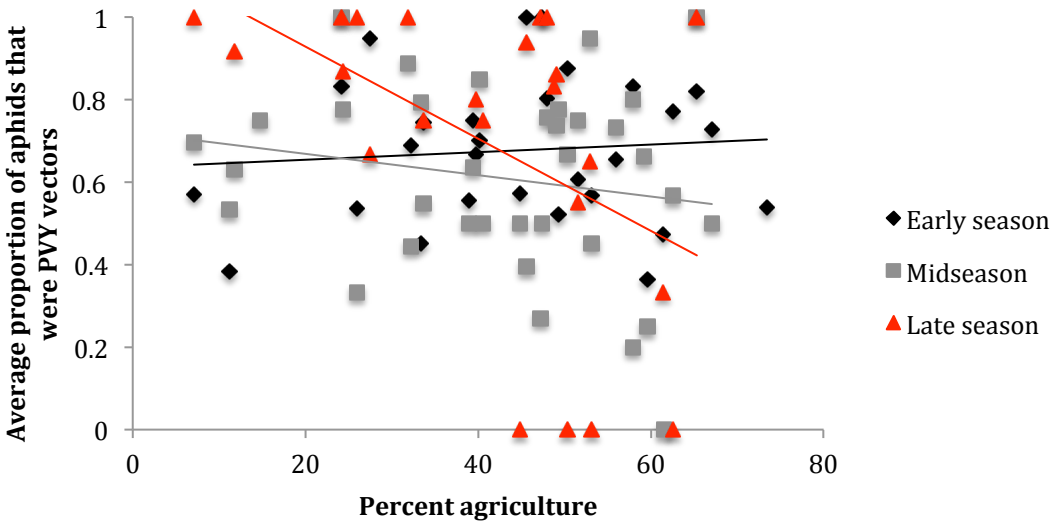

Figure 3: The average proportion of total aphids (excluding unidentified specimen) that were PVY vectors across a gradient of landscape composition in NY. Sites have been grouped by sampling week: early season (week 1-4), midseason (week 5-8), and late season (week 9-12). Lines show trends in the data, not statistical significance.

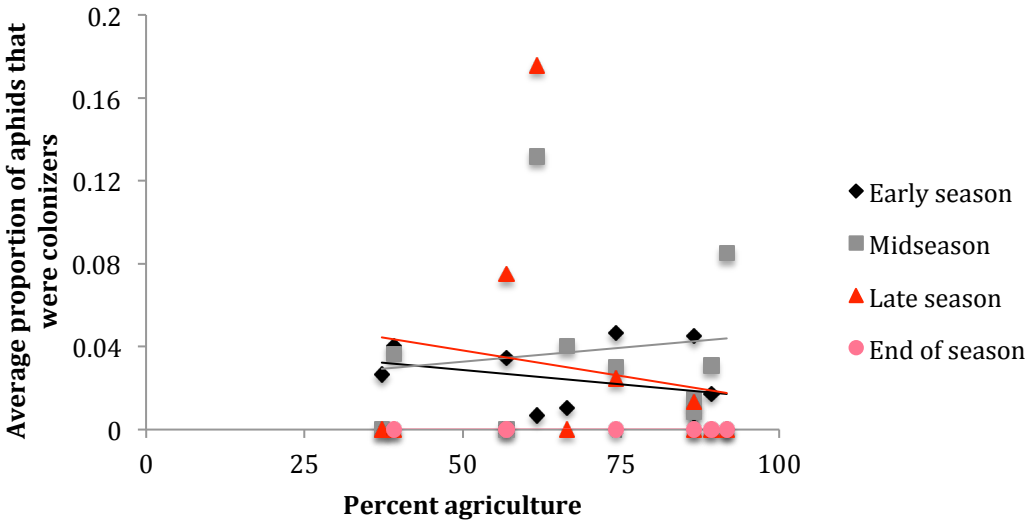

Figure 4: The proportion of aphids that were colonizing species or genera groups across a gradient of landscape composition in WI. Sites have been grouped by sampling week: early season (week 1-4), midseason (week 5-8), late season (week 9-12) and end of season (week 13-14). Lines show trends in the data, not statistical significance.

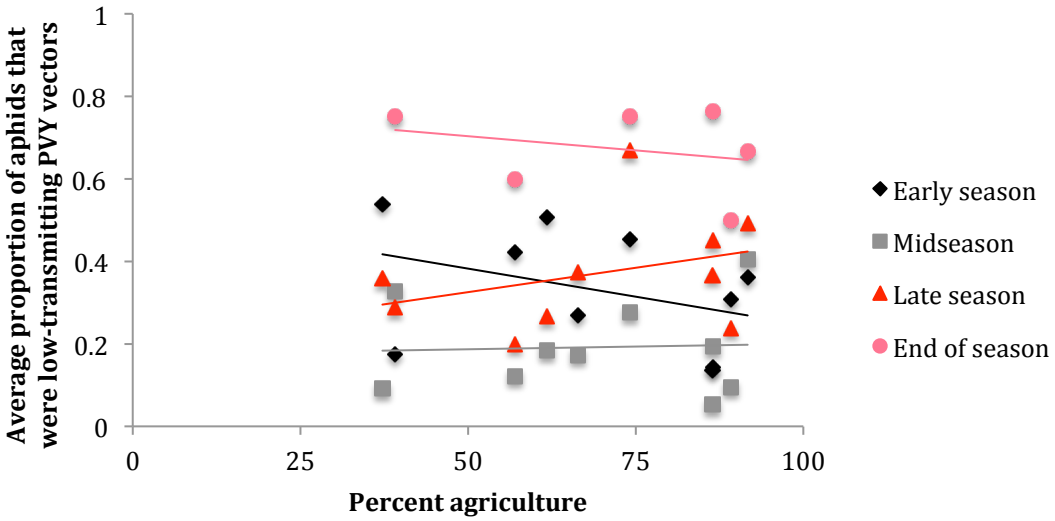

Figure 5: The proportion of aphids that were low-transmitting species or genera groups across a gradient of landscape composition in WI. Sites have been grouped by sampling week: early season (week 1-4), midseason (week 5-8), late season (week 9-12) and end of season (week 13-14). Lines show trends in the data, not statistical significance.

Independent effects of intra-annual variation and landscape composition on aphid abundance, species richness and functional community composition.

Intra-annual variation significantly interactively affected aphid abundance, species richness and functional community composition (Tables 3 and 4; Supplemental Tables 5, 7, 8, and 9; Supplemental Figures 1 and 2). It also significantly affected vector functional groups independently in both study regions. In WI, the proportion of aphids that were PVY vectors significantly increased through the season (Supplemental Figure 1c). In NY, the proportion of the aphids collected that were PVY vectors significantly increased as the season went on, and this effect was driven by the effect of intra-annual variation on the proportion of aphids that were unestimated PVY vectors. In NY, the proportion of aphids collected that were unestimated PVY vectors significantly increased over the season, explaining nearly 80% of the variation (Supplemental Table 9; Supplemental Figure 2a; R^2=0.79). Conversely, the proportion of aphids that were low-transmitting vectors (Supplemental Table 9; Supplemental Figure 2b; R^2=0.61) and high-transmitting vectors (Supplemental Table 9; Supplemental Figure 2c; R^2=0.023) significantly decreased over time. However, the latter relationships were inconsistent. The sampling week only significantly affected the proportion of low-transmitting vectors in the natural habitat model and the proportion of high-transmitting vectors in the percent agriculture model.

Landscape composition significantly interactively affected the aphid community in both WI and NY. In WI, landscape composition also significantly affected the proportion of aphids that were unestimated PVY vectors independent of intra-annual variation (Supplemental Table 9; Supplemental Figure 3). The proportion increased with

increasing amounts of agriculture in the surrounding area . However, landscape composition explained less than 1% of the observed variation.

Discussion

Overall, there was a great deal of species diversity among aphid alates trapped, including over 60 species in each year. Despite significant differences the surrounding landscape composition of the two study regions, several of the most abundant species in the WI study region, such as *A. pisum* and *C. eleagni*, were also the most common in the NY. In both regions, over 90% of specimen were non-colonizers, and over 66% were PVY vectors. The interaction of landscape composition and intra-annual variation affected aphid abundance, species richness, and functional community composition in both study regions. When evaluated independently, intra-annual explained a substantial amount of the observed variation, while landscape composition explained very little. Our results indicate that the landscape composition and seasonal variation interactively shape the aphid community in both diversified and simple cropping systems.

Interactive effects of intra-annual variation and landscape composition on aphid abundance, species richness and functional community composition.

The interactive effect of the landscape composition and intra-annual variation had a consistent, significant effect on aphid abundance, species richness, and aphid functional community composition in both study regions. However, while aphid abundance and species richness showed similar trends in both regions, aphid functional community composition was differentially affected in NY and WI.

Overall, aphid abundance and species richness decreased through the season in both study regions. The decrease in aphid abundance over time could be explained by climatic variables, with higher temperatures and lower precipitation, either directly (by decreasing aphid fitness) or indirectly (by decreasing host plant or ant mutualist fitness) reducing aphid abundance as the season progresses (Adler et al. 2007; Barton et al. 2014). Management strategies, such as greater irrigation or harvesting, may also reduce aphid abundance through the season (Matis et al. 2008). It is unsurprising that aphid species richness followed a similar pattern to aphid abundance. Aphid abundance and species richness are often strongly correlated, and the same factors driving one could influence the other.

The interactive effect of landscape composition and seasonality on aphid abundance and aphid species richness was remarkably consistent between the two study regions. The variance in the relationship between landscape composition and the aphid community could reflect the importance of different habitat types of aphid spread at different points in the season. Areas of natural habitat may be more important for early season aphid spread, providing overwintering hosts that allow for greater early season aphid spread in areas that are less agriculturally intense. Conversely, greater concentrations of cropland may increase long-distance aphid spread. Previous work demonstrates that increasing the amount of agricultural land increases aphid abundance (Gagic et al. 2012). Greater resource concentration could explain this relationship (Root 1973), with greater amounts of cropland attracting a larger aphid population. A greater amount of conventionally managed agricultural land and/or greater amounts of chemical inputs, such as insecticide treatments, in the surrounding landscape could also be driving

aphid abundance. Krauss et al. (2011) found that aphid abundance was significantly greater in conventional fields compared to organic, and that conventional fields that sprayed insecticides had higher late season aphid abundance than those that did not.

While there was a significant effect of landscape composition on aphid functional community composition that varies over time in both study regions, the interaction of landscape composition and intra-annual variation differentially affected aphid functional community composition in WI and NY. In NY, the relationship between the proportion of aphids that were PVY vectors and the percent agriculture appears to have become progressively more negative throughout the season. Again, these results may indicate that the source of aphids varies between areas of high and low intensity agriculture, with natural habitat serving as a greater source in low intensity areas, at least in the early season prior to long distance aphid spread. There was no interactive effect on the proportion of aphids that were PVY vectors in WI.

Conversely, there was a significant interactive effect on the proportion of aphids that were colonizing aphids and low-transmitting PVY vectors in WI, but not NY. In WI, it appears that the initial proportion of the aphid community comprised by potato colonizing species is roughly equivalent across sites, but that colonizing species increase relative to non-colonizers in different areas at different times before dwindling to virtually none by the end of the season. Because colonizing species are generally high-transmitting vectors, these results indicate that the timing of high-risk periods of PVY spread may differ between different landscapes. The proportion of aphids that were low-transmitting vectors appears to be higher at sites in less agricultural areas in both the early season and the end of the season. But while the proportion of aphids that were low-

transmitting vectors was initially moderate, by the end of the season, it was high across all sites. These results could reflect the species-specific effect of landscape complexity and climate on aphid abundance (Grez et al. 2008; Braschler et al. 2003; Banks 1998; Zhao et al. 2015).

The interactive effect of landscape composition and intra-annual variation on the aphid community has implications for aphid community composition and PVY prevalence. Our previous work in NY (working with the same dataset) shows that at these sites, greater amounts of agricultural land leads to higher final PVY prevalence, and that this effect is likely mediated by the effect of the landscape on the aphid community (Claflin, unpublished data). The results of the present study agree with the *landscape-moderated concentration and dilution hypothesis* (Tscharntke et al. 2012). This work suggests that changes in the landscape consistently shape the aphid community over time, and emphasize the importance of non-colonizing species and species spillover in determining aphid community structure.

Independent effects of intra-annual variation on aphid abundance, species richness and functional community composition.

In NY, intra-annual variation strongly affected functional community composition independent of landscape composition. However, the direction of its effect varied depending on the functional group. The effect of intra-annual variation on functional community composition could be a reflection of climatic variables, especially the increased frequency of higher temperatures later in the season. Ma et al. (2015) found

that increasing the number of extreme high temperature events changed the aphid community structure, with species-specific responses to increased temperature driving the shift. In this study, the overall proportion of PVY vectors increased through the season in both regions. However, in NY this trend obscured differences between vector groups. The proportion of unestimated vectors increased over the course of the season, but the proportion of low and high-transmitting vectors (both colonizing and non-colonzing) decreased significantly. This has significant management implications: as time progressed, the risk of PVY transmission becomes more difficult to estimate, as the transmission efficiencies of the vector species have not been quantified. These results also support the findings of DiFonzo et al. (1997) who found that non-colonizing aphid species, which typically have lower or unestimated transmission efficiencies, have the greatest abundance. Our findings suggest that the abundance of unestimated vector species may be driving late season PVY spread in NY. These results emphasize the importance of further studies assessing the transmission efficiencies of a greater number of species, as well as evaluating vectors with different capacities separately.

Independent effects of landscape composition on aphid abundance, species richness and functional community composition.

In this study, landscape composition significantly affected the aphid community. This agrees with previous work demonstrating the influence of landscape structure on local arthropod populations and communities (Chaplin-Kramer et al. 2011). The direction of these effects varied between study regions. For example, aphid abundance had a positive relationship with percent agriculture in NY, and a negative relationship in WI.

These differences indicate that there were significant differences in aphid community dynamics between regions.

In this study, we analyzed the relationship of landscape composition, intra-annual variation, and their interaction on local aphid communities in two regions: an agriculturally intense region in WI, and the more diverse Finger Lakes region of NY. We found a consistent, significant interactive effect of landscape composition and intra-annual variation on aphid abundance, species richness, and functional community composition in both study regions. Our work shows that intra-annual variation, in interaction with landscape composition, is an important driver of local aphid communities, and that spatiotemporal shifts in agroecosystems have significant implications for aphid functional community composition.

REFERENCES

Adler, LS, P De Valpine, J Harte, and J Call. (2007). Effects of long-term experimental warming on aphid density in the field. *Journal of the Kansas Entomological Society*, 80: 156-168.

Banks, JE. (1998). The scale of landscape fragmentation affects herbivore response to vegetation heterogeneity. *Oecologia*, 117: 239-246.

Barton, BT, and AR Ives. (2014). Direct and indirect effects of warming on aphids, their predators, and ant mutalists. *Ecology*, 95: 1479-1484.

Boquel, S, A Ameline, and P Giordanengo. (2011). Assessing aphids potato virus Y transmission efficiency: a new approach. *Journal of Virological Methods*, 178: 63-67.

Braschler, B, G Lampel, and B Baur. (2003). Experimental small-scale grassland fragmentation alters aphid populations dynamics. *Oikos*, 100: 581-591.

Brunt, AA. (2001). The main viruses infecting potato crops, In*:* G Loeben- stein, et al. (eds.), *Virus and Virus-like Diseases of Potatoes and Production of Seed-potatoes*. Kluwer Academic Publishers, Dordrecht. pp. 65-67.

Cardinale, BJ, JJ Weis, AE Forbes, KJ Tilmon, and AR Ives. (2006). Biodiversity as both a cause and consequence of resource availability: a study of reciprocal causality in a predator-prey system. *Journal of Animal Ecology*, 75: 497-505.

Chaplin-Kramer, R, ME O'Rourke, EJ Blitzer, and C Kremen. (2011). A meta-analysis of crop pest and natural enemy response to landscape complexity. *Ecology Letters*, 14: 922-932.

Cocu, N, R Harrington, MDA Rounsevell, SP Worner, M Hullé, and EXAMINE project participants. (2005). Geographical location, climate and land use influences on the phenology and numbers of the aphid, *Myzus periscae*, in Europe. *Journal of Biogeography*, 32: 615-632.

Davis, TS, JT Abatzoglou, NA Bosque-Pérez, S Halbert, K Pike, and SD Eigenbrode. (2014). Differing contributions of density dependence and climate to the population dynamics of three eruptive herbivores. *Ecological Entomology*, 39: 566-577.

Debinski, DM, and RD Holt. (2000). A survey and overview of habitat fragmentation experiments. *Conservation Biology*, 14: 342-355.

Dedryver, C-A, A Le Ralec, and F Fabre. (2010). The conflicting relationships between

aphids and men: a review of aphid damage and control strategies. *Comptes Rendus Biologies*, 333: 539-553.

DiFonzo, CD, DW Ragsdale, EB Radcliffe, NC Gudmestad, and GA Secor. (1997). Seasonal abundance of aphid vectors of potato virus Y in the Red River Valley of Minnesota and North Dakota. *Journal of Economic Entomology*, 90: 824-830.

Dixon, AFG, P Kindlmann, J Leps, and J Holman. (1987). Why there are so few species of aphids, especially in the tropics. *The American Naturalist*, 129: 580-592.

Gagic, V, S Hänke, C Thies, C Scherber, Z Tomanović, and T Tscharntke. (2012). Agricultural intensification and cereal aphid-parasitoid-hyperparasitoid food webs: network complexity, temporal variability and parasitism rates. *Oecologia*, 170: 1099-1109.

Gilabert, A, JC Simon, L Mieuzet, F Halkett, S Stoeckel, M Plantegenest, and CA Dedryver. (2009). Climate and agricultural context shape reproductive mode variation in aphid crop pest. *Molecular Ecology*, 18: 3050-3061.

Gray, S, S De Boer, J Lorenzen, A Karasev, J Whitworth, P Nolte, R Singh, A Boucher, and H Xu. (2010). Potato virus Y: an evolving concern for potato crops in the United States and Canada. *Plant Disease*, 94: 1384-1397.

Grez, A, T Zaviezo, L Tishcendorf, and L Fahrig. (2004). A transient, positive effect of habitat fragmentation on insect population densities. *Oecologia*, 141: 444-451.

Grez, AA, T Zaviezo, S Díaz, B Camousseigt, and G Cortés. (2008). Effects of habitat fragmentation on the abundance and species richness of aphidophagous beetles and aphids in experimental alfalfa landscapes. *European Journal of Entomology*, 105: 411-420.

Halbert, SE, DL Corsini, and MA Wiebe. (2003). Potato virus Y transmission efficiency for some common aphids in Idaho. *American Journal of Potato Research*, 80: 87-91.

Hooks, CRR, and A Fereres. (2006). Protecting crops from non-persistently aphid transmitted viruses: a review on the use of barrier plants as a management tool. *Virus Research*, 120: 1-16.

Hull, R. (2002). Matthew's Plant Virology. Academic Press, San Diego, California, p.1001.

Karasev, AV, and SM Gray. (2013). Continuous and emerging challenges of Potato virus Y in potato. Annual Review of Phytopathology 51: 571-586.

Krauss, J, I Gallenberger, and I Steffan-Dewenter. (2011). Decreased functional diversity

and biological control in conventional compared to organic crop fields. *PLOS One*, 6: e19502.

Lacroix, C, A Jolles, EW Seabloom, AG Power, CE Mitchell, and ET Borer. (2014). Nonrandom biodiversity loss underlies predictable increases in viral disease prevalence. *Journal of the Royal Society Interface*, 11: 20130947.

Loxdale, HD, J Hardie, S Halbert, R Foottit, NAC Kidd, and CI Carter. (1993). The relative importance of short- and long-range movement of flying aphids. *Biological Reviews*, 68: 291-311.

Ma, G, VHW Rudolf, and C-S Ma. (2015). Extreme temperature events alter demographic rates, relative fitness, and community structure. *Global Change Biology*, 21: 1794-1808.

Margosian, ML, KA Garrett, JMS Hutchinson, and KA With. (2009). Connectivity of the American agricultural landscape: Assessing the national risk of crop pest and disease spread. Bioscience 59: 141-151.

Matis, TI, MN Parajulee, JH Matis, and RB Shrestha. (2008). A mechanistic model based analysis of cotton aphid population dynamics data. *Agricultural and Forest Entomology*, 10: 355-362.

McVean, RIK, AFG Dixon, and R Harrington. (1999). Causes of regional and yearly variation in pea aphid numbers in eastern England. *Journal of Applied Entomology*, 123: 495-502.

Mello, AFS, RA Olarte, SM Gray, and KL Perry. (2011). Transmission efficiency of potato virus Y strains PVY^{O} and $PVY^{N\text{-}Wi}$ by five aphid species. *Plant Disease*, 95: 1279-1283.

Narayandas, GK, and AV Alyokhin. (2006). Interplant movement of potato aphid (Homoptera: Aphididae) in response to environmental stimuli. *Environmental Entomology*, 35: 733-739.

O'Rourke, ME, K Rienzo-Stack, and AG Power. (2011). A multi-scale, landscape approach to predicting insect populations in agroecosystems. *Ecological Applications*, 21: 1782-1791.

Pelletier, Y, X Nie, M McClure, S Whitney, and M-A Giguère. (2008). Behavior of bird cherry-oat aphid and green peach aphid in relation to potato virus Y transmission. *Journal of Economic Entomology*, 101: 728-735.

Pelletier, Y, X Nie, M-A Gigère, U Nanayakkara, E Maw, and R Foottit. (2012). A new approach for the identification of aphid vectors (Hemiptera: Aphididae) of potato virus Y. *Journal of Economic Entomology*, 105: 1909-1914.

Pike, KS, LL Boydston, and DW Allison. (2003). Aphids of Western North America North of Mexico. *Washington State University Extension*.

Radcliffe, EB, and DW Ragsdale. (2002). Aphid-transmitted potato viruses: the importance of understanding vector biology. *American Journal of Potato Research*, 79: 353-386.

Root, RB. (1973). Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*, 43: 95-124.

Rösch, V, T Tscharntke, C Scherber, and P Batáry. (2013). Landscape composition, connectivity, and fragment size drive effects of grassland fragmentation on insect communities. *Journal of Applied Ecology*, 50: 387-391.

Rusch, A, K Birkhofer, R Bommarco, HG Smith, and B Ekbom. (2014). Management intensity at field and landscape level affects the structure of generalist predator communities. *Oecologia*, 175: 971-983.

Salazar, LF. (1996). Potato Viruses and Their Control. CIP, Lima. pp. 214.

Scholthof, KB, S Adkins, H Czosnek, P Palukaitis, E Jacquot, T Hohn, B Hohn, K. Saunders, T Candresse, P Ahlquist, C Hemenway, and GD Foster. (2011). Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology*, 12: 938-954.

Sudderth, EA, and EB Sudderth. (2013). Quantifying aphid behavioral responses to environmental change. *Entomologia Experimentalis et Applicata*, 150: 7-18.

Thies, C, I Steffan-Dewenter, and T Tscharntke. (2008). Interannual landscape changes influence plant-herbivore-parasitoid interactions. *Agriculture, Ecosystems, and Environment*, 125: 266-268.

Torma, A., R. Gallé, and M. Bozsó. 2014. Effects of habitat and landscape characteristics on the arthropod assemblages (Aranea, Orthoptera, Heteroptera) of sand grassland remnants in Southern Hungary. *Agriculture, Ecosystems, and Environment*, 196:42-50.

Tscharntke, T, JM Tylianakis, TA Rand, RK Didham, L Fahrig, P Batáry, J Bengtsson, Y Clough, TO Crist, CF Dormann, RM Ewers, J Fründ, RD Holt, A Holzschuh, AM Klein, D Kleijn, C Kremen, DA Landis, W Laurance, D Lindenmayer, C Scherber, N Sodhi, I Steffan-Dewenter, C Thies, WH van der Putten, and C Westphal. (2012). Landscape moderation of biodiversity patterns and processes – eight hypotheses. *Biological Reviews*, 87: 661-682.

Tylianakis, JM, T Tsharntke, and OT Lewis. (2007). Habitat modification alters the

structure of tropical host-parasitoid food webs. *Nature*, 445: 202-205.

Tylianakis, JM, and CM Romo. (2010). Natural enemy diversity and biological control: making sense of the context-dependency. *Basic and Applied Ecology*, 11: 657-668.

Verbeek, M, PGM Piron, AM Dullemans, C Cuperus, and RAA van der Vlugt. (2010). Determination of aphid tranmission efficiencies for N, NTN, and Wilga strains of potato virus Y. *Annals of Applied Biology*, 156: 30-49.

Vialatte, A, JC Simon, CA Dedryver, F Fabre, and M Plantegenest. (2006). Tracing individual movement of aphids reveals preferential routes of population transfers in agroecosystems. *Ecological Applications*, 16: 839-844.

Weibull, A-C, and Ö Östman. (2003). Species composition in agroecosystems: the effect of landscape, habitat, and farm management. *Basic and Applied Ecology*, 4: 349-361.

Werling, BP, and C Gratton. (2010). Local and broadscale landscape structure differentially impact predation of two potato pests. *Ecological Applications*, 20: 1114-1125.

Woodcock, BA, C Harrower, J Redhead, M Edwards, AJ Vanbergen, MS Heard, DB Roy, and RF Pywell. (2014). National patterns of functional diversity and redundancy in predatory ground beetles and bees associated with key UK arable crops. *Journal of Applied Ecology*, 51: 142-151.

Zhao, Z-H, C Hui, D-H He, and B-L Li. (2015). Effects of agricultural intensification on ability of natural enemies to control aphids. *Scientific Reports*, 5: 1-7.

CHAPTER THREE

APHID DENSITY AND COMMUNITY COMPOSITION DIFFERENTIALLY AFFECT APHID MOVEMENT AND PLANT VIRUS TRANSMISSION

Abstract

1. Although many vector-borne pathogens are transmitted by an array of vector species, most studies do not account for the potential effects of species interactions.
2. By manipulating conspecific and heterospecific vector density in small experimental mesocosms, this study disentangled the impact of vector density and community composition on vector movement and plant virus transmission in the potato virus Y system.
3. We tested the following predictions: 1) Increasing aphid density will increase aphid movement and virus transmission, 2) Adding low-efficiency vectors and thereby decreasing the average transmission efficiency of the vector assemblage will decrease virus transmission, and 3) Aphid movement and the average vector transmission efficiency will mediate the effect of aphid density and community composition on virus transmission.
4. We found that initial density positively affected aphid movement, but had no effect on virus transmission, and that conspecific density was more important than heterospecific density. Conversely, community composition affected both aphid movement and virus transmission. These effects were driven by species identity, rather than species richness *per se*.

5. The results of this study emphasize the importance of accounting for vector behavior, and analyzing it within the context of the wider vector assemblage.

Introduction

Herbivorous insects afflict plants through both physical damage and disease transmission; insects vector 79% of all plant viruses (Power & Flecker, 2008), and aphids are the most common vectors, transmitting about 50% of plant viruses (Hooks & Fereres, 2006), making them a group of particular importance. Although many plant pathogens are transmitted by multiple vector species, our understanding of the effects of vector diversity is still rudimentary (Roche & Guégan, 2011). In experimental studies, simplifying the vector community is often necessary logistically; however, it paints an incomplete picture of vector behavior, as it eliminates the effect of community interactions. In particular, we have only a basic understanding of the relative importance of community composition and vector species diversity on pathogen spread (Roche & Guégan, 2011). Contrasting the effects of vector density and community composition on transmission and vector behavior could offer important insights into disease dynamics.

The density of both conspecifics and heterospecifics is known to affect aphid behavior, and could directly or indirectly influence aphid movement and other transmission behaviors, such as probing. Aphids can have a direct impact on each other through alarm pheromones and other anti-crowding behaviors that increase movement (Kunert *et al.*, 2005), and they can also interact indirectly through changes in plant quality (Petersen & Sandström, 2001) and apparent competition (Evans, 2008). These effects can occur between both hetero- and conspecifics, resulting in a web of potential

intra- and interspecific competitive interactions that spans the aphid community (Mehrparvar *et al.*, 2014; Smith *et al.*, 2008; Kaplan & Denno, 2007). For example, Mehrparvar *et al.* (2014) found that aphid host plant selection was significantly influenced not only by current plant occupancy by both hetero- and conspecifics, but also by previous infestation. The magnitude of these effects were species-specific, but reflected community competitive hierarchies.

Aphid movement is important for disease transmission, is species-specific, and dependent not only on aphid density but also a suite of environmental factors. Although long distance dispersal is possible and can have a significant effect on local aphid dynamics (Reynolds *et al.*, 2006), short-range dispersal is generally thought to be more important for within-field dynamics (Loxdale *et al.*, 1993; Vialatte *et al.*, 2007). Bailey *et al.* (1995) found that apterous (wingless) *R. padi* movement in oats was increased by direct, indirect, physical, and biological disturbances, including crowding and virus infection in host plants. Apterae, both colonizing (those that settle and reproduce on the plant) and non-colonizing (those that do not) species, can be extremely restless, increasing movement in response to very mild crowding (>20 adult aphids and their nymphs per plant), and changes in plant quality (Hodgson, 1991). Apterous *M. euphorbiae*, a potato colonizing species, move without perturbation in potato fields, and aphid movement increases when the canopy overlaps (Narayandas & Alyokhin, 2006). Compared directly, a non-colonizing aphid species, *R. padi*, moved off potato plants more than a potato colonizing species, *M. persicae* (Pelletier *et al.*, 2008). However, other transmission-related behaviors were similar between species. The *R. padi* individuals that remained on the potato took the same mean time to initiate their first

probe, and that probe lasted the same mean time as the *M. persicae* alates (Pelletier *et al.*, 2008).

Vector community composition may also have a significant effect on disease transmission. Behavior during plant probing, such as cell penetration, is critical for the acquisition and inoculation of many plant viruses, and has been shown to vary between aphid species (Boquel *et al.*, 2011). Generally, colonizing species have high transmission efficiencies and non-colonizing species are less effective vectors. Consequently, there is a wide range of transmission efficiencies among aphid species, which could mediate a direct relationship between community composition and virus prevalence. Models suggest that vector diversity could also influence disease transmission. Roche *et al.* (2013) developed a vector-borne disease model that illustrates the potential importance of vector species richness. They conclude that the effect of vector diversity will depend on how it impacts the average susceptibility (in the case of this study, the transmission efficiency, or the probability of host infection) of the vector and host assemblages: the greater the transmission efficiency, the greater the prevalence of disease.

The objective of the current study was to investigate the effects of vector community composition and movement behavior on the spread of an economically important virus in potatoes, *Potato virus Y* (PVY). In particular, we disentangled the effects of vector density, number of species, and species identity, and assessed the relative effect of a colonizing high-efficiency vector species and two non-colonizing low-efficiency vector species on disease transmission, and aphid movement. We tested the following predictions: 1) Increasing aphid density will increase aphid movement and virus transmission, 2) Decreasing the average transmission efficiency of the vector

assemblage by adding low-efficiency vectors will decrease virus transmission, and 3) The effect of aphid density and community composition on virus transmission will be mediated by aphid movement and the average vector transmission efficiency.

Materials and Methods

Study system

The PVY system is ideal for studying the role of the vector community in pathogen spread, as the virus is a vector generalist, and its vectors span a range of natural histories, host preferences, and transmission efficiencies (Gray *et al.*, 2010). These experiments included a suite of three vector species: a colonizing aphid vector species, the potato aphid (*Macrosiphum euphorbiae*), which settles and reproduces on potato; and two non-colonizing species, the bird cherry oat aphid (*Rhopalosiphon padi*) and the pea aphid (*Acrytosiphon pisum*), which do not settle or reproduce on potato. *M. euphorbiae* has a relatively high PVY transmission efficiency (approximately 27%), while the efficiencies of *R. padi* and *A. pisum* are quite low (approximately 3% and 7%, respectively; Boquel *et al.*, 2011). All three species are commonly found in potato fields in New York State (S. Claflin, unpublished data). Wingless morphs were used in these experiments, in order to allow for closer observations of behavior, and direct comparison between the effects of colonizing and non-colonizing species.

Study Design

We conducted two sequential experiments focusing on the colonizing species, *M. euphorbiae*. First, we carried out a caged mesocosm experiment in the field, which

evaluated the effects of initial aphid density and community composition on aphid movement (hereafter called the "movement experiment"). Second, informed by the results of the movement experiment, we conducted a greenhouse study that tested the effect of initial aphid density and community composition on both aphid movement and PVY transmission (hereafter called the "movement/transmission experiment").

Plant and insect care

Potato plants (*Solanum tuberosum*, cv. Yukon Gold, Andover and Superior) were planted in 10.2cm or 15.2cm diameter pots and grown in the greenhouse in commercial potting soil (Metro-Mix 360, Sun Gro Horticulture) with fertilizer applied at watering each day. Before being used in bioassays plants were allowed to grow for 3-4 weeks. Plants included in the movement experiment, cv. Yukon Gold and Andover, were then transplanted into $1m^3$ fine mesh cages in a field.

Plants included in the movement/transmission experiment were moved to the experimental greenhouse and placed in black plastic flats. The naïve recipient (uninfected) plants (cv. Yukon Gold) were grown from certified disease free tubers, and the PVY source plants (cv. Superior) were grown from infected tubers. PVY infection in the source plants was confirmed using enzyme-linked immunosorbent assay (ELISA) before inclusion in the greenhouse experiment (Ellis *et al.*, 1996).

Aphid colony maintenance was the same for both experiments. *Macrosiphum euphorbiae* were maintained in colonies on potato in a growth chamber at 20°C with 14 hours of light/day. *Acrytosiphom pisum* were maintained in colonies on fava beans (*Vicia*

faba), and *Rhopalosiphum padi* were maintained in colonies on barley (*Hordeum vulgare*, cv. Romulus) in growth chambers at 20˚C with 24 hour light.

Experimental design and procedure

The experiments in this study were designed to test the effects of aphid density and aphid community composition on movement and virus transmission. We included both low-density (30 aphids) and high-density (60 aphids) single-species controls in a design that included mixtures consisting of 60 total aphids. By comparing the low-density controls to the mixtures, it was possible to separate the effect of each species. In the movement experiment, each experimental unit was randomly assigned to one of seven aphid community treatments: *M. euphorbiae* only (ME+ME), a high-density (60 aphid) control; two two-species mixtures (30 aphids of each species, 60 aphids total): *M. euphorbiae* and *A. pisum* (ME+AP), and *M. euphorbiae* and *R. padi* (ME+RP); a three-species mixture (20 aphids of each species, 60 aphids total): *M. euphorbiae*, *A. pisum*, and *R. padi* (THREE); and three low-density (30 aphid) single-species controls, *M. euphorbiae* density control (ME), *A. pisum* density control (AP), and *R. padi* density control (RP).

Based on the preliminary results of the movement experiment, four treatments were included in the movement/transmission experiment: a high-density (60 aphid) *M. euphorbiae* control (ME+ME); two two-species mixtures (30 aphids of each species, 60 aphids total): *M. euphorbiae* and *A. pisum* (ME+AP), and *M. euphorbiae* and *R. padi* (ME+RP); and a *M. euphorbiae* low-density (30 aphid) control (ME).

By comparing treatments with the same aphid density, but different community compositions, we were able to examine the impact of community composition. By comparing treatments with the same composition but different densities, we were able to analyze the effect of density.

In both experiments, the experimental unit was an array of 6 potato plants, arranged in a single row. In the movement experiment, the plants were transplanted 10.2cm apart within $1m^3$ fine mesh cages in a field. The cages were arranged in 10 spatially separate blocks within the field. The experiment was replicated twice, with each replicate including 5 randomized blocks.

In a typical field situation, we would expect that colonizing aphids would generally be resident on plants throughout the season, whereas non-colonizers would visit briefly, and then depart. Hence, in the mixture and monospecific *M. euphorbiae* treatments, 30 wingless adult *M. euphorbiae* were placed on the first plant in the row, which was then bagged with a fine mesh fabric, and allowed to settle for 24 hours. In the monospecific *A. pisum* and *R. padi* low-density control treatments without *M. euphorbiae* (AP and RP), the first plant was bagged for the 24-hour settling period without aphids on it. After 24 hours, the mesh was removed and a second group of 30 wingless adult aphids was placed on the first plant, except in the *M. euphorbiae* low-density control treatment (ME), which had no second group of aphids. All plants were censused for aphids at four time points: 1, 3, 5, and 24 hours after the release of the second group of aphids. Aphid movement was quantified using two metrics: the proportion of aphids moving and plant occupancy (the number of aphids on recipient plants), which measures aphid-plant contact rate. The proportion of aphids moving is a relative measure of aphid movement,

assessing the number of aphids that had moved to recipient plants (plant occupancy) as a proportion of the total number of aphids found in the array.

In the movement/transmission experiment, the experimental procedure was similar to that described above, but the experimental set-up was slightly different. Plants were kept in pots and placed 15.2cm apart in a single row within black plastic flats. The PVY source plants were placed first in the row, and plants were arranged so that the leaves were touching The pots were filled to the brim with soil, causing the soil to overflow into adjacent pots, allowing for aphid movement between plants on the soil as well as direct movement from plant to plant. The experimental units were arranged in two blocks, one on either side of the greenhouse, and strips of cardboard painted with Tanglefoot (Contech, Goddard, KS 67052) separated each unit from those next to it, to prevent aphids from walking between arrays. After the completion of the bioassay, the PVY source plants were removed and composted and the recipient (initially uninfected) plants were sprayed with insecticide to kill all remaining aphids (Endeavor (EPA Reg.#100-913) and Avid (EPA Reg.#100-896)). The recipient plants continued to grow in the greenhouse for 4 weeks to allow the virus to replicate to detectable levels, at which time their foliage was sampled and frozen for later ELISA analysis.

Analysis

Data from both experiments were analyzed with generalized linear mixed effects models. A poisson distribution was used in analyses with plant occupancy and the number of infected plants as the response variables, and a binomial distribution was used in analyses with the proportion of aphids moving as the response variable. For the

movement experiment, the time point, treatment, the interaction between time point and treatment, and replicate were included as fixed effects; block nested within replicate and array nested within block were included as random effects. For the movement/transmission experiment, time point, treatment, and block were included as fixed effects, and array nested within block was included as a random effect. In the preliminary analysis of the movement/transmission experiment, average plant occupancy was included as a covariate in the model exploring effects on the number of infected plants. However, the effect of average plant occupancy was not significant ($p=0.612$), and so was excluded from the final model.

A number of treatment subsets were analyzed separately to explore the effects of density and diversity in both experiments. The density subset included the *M euphorbiae* high-density control treatment (ME+ME) and the low-density control (ME), comparing the effect of aphid densities of 60 and 30 aphids, respectively. The diversity subset included treatments with a density of 60 aphids, but varying proportions of the different aphid species: ME+ME, ME+AP, ME+RP, and THREE (for the movement experiment) or ME+ME, ME+AP, and ME+RP (for the movement/transmission experiment). The single species subset evaluated the effect of each species at low density: ME, AP, and RP.

A generalized linear mixed effect model was used to assess the effect of time and treatment on the average transmission efficiency of the vector assemblage in the movement/transmission experiment. Time point, treatment, their interaction, and block were included as fixed effects, and array nested within block was included as a random effect. To use a GLMM with a poisson distribution it was necessary to convert transmission efficiency to a whole integer, therefore the ratio of the published

transmission efficiencies of the three aphid species (ME=0.267, AP=0.067, RP=0.034) was used for calculations (ME=8, AP=2, and RP=1). The model included the log of the total number of aphids found as an offset variable, and used a poisson distribution. In all models, differences between treatments were analyzed using z-values. All analyses were conducted in R (version 3.2.1).

Results

Both experiments demonstrated that, as expected, higher initial aphid density had a positive effect on aphid movement. The movement of the colonizing aphid species, *M. euphorbiae*, was primarily determined by conspecific density, and to a lesser degree, by the density of heterospecifics or total aphid density. Aphid community composition also affected both aphid movement and PVY infection, possibly by affecting *M. euphorbiae* transmission-related behaviors.

Plant occupancy

As expected, aphid density increased aphid movement. The density subsets of both experiments demonstrate that the total number of aphids occupying recipient plants was greater in the high-density ME+ME treatment than the low-density ME treatment (movement: $p<0.001$; movement/transmission: $p=0.0028$). In the movement experiment, the greatest occupancy by both total aphids and *M. euphorbiae* was in the high-density ME+ME treatment (Supplemental Table 1 and 2; Figure 1a and 1b). In the movement/transmission experiment, total aphid density was the main driver of total plant occupancy. Although total recipient plant occupancy in the ME+ME high-density control

was more than 7-fold and 9-fold greater than the ME+RP and ME+AP mixture treatments, respectively, the ME+ME treatment was only significantly different from the ME low-density control (Supplemental Table 1; Figure 1d). However, our results did not entirely support our expectation that aphid movement would be driven by total aphid density. Instead, and in some cases, plant occupancy was driven by the initial density of *M. euphorbiae* included in the treatment, not the total aphid density. The aphid community composition also had an effect.

Aphid community composition affected plant occupancy in both experiments. In the movement experiment, plant occupancy was partially driven by the greater amounts of movement by *A. pisum* compared to *R. padi*. The number of aphids on recipient plants was lowest in the RP and ME low-density treatments, with the ME+AP and THREE treatments significantly lower than the ME+ME treatment, but significantly greater than the ME and RP treatments. The ME+RP and AP treatments were intermediate between the ME+AP and THREE treatments and the RP and ME low-density treatments (Supplemental Table 1 and 2; Figure 1b). In the movement/transmission experiment, *M. euphorbiae* plant occupancy in the ME+ME and ME+AP treatments were significantly higher than in the ME low-density control, with the ME+RP treatment intermediate between the ME+AP and the low-density ME treatments, and significantly lower than in the ME+ME high-density treatment (Supplemental Table 2; Figure 1c).

The diversity subsets also show that aphid community composition had an effect on plant occupancy in both experiments, and demonstrate the importance of initial *M. euphorbiae* density on plant occupancy. In the movement experiment, the ME+ME treatment had significantly greater recipient plant occupancy than the ME+AP ($p<0.001$),

ME+RP ($p<0.001$), and THREE ($p<0.001$) mixture treatments, despite equal initial total aphid densities (but varying densities of *M. euphorbiae*). This is further supported by the analysis of the single species low-density treatments; the number of aphids occupying recipient plants was significantly higher in the ME treatment compared to the RP and AP treatments (RP: $p<0.001$; AP: $p=0.006$), despite equal initial densities. In the movement/transmission experiment diversity subset, the total plant occupancy was unaffected by treatment, but the *M. euphorbiae* plant occupancy was significantly affected. As expected, given the twofold higher initial *M. euphorbiae* density in the ME+ME treatment, the ME+ME occupancy was significantly higher than the ME+RP occupancy ($p=0.025$), with ME+AP intermediate ($p=0.21$).

Time had a negative effect on plant occupancy, particularly for non-colonizing species, a significant number of which left the plants for the soil or died. In the movement experiment, the number of aphids occupying recipient plants decreased significantly after the first time point (one hour after release), particularly in the RP and AP treatments, where the average recipient plant occupancy at 24 hours was 0.15 and 0.07, respectively (Supplemental Table 1).

Proportion of aphids moving

Contrary to our expectations, conspecific density (and not total aphid density) determined the proportion of *M. euphorbiae* moving in both experiments. The density subset showed that the proportion of aphids moving in the high-density ME+ME treatment was 10 times higher than the low-density ME treatment (movement: $p=0.008$;

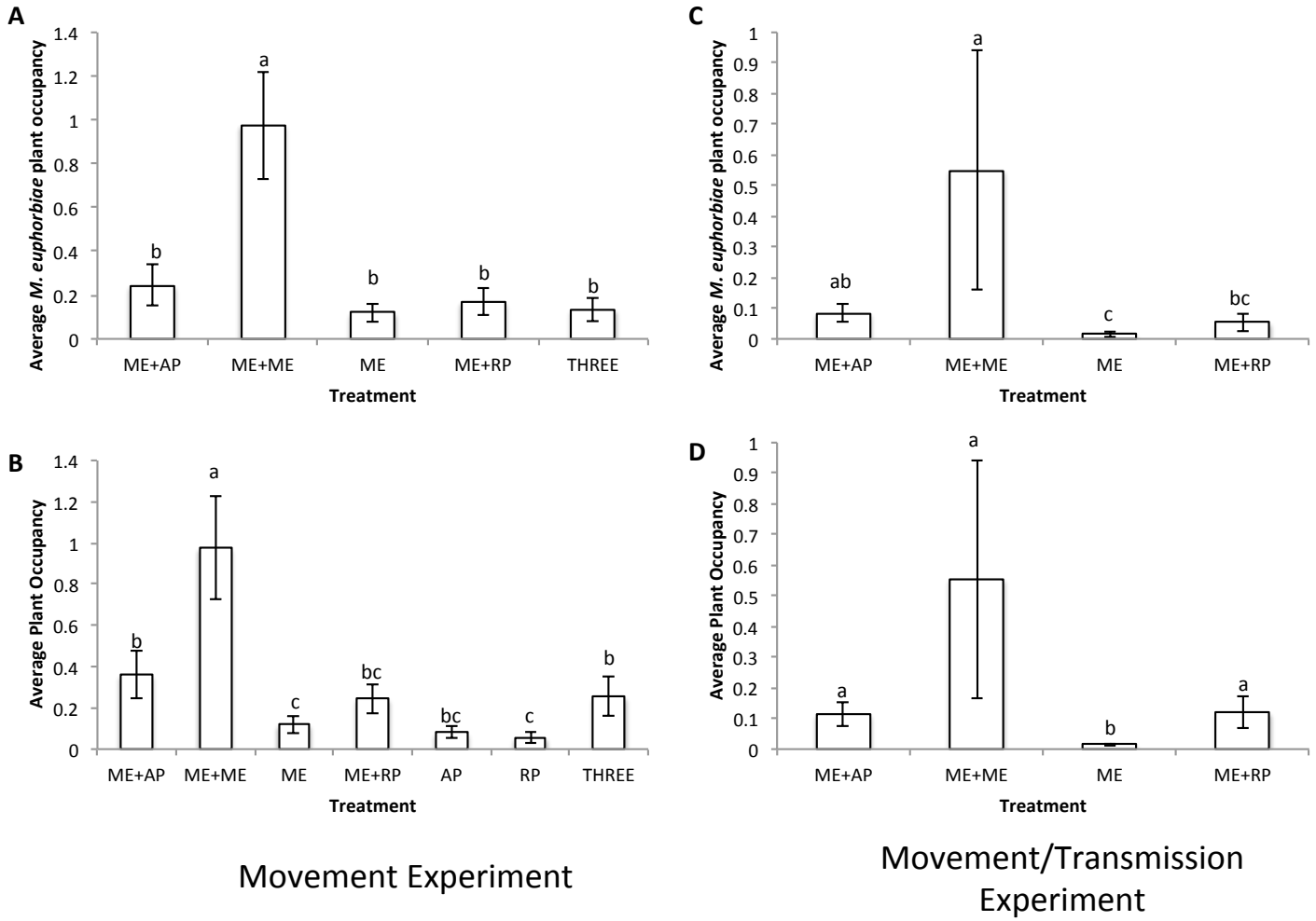

Figure 1: Average A) *M. euphorbiae* and B) total aphid plant occupancy in the movement only, and the average C) *M. euphorbiae* and D) total aphid plant occupancy in the movement/transmission experiment. Letters indicate significant differences, which were determined using z-values from a generalized linear mixed effects model.

movement/transmission: p=0.030; Figure 2b and 2d). In the movement experiment, a greater proportion of *M. euphorbiae* moved in the high-density ME+ME treatment compared to the other treatments containing the species, which had half the initial *M. euphorbiae* density of the ME+ME treatment (Supplemental Table 3 and 4; Figure 2a). In the movement/transmission experiment, the high-density ME+ME treatment had a significantly greater proportion of total aphids moving compared to the low-density ME treatment, with the ME+RP and ME+AP mixture treatments intermediate (Supplemental Table 3; Figure 2d). The proportion of *M. euphorbiae* moving in the movement/transmission experiment followed an identical pattern. *M. euphorbiae* movement also significantly increased over time, with the average number of aphids moving more than doubling between 1 hour and 24 hours after release (Supplemental Table 4; Figure 2c).

As with plant occupancy, aphid community composition also had an impact on the proportion of aphids moving. In the movement experiment, the proportion of aphids moving appears to have also been driven by species-specific effects, not only *M. euphorbiae* initial density. The ME+RP mixture and low-density ME treatments had the lowest proportion of total aphids moving, with the rest of the treatments (ME+ME, ME+AP, THREE, AP and RP) having a significantly higher proportion of aphids moving. The high proportion of aphid movement in the single-species low-density controls (the AP and RP treatments) is likely explained by the low retention of non-colonists in the bioassay, as discussed above. In the movement experiment diversity subset, the proportion of aphids moving was significantly greater in the ME+ME and ME+AP treatments than the ME+RP treatment (ME+ME: $p<0.001$; ME+AP: $p=0.04$),

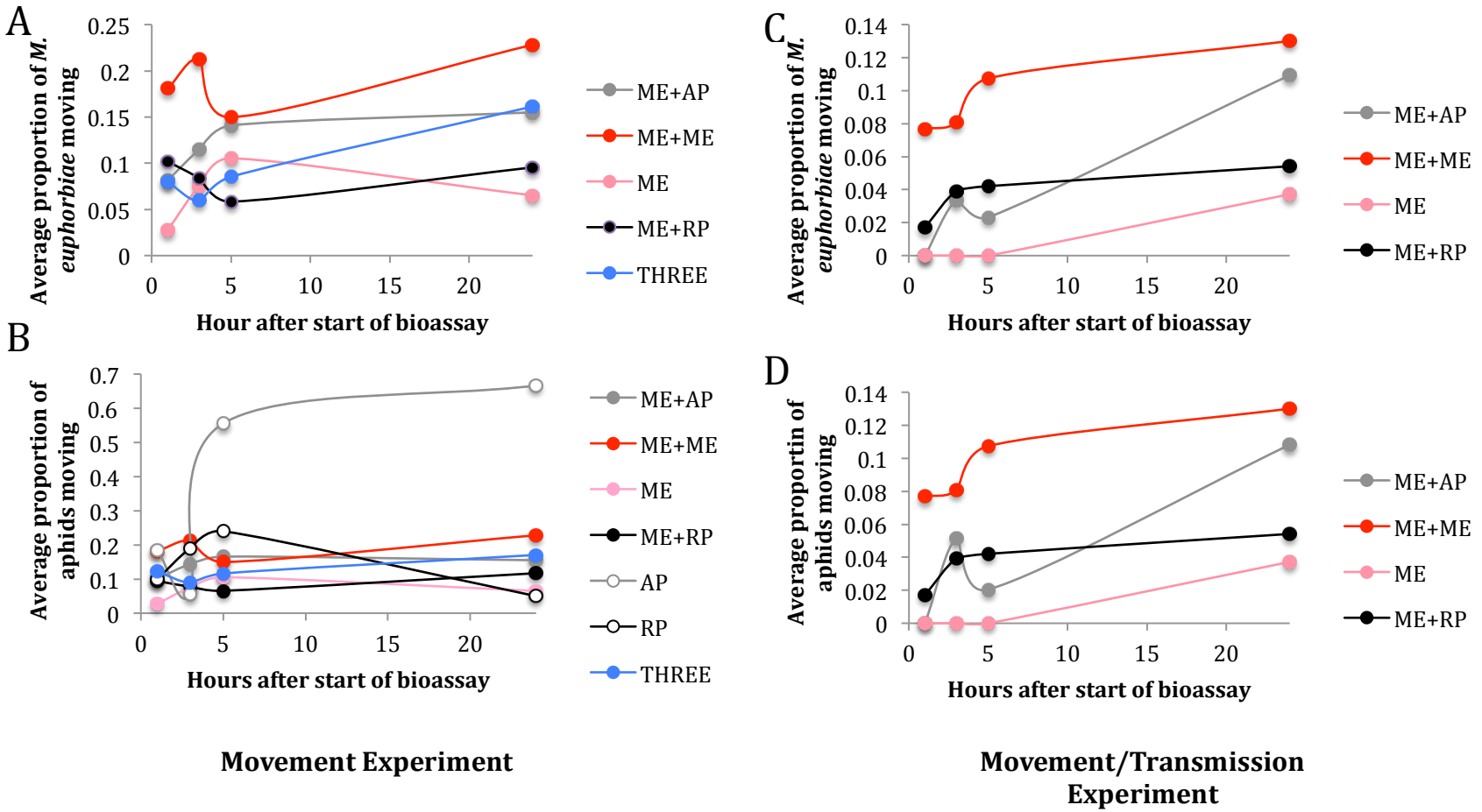

Figure 2: Average proportion of A) *M. euphorbiae* and B) total aphids moving in the movement only and the average proportion of C) *M. euphorbiae* and D) total aphids moving in the movement/transmission experiments over 4 census time points (1, 3, 5, and 24 hours after the start of the bioassay).

with the THREE treatment intermediate (p=0.12). In both cases, the significant difference between the ME+AP and ME+RP treatments, despite equal initial densities and species number, and similarly low retention of non-colonists, suggests that *A. pisum* and *R. padi* affect overall aphid movement differently by differentially affecting *M. euphorbiae*.

Average vector transmission efficiency

As expected, in the movement/transmission experiment, the average vector assemblage transmission efficiency was primarily driven by the proportion of the vector assemblage composed of *M. euphorbiae* (the most efficient vector species). The average transmission efficiency of the ME+ME treatment was not significantly different from the low-density ME treatment. However, average transmission efficiency of the ME+ME treatment was significantly higher than the species mixtures, ME+AP and ME+RP. Additionally, the ME+AP treatment had a significantly higher average transmission efficiency compared to the ME+RP treatment ($p<0.001$; Figure 3). This results from the difference in transmission efficiency between *A. pisum* and *R. padi*; *A. pisum* has a transmission efficiency that is about twofold higher than *R. padi*. It also reflects the greater retention of *R. padi* throughout the bioassay. The average transmission efficiency in the ME+AP and ME+RP treatments significantly increase throughout the bioassay, but time had no effect in the single species ME and ME+ME treatments (Supplemental Table 5). The increased average transmission efficiency in the species mixture treatments is likely the result of the reduced proportion of non-colonizing low-efficiency vectors in the assemblage over time, meaning a greater proportion of the vectors occupying recipient plants are *M. euphorbiae* as time goes on.

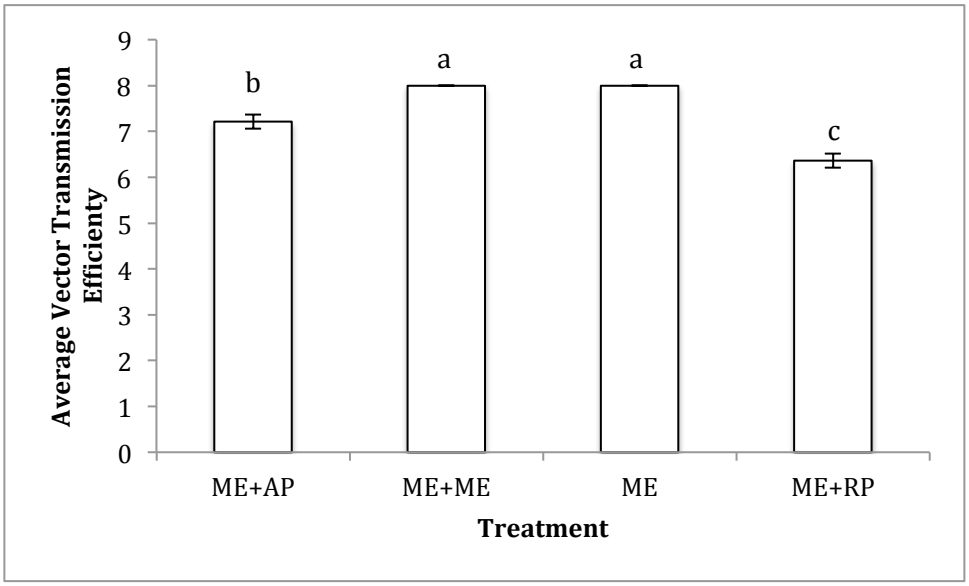

Figure 3: Average vector transmission efficiencies (±standard error) in the movement/transmission experiment: *M. euphorbiae* high-density control (ME+ME), *M. euphorbiae* and *A. pisum* (ME+AP) mixture, *M. euphorbiae* and *R. padi* mixture (ME+RP), and the *M. euphorbiae* low-density control (ME). Letters indicate significant differences, which were determined using z-values from a generalized linear mixed effects model.

PVY infection

Although the overall number of infections was low, PVY infection was significantly affected by treatment (Figure 4). However, contrary to our prediction, vector community composition, rather than *M. euphorbiae* density, drove this effect, with the majority of infections occurring in the ME+RP treatment. The ME+RP treatment had more than four times (n=9) the number of infections of the next highest (n=2). ME+RP had significantly greater amounts of infection than any other treatment (Figure 4). This was contrary to our expectations. We expected the highest transmission to occur in the ME+ME treatment, which has both a high average transmission efficiency and high density of the most efficient vectors. The fact that the ME+RP treatment also had significantly greater infection than the ME+AP treatment, which had equal initial density

and species number, indicates that the impact of adding another species to the vector community is species-specific. The low retention of non-colonists in the bioassay suggests that this effect was mediated by *M. euphorbiae* behavior.

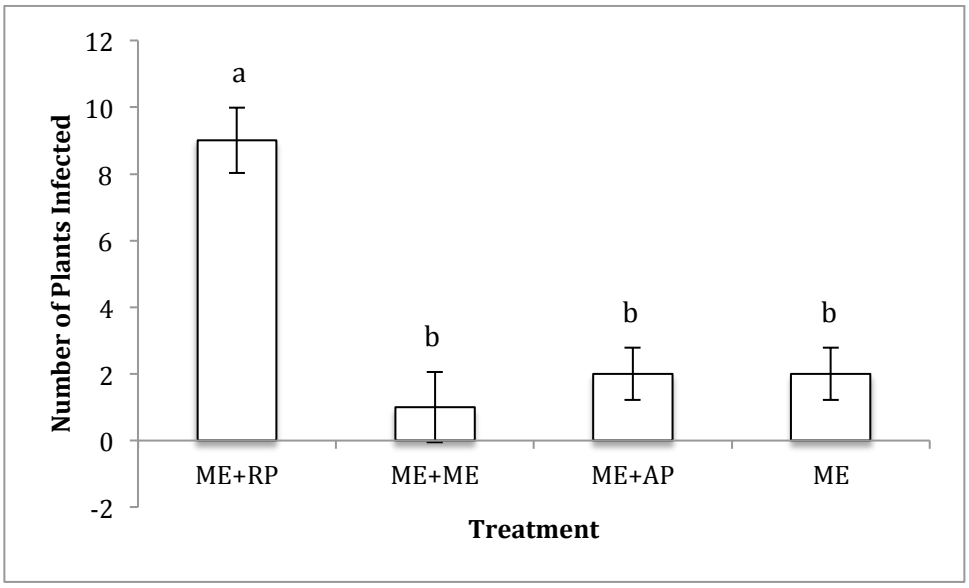

Figure 4: Number of plants infected (±standard error) in the movement/transmission experiment: *M. euphorbiae* high-density control (ME+ME), *M. euphorbiae* and *A. pisum* (ME+AP) mixture, *M. euphorbiae* and *R. padi* mixture (ME+RP), and the *M. euphorbiae* low-density control (ME). Letters indicate significant differences, which were determined using z-values from a generalized linear mixed effects model.

Discussion

Initial aphid density and community composition differentially affected aphid behavior and PVY transmission. While both initial aphid density and aphid community composition affected aphid movement, only the composition of the vector community was important for virus transmission. We hypothesize that the effect of vector community composition on disease spread may be mediated by interactions between *M. euphorbiae* and *R. padi*.

Prediction 1: Increasing initial density will increase aphid movement and virus transmission.

Although initial aphid density had a significant positive effect on aphid movement, it had no effect on virus transmission. Because of the low numbers of non-colonizing species occupying plants, the relationship between density and movement was primarily driven by the effect of density on *M. euphorbiae*, the colonizing species. The impact of aphid density on aphid movement and dispersal is well studied, and our findings are consistent with a large body of research that demonstrates the influence of crowding, even at low densities (20 adult aphids; e.g. Bailey *et al.*, 1995; Hodgson, 1991). Interestingly, it seems that the effect of density is more significant among conspecifics; in the movement/transmission experiment, *M. euphorbiae* movement was greatest in the ME+ME treatment, despite having the same initial total aphid density as both mixture treatments, ME+AP and ME+RP. This could be a reflection of the specificity of aphid pheromones; conspecific pheromones may elicit a stronger response (e.g. Guldemond *et al.*, 1993), and are known to be density-dependent (Kunert *et al.*, 2007). Alternatively, it could be evidence of indirect competition through changes in plant quality (Petersen & Sandström, 2001) or the shorter duration of occupancy on the source plant by non-colonizing species.

The absence of a relationship between initial density and disease transmission contrasts with the many studies that demonstrate a relationship between vector abundance and disease prevalence, both in the laboratory and in the field (e.g. Carrière *et al.*, 2014). It appears that in this experimental system, initial vector density is not the greatest driver of disease transmission.

Prediction 2: Decreasing the average vector transmission efficiency will decrease virus transmission.

The vector community composition affected aphid movement and greatly impacted virus transmission, though not as predicted. In this study, aphid community composition, through species identity, had an effect on aphid movement. The effect of community composition on disease transmission was also species-specific: the ME+RP treatment had significantly more infections than any other treatment, indicating that it is not the number of species, but the specific combination of species that elicited the response. While these findings are consistent with some predictions from the model of Roche *et al.* (2013), demonstrating an increase in disease prevalence in one of our treatments with two vector species, our results are not consistent with other model predictions. Contrary to the prediction that greater average susceptibility would correspond with an increase in disease prevalence, we found that the relationship between prevalence and average vector transmission efficiency was negative. Surprisingly, the treatment with the lowest average transmission efficiency (which had a higher proportion of non-colonizing low-efficiency aphids) had the highest disease transmission.

There are multiple potential drivers for the higher virus prevalence in the ME+RP treatment. First, the low average plant occupancy of both *R. padi* and *M. euphorbiae* in the ME+RP treatment (0.65 and 0.55, respectively, occupied recipient plants) could reflect rapid *R. padi* movement, spurred by the combined effects of species interactions and non-preferred host plants. This movement may have occurred during the first hour and thus have been quick enough that it was not adequately captured by this study.

Claflin *et al.* (2015) found that wingless *R. padi* were capable of rapid movement in similar arrays of potato plants. Second, low occupancy may reflect greater *R. padi* loss to the soil prior to movement through the array. If this is the case, it suggests that the greater virus prevalence in the ME+RP treatment may have been mediated by *M. euphorbiae* transmission behaviors. Interaction with *R. padi* on the infected source or recipient plants could influence the movement and probing behavior of *M. euphorbiae*, which determines both virus acquisition and inoculation (Symmes *et al.*, 2008), and may have led to greater amounts of transmission. By increasing *M. euphorbiae* intraplant movement, *R. padi* could decrease *M. euphorbiae* acquisition access period (the time from first contact with the infected plant to the first intracellular puncture via probing), which is critical for PVY transmission (Boquel *et al.* 2011). The low *M. euphorbiae* recipient plant occupancy can be explained by the high average retention of *M. euphorbiae*. The average retention of *M. euphorbiae* was more than three times higher than that of *R. padi* (an average of 0.64 *R. padi* were retained in the ME+RP treatment overall compared to 2.05 *M. euphorbiae*), meaning that many more *M. euphorbiae* than *R. padi* were found on the infected source plant.

Prediction 3: The average vector transmission efficiency and aphid movement will mediate the effect of density and vector community composition on virus transmission.

Although vector community composition affected both aphid movement and virus transmission, we did not detect a relationship between aphid movement and virus transmission. There are two possible explanations for these results: we may have failed to detect a relationship that was present or another behavior may mediate virus

transmission. This study may not have had the statistical power to detect an effect of aphid movement on transmission. However, other vector behaviors and functional traits, such as probing duration and type, could also mediate the relationship between vector community composition and PVY transmission in in this system. There was also a surprising absence of any correlation between the average vector transmission efficiency of the treatment and disease transmission, but this may be due to an overriding effect of aphid behavior on the probability of virus transmission to a recipient plant.

The results of this study draw a picture of transmission that contradicts our expectations. Despite reducing the average transmission efficiency, plant occupancy, and movement of the vector assemblage compared to the high-density *M. euphorbiae* treatment, the presence of *R. padi* resulted in significantly higher transmission. While the low number of *R. padi* occupying plants indicates that *M. euphorbiae* behavior drove infection in this study, the increased transmission in the presence of *R. padi* emphasizes the importance of considering species interactions within the entire vector assemblage.

If corroborated by further studies, these findings may have significant implications for PVY management. DiFonzo *et al.* (1997) hypothesized that the greater abundance of low-efficiency non-colonizing aphid species compared to high-efficiency colonizing species could make them the main driver of late season disease spread. Our work suggests that at least some non-colonizing species may impact disease spread, not only through sheer numbers, but also through their effect on the transmission behaviors of resident high-efficiency colonizing species. This study illustrates the importance of vector species interactions on disease transmission and demonstrates the potential influence of community ecology in this plant pathosystem. Our results highlight the need

for further work assessing the impact of common vector species with low transmission efficiencies on disease dynamics.

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REFERENCES

Bailey, SM, ME Irwin, GE Kampmeier, CE Eastman, and AD Hewings. (1995). Physical and biological perturbations: their effect on the movement of apterous Rhopalosiphum padi (Homoptera: Aphididae) and localized spread of barley yellow dwarf virus. *Environmental Entomology*, 24: 24-33.

Boquel, S, A Ameline, and P Giordanengo. (2011). Assessing aphids potato virus Y-transmission efficiency: a new approach. *Journal of Virological Methods*, 178: 63-67.

Carrière, Y, B Degain, KA Hartfield, KD Nolte, SE Marsh, C Ellers-Kirk, *et al.*. (2014). Assessing transmission of crop diseases by insect vectors in a landscape context. *Journal of Economic Entomology*, 107: 1-10.

Claflin, SB, AG Power, and JS Thaler. (2015). Predators, host abundance, and host spatial distribution affect the movement of wingless non-colonizing vector *Rhopalosiphum padi* (L.) and PVY prevalence in an oat/potato system. *Arthropod-Plant Interactions*, 9: 301-309.

DiFonzo, C, DW Ragsdale, EB Radcliffe, NC Gudmestad, and GA Secor. (1997). Seasonal abundance of aphid vectors of potato virus Y in the Red River Valley of Minnestora and North Dakota. *Journal of Economic Entomology*, **90**, 824-831.

Ellis, P, R Stace-Smith, G Bowler, and DJ Mackenzie. (1996). Production of monoclonal antiboides for dection and identification of strains of potato virus y. *Canadian Journal of Plant Pathology*, 18: 64-70.

Evans, EW. (2008). Multitrophic interactions among plants, aphids, alternate prey and shared natural enemies- a review. *European Journal of Entomology*, 105: 369-380.

Gray, S, S De Boer, J Lorenzen, A Karasev, J Whitworth, P Nolte, *et al.* (2010). Potato virus y: an evolving concern for potato crops in the United States and Canada. *Plant Disease*, 94: 1384-1397.

Guldemond, JA, AFG Dixon, JA Pickett, LJ Wadhams, CM Woodcock. (1993). Specificity of sex pheromones, the role of host plant odour in the olfactory attraction of males, mate recognition in *Cryptomyzus*. *Physiological Entomology*, 18: 137-143.

Hooks, CRR, and A Fereres. (2006). Protecting crops from non-persistently aphid-transmitted viruses: a review on the use of barrier plants as a management tool. *Virus Research*, 120: 1-16.

Hodgson, C. (1991). Dispersal of apterous aphids (Homoptera: Aphididae) from their

host plant and its significance. *Bulletin of Entomological Research*, 81: 417-427.

Kaplan, I, and RF Denno. (2007). Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters*, 10: 977-994.

Kunert, G, J Trautsch, and WW Weisser. (2007). Density dependence of the alarm pheromone effect in pea aphid *Acyrthosphon pisum* (Sternorrhyncha: Aphididae). *European Journal of Entomology*, 104: 47-50.

Kunert, G, S Otto, USR Rösse, J Gershenzon, and WW Weisser. (2005). Alarm pheromone mediates production of winged dispersal morphs in aphids. *Ecology Letters*, 8: 596-603.

Loxdale, HD, J Hardie, S Halbert, R Foottit, NAC Kidd, and CI Carter. (1993). The relative importance of short- and long-range movement of flying aphids. *Biological Reviews*, 68: 291-311.

Mehrparvar, M, SM Mansouri, and WW Weisser. (2014). Mechanisms of species sorting: effect of habitat occupancy on aphids' host plant selection. *Ecological Entomology*, 39, 281-289.

Narayandas, G.K. & Alyokhin, A.V. (2006) Interplant movement of potato aphid (Homoptera: Aphididae) in response to environmental stimuli. *Environmental Entomology*, 35: 733-739.

Pelletier, Y, X Nie, M McClure, S Whitney, and M-A Giguère. (2008). Behavior of bird cherry-oat aphid and green peach aphid in relation to potato virus Y transmission. *Journal of Economic Entomology*, 101: 728-735.

Petersen, MK, and JP Sandström. (2001). Outcome of indirect competition between two aphid species mediated by responses in their common host plant. *Functional Ecology*, 15: 525-534.

Power, AG, and AS Flecker. (2008). The role of vector diversity in disease dynamics. In: RS Ostfeld, et al. (eds.), *Infectious disease ecology: The effects of ecosystems on disease and of disease on ecosystems*. Princeton University Press, Princeton, NJ, pp.30-47.

Reynolds, DR, JW Chapman, and R Harrington. (2006). The migration of insect vectors of plant and animal viruses. *Advances in Virus Research*, 67: 453-517.

Roche, B, and JF Guégan. (2011). Ecosystem dynamics, biological diversity, and emerging infectious disease. *Comptes Rendes Biologies*, 334: 385-392.

Roche, B, P Rohani, AP Dobson, and JF Guégan. (2013). The impact of community organization on vector-borne pathogens. *The American Naturalist*, 181.

Smith, RA, KA Mooney, and AA Agrawal. (2008). Coexistence of three specialist aphids on common milkweed, Asclepias syriaca. *Ecology*, 89: 2187-2196.

Symmes, EJ, GP Walker, TM Perring. (2008). Stylet penetration behavior of *Myzus persicae* related to transmission of Zucchini yellow mosaic virus. *Entomologia Experimentalis et Applicata*, 129: 258-267.

Vialatte, A, M Plantegenest, J-C Simon, and C-A Dedryver. (2007). Farm-scale assessment of movement patterns and colonization dynamics of the grain aphid in arable crops and hedgerows. *Agricultural and Forest Entomology*, 9: 337-346.

CHAPTER 4

PREDATORS, HOST ABUNDANCE, AND HOST SPATIAL DISTRIBUTION AFFECT THE MOVEMENT OF WINGLESS NON-COLONIZING VECTOR RHOPALOSIPHUM PADI (L.) AND PVY PREVALENCE IN AN OAT/POTATO SYSTEM[1]

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Abstract

In the study of insect-vectored plant viruses, colonizing vector species remain the focus. However, non-colonizing vector species, those that do not settle and reproduce on the viral plant host, are often the most abundant in the field and may be the largest contributors to disease spread. While non-colonists may have a substantial effect on disease prevalence, the factors influencing their movement and transmission on non-host plants have been little studied. Here we evaluated how a common biological control agent (*Hippodamia convergens*), host and non-host plant abundance, and plant spatial distribution impact the movement and density of a wingless non-colonizing vector (*Rhopalosiphum padi* (L.)) and transmission of Potato virus Y (PVY) in potatoes in experimental arenas. The results of this work illustrate the importance of plant species function (host or non-host) and distribution to vector behavior and disease spread. Predation, host plant abundance, and plant spatial distribution interactively affected viral prevalence within infected arenas. Increasing the number of vector non-host plants

increased the distance and frequency of aphid movement, and the effect was influenced by plant spatial distribution, the arrangement of plant species in the experimental arena. Increasing the number of vector host plants increased the density of aphids. Although the interaction of the plant and predator treatments affected the proportion of potato plants infected in arenas where infection occurred, and host abundance and spatial distribution impacted vector movement and viral prevalence, aphid movement did not appear to mediate the effect of plant and predator treatments on PVY prevalence. This work demonstrates that both wingless non-colonizing vector behavior and transmission are aggregated responses to multiple environmental drivers.

Introduction

Insect vectors are an important component of many plant pathosystems; 79% of plant viruses are vectored by insects (Power and Flecker 2008). Plant viruses are often transmitted by more than one species of insect vector, including many species that do not colonize (settle and reproduce on) the virus's host plant (Gray et al. 2010). Although these non-colonizing vector species do not engage in committed feeding on the viral host and generally have lower transmission efficiencies than colonizing species, they will probe non-host plants and are often the most abundant species in the field (Boquel et al. 2011). Though they may have a significant impact on virus prevalence, non-colonizing species are understudied relative to their potential importance in disease systems. Greater knowledge of the behavior of non-colonizing vectors will improve our understanding of plant virus epidemiology.

Vector behavior, especially movement and probing, is fundamental for both virus acquisition and inoculation (Pirone and Perry 2002) and has a direct impact on virus spread (Fereres and Moreno 2008; Power 1991; Singh et al. 1988). A number of environmental factors have been demonstrated to affect colonizing vector behavior, including host plant diversity and predation (Bailey et al. 1995; Narayandas and Alyokhin 2006). A recent review shows that host diversity can have varying effects on pathogen transmission; increasing host diversity may amplify, dilute, or leave disease prevalence unchanged (Ostfeld and Keesing 2012). In plant communities both hosts and non-hosts of the vector, species diversity and spatial distribution, could alter vector behavior through visual, olfactory, or tactile cues; encountering vector non-hosts may encourage increased vector movement and probing until an appropriate host is discovered. For vector species with limited mobility, such as wingless aphids, the fine-scale spatial structure of the plant community, such as the row arrangement of plant species, may also influence movement. If plant species differ in competence as hosts of the virus, the impact of plant diversity on vector movement could have a cascading effect on viral prevalence in the system (e.g. Power 1987, Bottenburg and Irwin 1992).

Predators can affect vector population size and vector behavior (Nelson et al. 2004), but their effect on disease prevalence varies (Finke 2012). In plant pathosystems, predators of vectors have the potential to impact viral prevalence through both consumptive (e.g. reducing vector abundance) and non-consumptive (e.g. modifying vector behavior) effects (Preisser and Bolnick 2008; Finke 2012; Kaplan and Thaler 2012). Predators frequently suppress vector populations and elicit sedentary anti-predator behavioral responses (e.g. hiding), which have the potential to reduce virus prevalence

(Moore et al. 2009). However, predators can also elicit active anti-predator behavioral responses (e.g. dropping), which may increase disease prevalence by increasing movement of vectors onto uninfected hosts (Roitberg and Myers 1978). Additionally, the effects of predation, host abundance and host spatial structure on vector movement and virus prevalence may interact. This short-term greenhouse study assesses the importance of 1) host abundance and spatial distribution, 2) predation, and 3) their interaction for non-colonizing vector movement, and virus prevalence.

Hypothesis and predictions

Because of the importance of vector behavior for virus transmission, we hypothesize that the effect of plant abundance and distribution, and predation on virus prevalence will be determined by their effect on aphid movement. To test this hypothesis, we assessed the following four predictions: Prediction 1) the presence of predators will increase aphid movement and PVY prevalence Prediction 2) increasing the vector non-host plant (virus host plant) abundance will increase aphid movement and PVY prevalence, Prediction 3) increasing the distance between vector host plants will increase aphid movement and PVY prevalence, and Prediction 4) vector host plant abundance, spatial structure, and predation will interactively affect aphid movement and PVY prevalence. We investigated these predictions by quantifying wingless adult *R. padi* movement and PVY prevalence in a fully factorial greenhouse experiment, crossing four plant species treatments with two predator treatments (presence or absence of predators). The plant species treatments included an oat monoculture (virus non-host, vector host) a potato monoculture (virus host, vector non-host), and two species mixtures (Fig. 1). In

the mixtures the position of the host and non-host plants were manipulated, which allowed for the comparison of the effects of host plant spatial structure on aphid movement.

Materials and methods

Study system

Small increases in PVY prevalence can have a profound effect on commercial potato crops; with every 1% increase in PVY in the seed stock, yield is reduced by 0.18 t/ha (Scholthof et al. 2011; Nolte et al. 2004). DiFonzo et al. (1997) hypothesized that, because of their abundance, non-colonizing species were the main drivers of Potato Virus Y (PVY) spread in potato fields. This project evaluated PVY transmission and factors affecting the short distance movement of one of the most abundant non-colonizing aphid species landing in potato fields, the bird cherry oat aphid, *Rhopalosiphum padi* (DiFonzo et al. 1996). Although winged non-colonizing vector adults are most common in agricultural systems, wingless adults were used in this experiment in order to increase aphid-plant interaction, allow for monitoring of interplant movement, and make this study comparable to other work in the area, which generally uses wingless adults of a colonizing species (e.g. Narayandas and Alyokhin 2006). The virus and vector hosts are not congruent in this system; potato is the virus host, oat is the virus non-host, while potato is the vector non-host and oat is the vector host.

PVY causes an array of symptoms ranging from leaf mottling to necrosis in solanaceous crops, including potatoes, tomatoes, and tobacco. PVY is a stylet-borne virus that does not replicate within its aphid vectors. It is transmitted in less than a minute at

different transmission efficiencies by at least 40 species of aphid, the majority of which are non-colonizing species (Gray et al. 2010; Mello et al. 2011). The virus is lost rapidly with vector probing, leaving the vector unable to transmit after feeding on a few plants.

Additionally, as there is a long history of biological control efforts directed at aphids, the effect of coccinellid predators on aphid behavior is well documented (Obrycki and Kring 2009). In this study, we used a readily available adult coccinellid predator marketed as a biological control agent, *Hippodamia convergens*, and PVY^{NTN}, a necrotic strain of PVY that has increased in incidence in the United States and Canada over the last decade (Gray et al. 2010).

Plant and insect care

Potato plants (*Solanum tuberosum*, cv. Yukon Gold) were planted in 10.2cm diameter pots and grown in the greenhouse in a commercial potting soil (Metro-Mix 360, Sun Gro Horticulture) with fertilizer applied at watering each day. Oats (*Avena sativa*, Sunmark Seeds) were planted in $120cm^3$ cells and grown in similar conditions. Before being used in bioassays plants were allowed to grow for 2-3 weeks. To produce infected tissue, some potato plants were inoculated with PVY after 2-3 weeks of growth. On each plant, 3-4 new leaves were lightly sprinkled with carborundum and the PVY^{NTN} isolate (source plant: *Nicotiana tabacum*; S. Gray, Cornell University) was manually spread on the leaves using a cotton swab approximately thirty minutes later. Thirty minutes after exposure to the isolate, the leaves were rinsed with water. The virus was allowed to replicate for at least 14 days before plant material was collected for aphid acquisition of the virus.

Rhopalosiphum padi were maintained in colonies on barley (*Hordeum vulgare*, cv. Romulus) in growth chambers at 20˚C with 24 hour light. This colony was founded in the 1960s from a New York State population, and has been maintained for use in virus transmission assays. *R. padi* do not colonize (feed or reproduce on) potatoes, but they probe potato plants and other non-hosts, as this behavior is triggered by encountering smooth surfaces such as leaves and is not host-specific (Döring et al. 2004). This superficial probing is sufficient to transmit PVY.

Prior to inclusion in a bioassay, adult coccinellid predators (*Hippodamia convergens*, from Rincon-Vitova Insectary) were kept at 4°C and fed a mixture of honey and water once a week. To prevent flight and encourage on-plant movement, predators had their wings lacquered with clear nail polish before being introduced to the arena.

Experimental procedure

Plants were transplanted into potting soil (Metro-Mix 360, Sun Gro Horticulture) in 50.8x25.4cm black plastic flats in two rows of five plant positions (10 total plant positions/arena). The rows were approximately 15cm apart, plant positions within rows were approximately 10cm apart, and all remaining empty space was filled with potting soil. Plants were arranged in one of four treatments: oat monoculture (OM), potato monoculture (PM), separate rows of plant species (SR), or mixed rows, with alternating plant species within rows (MR; Fig. 1). The plants were placed so that they did not touch between rows, but did touch within rows. In mixture treatments the pattern of the plants was randomized so that the same species was not always located in the same position in the arena. To approximately equalize the amount of plant material between species,

potato pots were thinned to include a single shoot and oat pots were thinned to include 6 shoots per pot before inclusion in the bioassay. Potato shoots were approximately 1cm in diameter and 20-35cm tall. Oat shoots were approximately 1-2mm in diameter and approximately 20 cm tall.

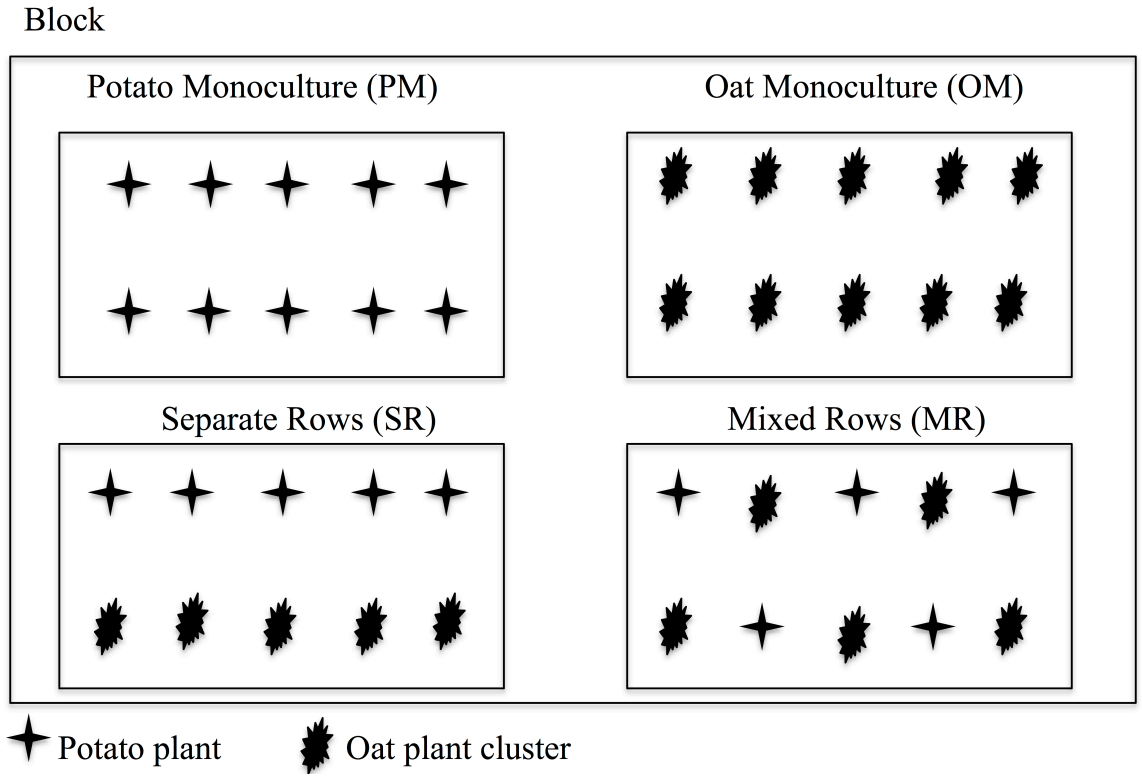

Figure 1: Schematic of plant treatments: oat monoculture (OM), potato monoculture (PM), separate rows (SR), and mixed rows (MR). The border signifies the block, which was assigned one of two predator treatment levels (presence or absence of predators).

The experimental unit was a flat of 10 plant positions, which received one of four plant treatments: oat monoculture, potato monoculture, separate rows or mixed rows (Fig. 1). A block included one experimental unit of each plant treatment that was conducted at the same time and each pair of sequential blocks was randomly assigned a level of predator treatment (presence or absence of predators). Twenty blocks, 10 with predators and 10 without, were conducted over the course of 17 nonconsecutive days.

For each flat, thirty adult wingless aphids were allowed to acquire PVY on infected tissue in a closed petri dish for at least one hour before beginning the bioassay. This number of aphids was selected in order to limit the risk of all plants in the arenas becoming infected. The aphids were placed between the two rows, 7.6cm from the first plant in each row. Aphids were introduced on the soil to avoid biasing their behavior by introducing them on either a host or non-host plant. All plants were censused for aphids at 20, 40, 60, 120, and 180 minutes from the time of release: the highest possible monitoring frequency within the first hour, and then hourly until the completion of the assay. The final census included a Euclidean distance (linear distance) measurement of aphid distance from the point of introduction (marked with a toothpick) for all aphids found at the final time point. Because PVY transmission occurs so rapidly, the bioassay was kept brief to capture the initial effect of host plant abundance and distribution on transmission. To allow for observations between censuses, and close monitoring of aphid movement with minimal disturbance, cages were not used. In the predator inclusion treatments, two predators were randomly placed in the flat at the same time as the aphid introduction. During the bioassay, they often left the arena by dropping from overhanging plants and, if so, were returned to the arena at a random location during the next census. Predators were unable to access an arena independently. Predators were not observed consuming aphids. Assays were conducted in a windowless room with constant overhead fluorescent lighting, to eliminate variation in light conditions.

Measuring aphid density and movement

Aphid density was calculated from the number of aphids found per plant position during the census. By adding up the positive differences between census counts (i.e. times when there were more aphids on a plant at a given time point compared to the previous census count) we estimated the number of unique aphids that were on each plant throughout the census.

The census data were also aggregated into two measures of aphid movement: 1) *linear distance* (cm) travelled by aphids counted on plants at the final census time point from the introduction point, and 2) *proportion of aphids leaving plants*. To account for the difference in aphid density between plant species, we calculated the *proportion of aphids leaving plants*, dividing the number leaving (the sum of the negative differences between census time points, when there were fewer aphids on a plant than at the previous time point) by the total number of aphids found on the plant. Each measurement was calculated for an individual plant and averaged across the flat. There was a lot of aphid movement in the plots, but because many aphids were never found on plants much of that movement occurred on the soil. Therefore census measurements are a conservative estimate of total aphid movement.

Measuring viral prevalence

After the aphid movement bioassay, all potato plants were removed from the flat and returned to their 10.2cm pot, bagged in a water and light permeable aphid-proof fabric (Agrifabric Pro-17), and were kept for at least 14 days in a growth chamber (27°C during the day and 25°C at night), when foliar samples were taken from each potato plant for enzyme-linked immunosorbent assay (ELISA) analysis (Ellis et al. 1996). To

compensate for the fact that treatments differed in the number of potato plants (susceptible hosts), we calculated the viral prevalence as the proportion of potato plants infected per flat, as well as the number of infected plants per flat.

Analysis

In all analyses, plant treatment and predator treatment were included as fixed effects, and block and plant treatment nested within block were included as a random effects. We performed model simplification (Crawley 2007) on the aphid census data, using $\alpha \leq 0.05$ as the threshold of significance. To assess *aphid density* trends throughout the census, the number of aphids per plant was analyzed using a repeated measures generalized linear mixed effects model with a Poisson distribution, plant and predator treatment as fixed effects, and block as a random effect. *Linear distance* was analyzed with a linear mixed effects model, and the average *proportion of aphids leaving* was analyzed using a mixed effects model. Both models included plant treatment and predator treatment as main effects and their interaction. When differences due to plant treatment or the interaction were found, Tukey's HSD tests were performed. The proportion of aphids on potato plants in the two plant mixture treatments were square root transformed and compared with a linear mixed model.

Because of the high number of zero values, a logistic regression was performed on the viral prevalence data. The proportion of potato plants infected was arcsine square root transformed to improve the normality of the data. The logistic regression of the full model, which included predator and plant treatments as factors, indicated that there were no significant differences in PVY prevalence between plant or predator treatments. To explore transmission once inoculation occurs, we analyzed the non-zero values. Flats

with infection (n=15; Table 2) were further analyzed using a mixed effects model with plant treatment and predator treatment as main factors and their interaction, in order to assess the effect of the treatment on the proportion of susceptible hosts infected. When differences due to treatment or the interaction were found, Tukey's HSD tests were performed. Analyses were conducted in R (version 2.14.0) and JMP Pro 10.

Results

Prediction 1: The effect of the predator treatment.

The predator treatment had no effect on any measurement of aphid behavior. The presense of predators did not affect *aphid density* ($F_{1,18}$=0.33, p=0.57; Table 2), *linear distance* ($F_{1,14}$=0.26, p=0.62), the *proportion of aphids leaving* ($F_{1,18}$=0.49, p=0.49), the *proportion of potato plants infected* ($F_{1,9}$=2.11, p=0.18), or the average *number of plants infected* ($F_{1,9}$=2.99, p=0.12).

Prediction 2 and 3: The effect of the plant treatment.

After model simplification, time point and plant treatment were the only explanatory factors related to the number of aphids per plant over the course of the census (Table 1). Plant treatment had a strong effect on the *aphid density* ($F_{3,54}$=7.41, p<0.001; Fig. 2); the number of aphids per plant in the potato monoculture was about half the number found in the other plant treatments over the course of the census. On average across plant treatments, oat plants recruited twice as many aphids as potato plants over the course of the bioassay ($F_{1,779}$=20.84, p<0.0001; Table 2).

Table 1: Model simplification from the full model of the aphid census data.

Model 1	Model 2	Δlog-likelihood	Chi Square	df	p value	Action
Full model	w/o 3-way interaction	2.8	5.7053	3	0.1269	**Proceed**
w/o 3-way interaction	w/o 3-way interactoin or Time.point:Plant	1.3	2.4769	3	0.4795	**Proceed**
	w/o 3-way interaction or Pred:Plant	2.2	4.3076	3	0.2301	**Proceed**
	w/o3-way interaction or Pred:Time.point	0.1	0.1487	1	0.6997	**Remove**
w/o 3-way interaction or Pred: Time.point	w/o 3-way interaction or Pred:Time.point or Pred:Plant	2.2	4.2937	3	0.2314	**Proceed**
	w/o 3-way interaction or Pred:Time.point or Time.point:Plant	1.2	2.3892	3	0.4957	**Remove**
w/o 3-way interaction or Pred: Time.point or Time.point:Plant	w/o 2-way interactions	2.2	4.2937	3	0.2314	**Remove**
w/o 3-way or 2-way interactions	w/o 3-way or 2-way interactions or Plant	61.3	122.44	4	2.20E-16	**Proceed**
	w/o 3-way or 2-way interactions or Pred	0.7	1.5895	1	0.2074	**Remove**
	w/o 3-way or 2-way interactions or Time.Point	72.7	145.52	1	2.20E-16	**Proceed**
w/o 3-way or 2-way interactions or Pred	**w/o Treat**	**85**	**169.87**	**5**	**2.20E-16**	**Keep**
	w/o Time.Point	**72.8**	**145.52**	**2**	**2.20E-16**	**Keep**

Table 2: Summary statistics for each predator and plant treatment: separate row (SR), mixed row (MR), potato monoculture (PM), and oat monoculture (OM); each column lists the cumulative number of aphids found in all ten replicates of each treatment.

Treatment	Total number of aphids	Total number of aphids on oat plants	Total number of aphids on potato plants	Total number of infected plants
Predator				
SR	114	71	43	3
MR	70	51	19	3
PM	42	NA	42	9
OM	62	62	NA	NA
No Predator				
SR	95	70	25	3
MR	80	58	22	3
PM	42	NA	42	4
OM	93	93	NA	NA

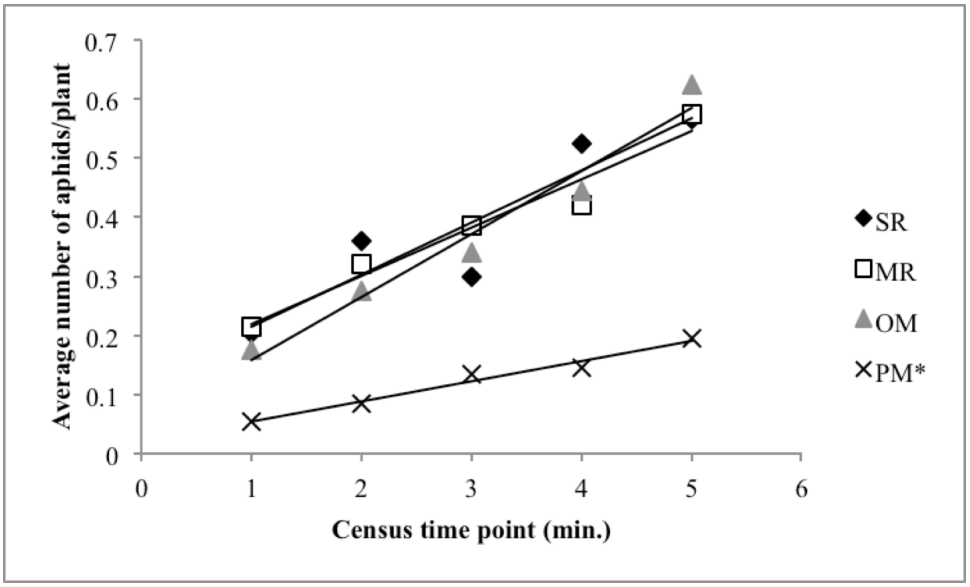

Figure 2: The average number of aphids on a plant across five census points (20, 40, 60, 120, and 180 minutes) during the course of three hours in four plant treatments: oat monoculture (OM), potato monoculture (PM), separate rows (SR), and mixed rows (MR). Lines are trendlines and asterisks indicate a signficant difference.

Increasing the number of non-host plants significantly increased aphid movement. Aphids moved twice as far in *linear distance* in the potato monoculture compared to the other plant treatments ($F_{3,46}$=3.22, p=0.0024; Fig. 3) and aphids tended to move farther in

the mixed row treatment than in the oat monoculture (p=0.058), increasing the average distance by 22%. The separate row treatment and the potato monoculture had the most aphids leaving. The average *proportion of aphids leaving plants* was two and a half times lower in the oat monoculture than the separate row treatment and the potato monoculture, with the mixed row treatment intermediate ($F_{3,54}$=5.124, p=0.0034; Fig. 4), indicating that plant spatial distribution affected the frequency of aphid movment.

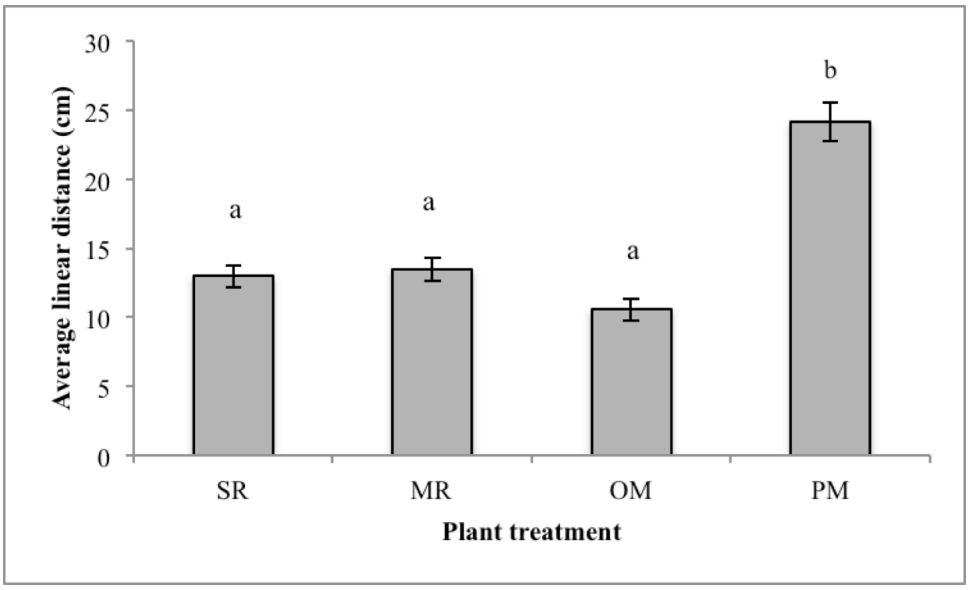

Figure 3: Average linear distance (cm) travelled by aphids after 3 hours in four plant treatments ± SE: oat monoculture (OM), potato monoculture (PM), separate rows (SR), and mixed rows (MR). Letters indicate significant differences.

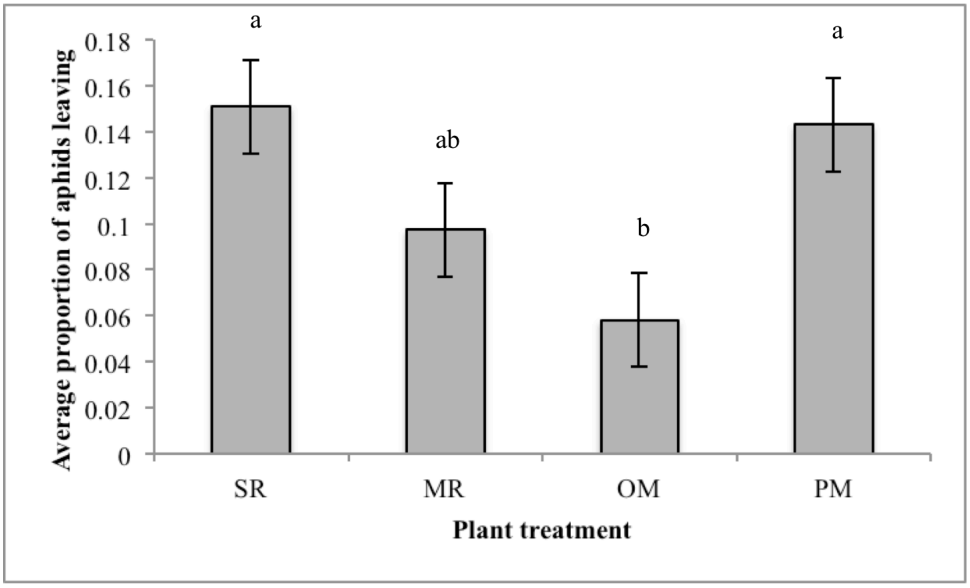

Figure 4: The average proportion of aphids leaving by plant treatment ± SE: oat monoculture (OM), potato monoculture (PM), separate rows (SR), and mixed rows (MR). Letters indicate significant differences.

Plant treatment had a significant effect on both the *proportion of plants infected* ($F_{1,9}$=5.89, p=0.023) and the average *number of plants infected* ($F_{2,9}$=5.33, p=0.03). The *proportion infected* was 56% greater in the separate row treatment than either the potato monoculture or the mixed row treatment, and the *number of plants infected* was half as great in the mixed row treatment versus the other two treatments.

Prediction 4: The interactive effect of plant and predator treatment.

Although the interactive effect of the plant and predator treatments did not influence aphid density or movement, it did significantly impact PVY prevalence. The interaction between treatments did not affect aphid density ($F_{3,54}$=0.88, p=0.46; Table 2) or movement, in *linear distance* ($F_{3,46}$=0.36, p=0.78) or the *proportion of aphids leaving* ($F_{3,54}$=0.911, p=0.44). The *proportion of potato plants infected* was significantly affected by the interaction between plant and predator treatment ($F_{2,9}$=5.67, p=0.026; Fig. 5), as

was the *number of plants infected* ($F_{2,9}$=4.55, p=0.043; Fig. 6). The presence of the predator doubled the proportion of infection in the potato monoculture and the separate row treatment, while it reduced the proportion of plants infected in the mixed row treatment by a third. The *number of plants infected* was half as great in the mixed row treatment versus the other two treatments.

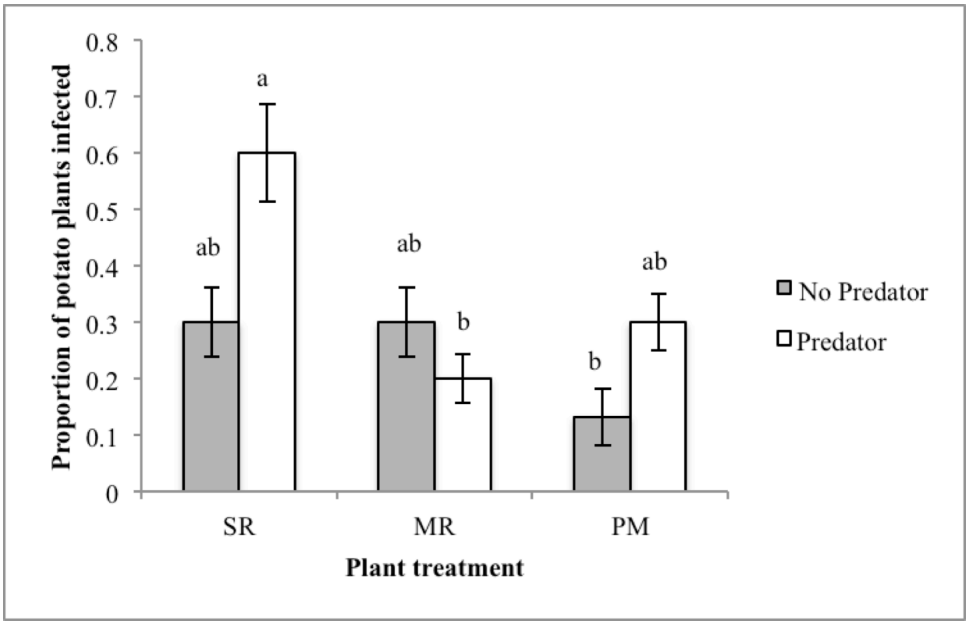

Figure 5: The proportion of potato plants infected in two predator treatment levels (presence and absence of predators) by plant treatment, excluding the oat monoculture (where no infection is possible) and uninfected plots ± SE: potato monoculture (PM), separate rows (SR), and mixed rows (MR). Letters indicate significant differences.

Hypothesis (aphid movement and virus transmission): When analyzed together, none of the metrics of aphid movement or density correlated with the average *proportion of potato plants infected* (*linear distance*, r=-0.21, p=0.14; *proportion leaving*, r=0.16, p=0.23; *aphid density*, r=-0.31, p=0.72) or the *number of plants infected* (*linear distance*, r=-0.12, p=0.39; *aphid density*, r=-0.14, 0.60). However, there was a marginal correlation between the proportion of aphids leaving and the number of plants infected (r=0.24, p=0.07).

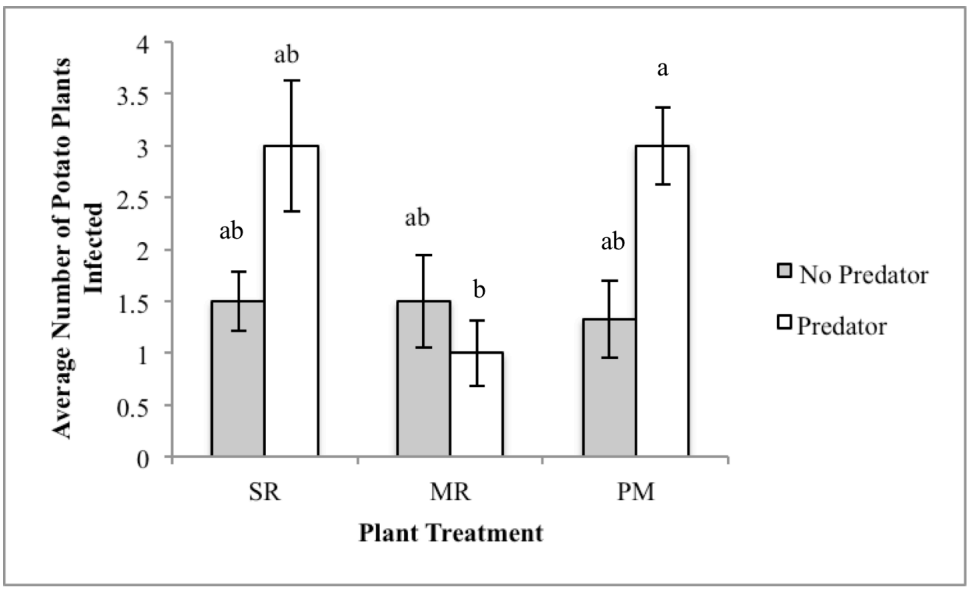

Figure 6: The number of potato plants infected by plant treatment, excluding the oat monoculture (where no infection is possible) and uninfected plots ± SE: potato monoculture (PM), separate rows (SR), and mixed rows (MR). Letters indicate significant differences.

Discussion

In this highly simplified plant pathosystem, the abundance of vector non-host (virus host) plants significantly affected wingless non-colonizing vector movement and density. In arenas with infection, PVY prevalence was influenced by the interactive effect of the predator and plant treatments. The presence of predators reduced virus prevalence in arenas with a more complex host plant distribution (i.e. the mixed row treatment) and increased prevalence in areans with simpler plant distributions (i.e. the separate row and potato monoculture treatments). Contrary to our hypothesis, this study did not find evidence that vector movement mediated the relationship between predator presense, host plant abundance and spatial structure, and PVY prevalence.

Increasing vector non-host plant abundance had a large effect on aphid density and movement (Prediction 2). The potato monoculture had about half the aphid density

and more than twice the aphid movement of the oat monoculture. This is consistent with the results of other studies (e.g. Srinivasan et al. 2013) that demonstrate that introducing a preferred host of the aphid increased the density of aphids on plants throughout the system. However, while the duration of aphid movement (the *linear disetance* traveled) was also primarily driven by vector non-host frequency, the rate of aphid movement (the *proportion of aphids leaving*) was determined by both the frequency and spatial distribution of the non-host plant (Prediction 3). Increasing the frequency of the non-host plant increased movement, and this effect was enhanced by separating the plant species by rows. The increased movement in arenas with more vector non-hosts may be a response to the poor quality of the non-host plants, and has been found in other aphid-plant systems (e.g. Sudderth and Sudderth 2014). The effect of host plant spatial distribution, with movement marginally greater in the separate row treatment than the mixed row treatement, indicates that host plant apparency may also play a role.

The predator treatment had no effect on the measures of aphid movement used in this study, consistent with the findings of Narayandas and Alyokhin (2006; Prediction 1). Despite this, the interaction between predator and plant treaments affected the *proportion of plants infected* with PVY in plots where infection occurred (Prediction 4). In accordance with the results of other studies (e.g., Hodge et al. 2011; Roitberg et al. 1978), the presense of predators increased virus prevalence in arenas with simpler host plant spatial distributions, the potato monoculture and separate row treatments. However, it had the opposite effect in the mixed row treatment, where the host plant spatial distribtuion was more complex. The difference in the effect of predators across plant treatments could reflect the distribution of aphids between the plant species in each mixture, as the

proportion of aphids on potato plants was greater in the separate row treatment than the mixed row treatment. A greater density of aphids on potato plants may have resulted in an amplification of transmission. Because the predators were not observed consuming aphid vectors, they may have been influencing the aphids through non-consumptive pathways (Nelson et al. 2004). These non-consumptive effects may include increased or altered probing behavior by the vectors. It is possible that the presence of predators encourages a 'hit-and-run' approach to probing; superficial probes may allow vectors to maintain greater mobility. If predators elicit increased briefer, epidermal probing, which is required for PVY transmission (Boquel et al. 2011), this could explain the corresponding jump in viral prevalence.

The *number of potato plants infected* followed a similar pattern to the *proportion of plants infected*. However, while the *proportion of infected plants* was slightly different in the potato monoculture and separate row treatments, the *number of plants infected* was the same. With no continuous virus source, vectors rapidly lose their ability to transmit the virus after probing one plant (Nault 1997), and it may be that the maximum amount of transmission occurred in both the potato monoculture and the separate row treatment, resulting in the same number of plants infected and the two-fold difference in proportion of plants infected.

While our findings have implications for future work, the simplified design and the use of wingless adult aphids in this study distances the results from agricultural application. This work should be replicated with winged adults and in a field setting before conclusions can be drawn for management and mitigation practices. Although aphid movement and density had no clear relationship to PVY prevalence, counter to our

initial hypothesis, the effects of predators and host plant abundance may be mediated by other vector behaviors, such as probing (Boquel et al. 2011), and may be dependent on intraplant movement. The results of this study demonstrate that wingless non-colonizing vector behavior and transmission of PVY are aggregated responses to multiple environmental drivers and emphasize the behavioral complexity of viral inoculation. Disentangling the relative importance of these factors, particularly in winged non-colonizing vector species, warrents further investigation.

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REFERENCES

Bailey, SM, ME Irwin, GE Kampmeier, CE Eastman, and AD Hewings. (1995). Physical and biological perturbations: their effect on the movement of apterous *Rhopalosiphum padi* (Homoptera: Aphididae) and localized spread of barley yellow dwarf virus. *Population Ecology*, 24: 24-33.

Boquel, S, A Ameline, and P Giordanengo. (2011). Assessing aphids potato virus y transmission efficiency: a new approach. *Journal of Virological Methods*, 178: 63-67.

Bottenburg, H, and ME Irwin. (1992). Using mixed cropping to limit seed mottling induced by soybean mosaic virus. *Plant Disease*, 76(3): 304-306.

Crawley, MJ. (2007). *The R Book*, 1st ed. John Wiley & Sons, West Sussex, England

DiFonzo, CD, DW Ragsdale, EB Radcliffe, NC Gudmestad, and GA Secor. (1997). Seasonal abundance of aphid vectors of potato virus y in the Red River Valley of Minnesota and North Dakota. *Journal of Economic Entomology*, 90: 824-831.

DiFonzo, CD, DW Ragsdale, EB Radcliffe, NC Gudmestad, and GA Secor. (1996). Crop borders reduce potato virus y incidence in seed potato. *Annals of Applied Biology*, 129: 289-302.

Döring, TF, SM Kirchner, S Kühne, and H Saucke. (2004). Response of alate aphids to green targets on coloured backgrounds. *Entomologia Experimentalis et Applicata*, 113: 53-61.

Ellis, P, R Stace-Smith, G Bowler, and DJ Mackenzie. (1996). Production of monoclonal antibodies for detection and identification of strains of potato virus y. *Canadian Journal of Plant Pathology*, 18: 64-70.

Fereres, A, and A Moreno. (2008). Behavioural aspects influencing plant virus transmission by homopteran insects. *Virus Research*, 141: 158-168.

Finke, D. (2012). Contrasting the consumptive and non-consumptive cascading effects of natural enemies on vector-borne pathogens. *Entomologia Experimentalis et Applicata*, 144: 1-11.

Gray, S, S De Boer, J Lorenzen, A Karasev, J Whitworth, P Nolte, R Singh, A Boucher, and H Xu. (2010). Potato virus y: An evolving concern for potato crops in the United States and Canada. *Plant Disease*, 94: 1384-1397.

Hodge, S, J Hardie, and G Powell. (2011). Parasitoids aid dispersal of a nonpersistently transmitted plant virus by disturbing the aphid vector. *Agricultural and Forest Entomology*, 13: 83-88.

Kaplan, I, and JS Thaler. (2012). Phytohormone-mediated plant resistance and predation risk act independently on the population growth and wing formation of potato aphids, *Macrosiphum euphorbiae*. *Arthropod-Plant Interactions*, 6: 181-186.

Mello, AFS, RA Olarte, SM Gray, and KL Perry. (2011). Transmission efficiency of potato virus y strains PVY^{O} and $PVY^{N\text{-}Wi}$ by five aphid species. *Plant Disease*, 95: 1279-1283.

Moore, SM, ET Borer, and PR Hosseini. (2009). Predators indirectly control vector borne disease: linking predator-prey and host-pathogen models. *Journal of the Royal Society Interface*, 7: 161-176.

Narayandas, GK, and AV Alyokhin. (2006). Interplant movement of potato aphid (Homoptera:Aphididae) in response to environmental stimuli. *Environmental Entomology*, 35: 733-739.

Nault, LR. (1997). Arthropod transmission of plant viruses: a new synthesis. *Annals of the Entomological Society of America*, 90: 521-541.

Nelson, EH, CE Matthews, and JA Rosenheim. (2004). Predators reduce prey population by inducing changes in prey behavior. *Ecology*, 85: 1853-1858.

Nolte, P, JL Whitworth, MK Thornton, and CS McIntosh. (2004). Effect of seed-borne potato virus y on performance of Russet Burbank, Russet Norkotah, and Shepody potato. *Plant Disease*, 88: 248-252.

Obrycki, JJ, and TJ Kring. (2009). Aphidophagy by coccinellidae: Application of biological control in agroecosystems. *Biological Control*, 51: 244-254.

Ostfeld, RS, and F Keesing. (2012). Effects of host diversity on infectious disease. *Annual Review of Ecology, Evolution, and Systematics*, 43: 157-182.

Pirone, TP, and KL Perry. (2002). Aphids: Non-persistent transmission. *Advances in Botanical Research*, 36: 1-19.

Power, AG. (1991). Virus spread and vector dynamics in genetically diverse plant populations. *Ecology*, 72: 232-241.

Power, AG, and AS Flecker. (2008). The role of vector diversity in disease dynamics. In: RS Ostfeld, et al. (eds.), *Infectious disease ecology: The effects of ecosystems on disease and of disease on ecosystems*. Princeton University Press, Princeton, NJ, pp.30-47.

Preisser EL, and DI Bolnick. (2008). The many faces of fear: comparing the pathways and impacts of nonconsumptive predator effects on prey populations. *PLOS One*,

3: e2465.

Roitberg, BD, and JH Myers. (1978). Effect of adult coccinellidae on the spread of a plant virus by an aphid. *Journal of Applied Ecology*, 15: 75-779.

Scholthof, KG, S Adkins, H Czosnek, P Palukaitis, E Jacquot, T Hohn, B Hohn, K Saunders, T Candresse, P Ahlquist, C Hemenway, and GD Foster. (2011). Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology*, 12: 938-954.

Singh, MN, SMP Khurana, BB Nagaich, and HO Agrawal. (1988). Environmental factors influencing aphid transmission of potato virus y and potato leafroll virus. *Potato Research*, 31: 501-509.

Srinivasan, R, JM Alvarez, and F Cervantes. (2013). The effect of an alternate weed host, hairy nightshade, Solanum sarrachoides (Sendtner) on green peach aphid distribution and Potato leaf roll virus incidence in potato fields of the Pacific Northwest. *Crop Protection*, 46: 52-56.

Sudderth EA, and EB Sudderth. (2014). Quantifying aphid behavioral responses to environmental change. *Entomologica Experimentalis et Applicata*, 150: 7-18.

APPENDIX

Chapter 1 Supplementary Material:

Supplementary Table 1: The results of Mantel tests evaluating the relationship between the Euclidean distance of the NY sites (determined by their latitudes and longitudes) and the percent agricultural land surrounding them within the measured scales.

Year	Scale (m)	r	Significance
All farms			
2012	500	0.031	0.348
2012	1000	0.003	0.444
2013	500	0.009	0.46
2013	1000	0.041	0.318
2013	1500	0.023	0.382
Infected farms only			
2012	500	-0.051	0.562
2012	1000	0.056	0.324
2013	500	-0.077	0.562
2013	1000	-0.042	0.525
2013	1500	-0.084	0.643

Supplementary Table 2: Spearman correlations between the percent agriculture and percent natural habitat at the three scales of analysis in 2012 and 2013 in both the reduced and full datasets.

Dataset	Year	Scale	p-value	Direction
Reduced Dataset	2012	500	**<0.0001**	Negative
Reduced Dataset	2012	1000	**0.0035**	Negative
Reduced Dataset	2012	1500	**0.0152**	Negative
Full Dataset	2012	500	**<0.0001**	Negative
Full Dataset	2012	1000	**<0.0001**	Negative
Full Dataset	2012	1500	**<0.0001**	Negative
Reduced Dataset	2013	500	**<0.0001**	Negative
Reduced Dataset	2013	1000	**<0.0001**	Negative
Reduced Dataset	2013	1500	**<0.0001**	Negative
Full Dataset	2013	500	**<0.0001**	Negative
Full Dataset	2013	1000	**<0.0001**	Negative
Full Dataset	2013	1500	**<0.0001**	Negative

Supplementary Table 3: Results from binomial positive counts models (step 2 in the sequential modeling process) evaluating the effect of landscape composition, measured as percent natural habitat, on final PVY prevalence, and the results from a linear poisson-family models evaluating the effect of landscape composition, measured as percent natural habitat, on aphid species richness (number of different species captured at each site). Analysis is of the reduced dataset, and bold type shows significant p-values. For results from the full dataset, please see *Supplementary Table 5 and 6.*

Response variable	Explanatory scale (m)	df	estimate	p	AIC
2012 PVY prevalence	500	2	-0.032	**3.65E-05**	53.015
	1000	2	-0.044	**1.23E-05**	50.17
	1500	2	-0.038	**0.00136**	59.97
2013 PVY prevalence	500	2	-0.016	**0.000165**	101.6
	1000	2	-0.026	**0.000165**	101.6
	1500	2	-0.029	**0.000769**	104.66
2012 Aphid species richness	500	2	0.00934	**0.00321**	91.11
	1000	2	0.0113	**0.00393**	91.58
	1500	2	0.010155	**0.0207**	94.6
2013 Aphid species richness	500	2	0.0113	**0.00393**	112.5
	1000	2	0.005254	**0.0407**	113.7
	1500	2	0.004839	**0.121**	115.4

Supplementary Table 4: Results from binomial positive counts models (step 2 in the sequential modeling process) evaluating the effect of landscape composition, measured as percent agriculture, on final PVY prevalence, and the results from a linear poisson-family models evaluating the effect of landscape composition, measured as percent agriculture, on aphid species richness (number of different species captured at each site). Analysis is of the reduced dataset, and bold type shows significant p-values. For results from the full dataset, please see *Supplementary Table 5 and 6.*

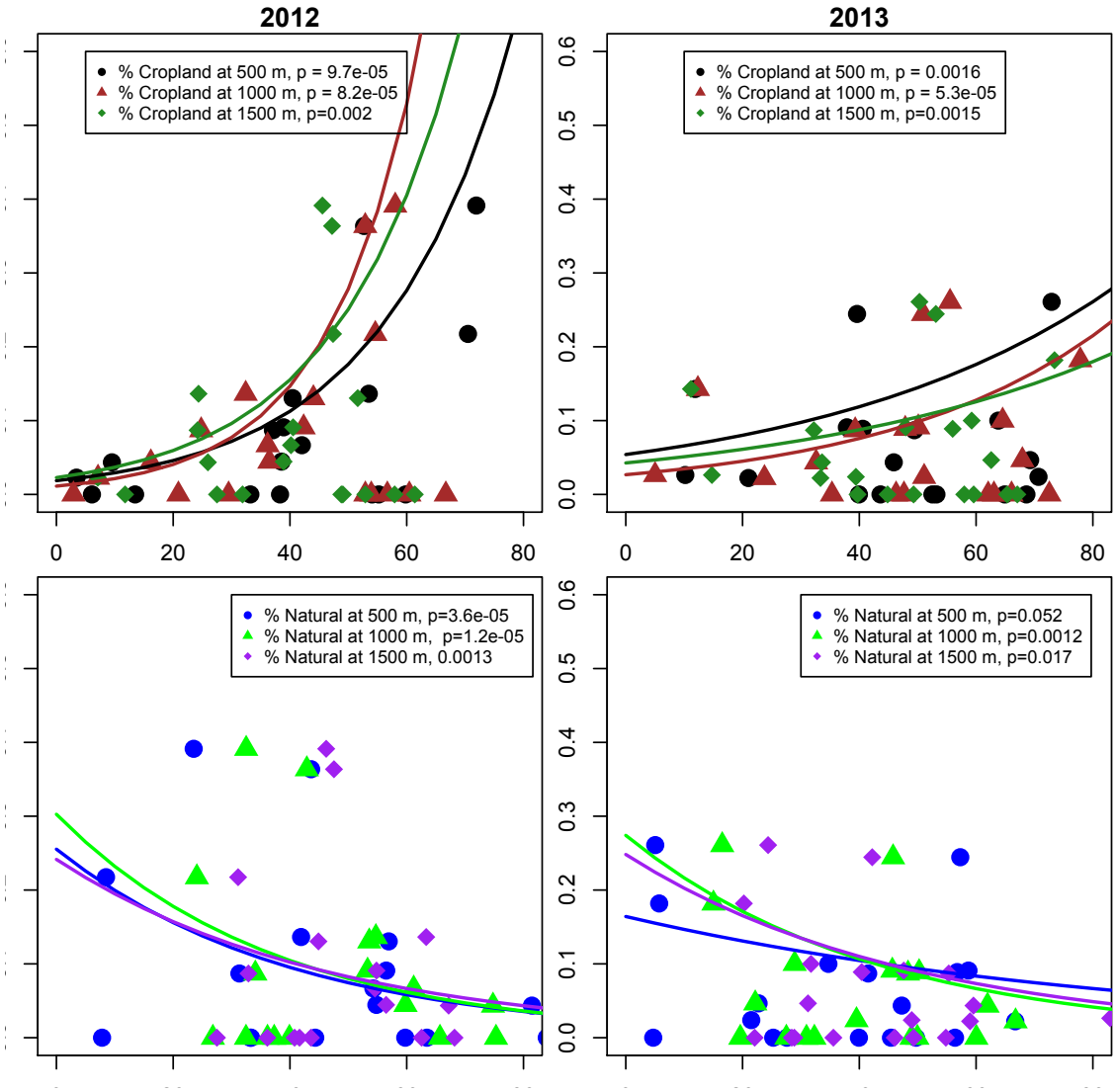
Response variable	**Explanatory scale (m)**	**df**	**estimate**	**p**	**AIC**
2012 PVY prevalence	500	2	0.045	**5.17E-06**	46.56
	1500	2	0.048	**0.00114**	59.05
2013 PVY prevalence	500	2	0.02	**0.0017**	107.4
	1500	2	0.028	**0.0016**	106.7
2012 Aphid Species Richness	500	2	-0.009536	**0.0077**	92.75
	1500	2	-0.010777	**0.0499**	96.01
2013 Aphid Species Richness	1000	2	-0.005755	**0.0448**	113.8
	1500	2	-0.003808	0.283	116.6

Supplementary Table 5: The results from binomial 1/0 (presence/absence) models (step 1 in the sequential modeling process) evaluating the effect of landscape composition on the presence or absence of disease in the landscape.

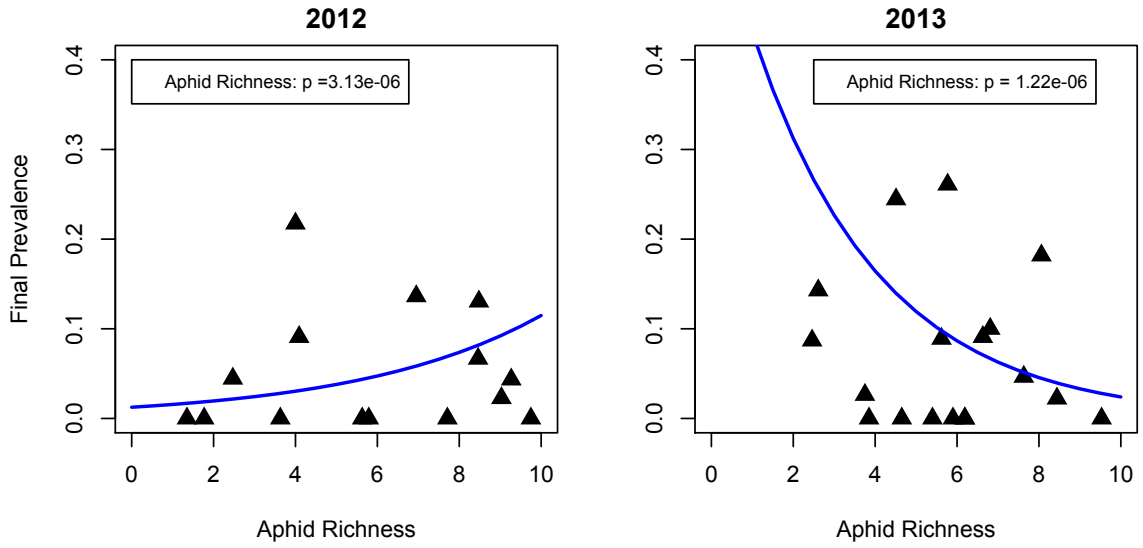
Response variable	Explanatory variable	df	estimate	p	AIC
2012 PVY prevalence	Percent cropland within 500 m	2	-0.003731	0.858	29.8
	Percent cropland within 1000 m	2	-0.01924	0.471	29.3
	Percent cropland within 1500 m	2	-0.03404	0.323	28.8
2012 PVY prevalence	Percent natural habitat within 500 m	2	-0.004042	0.831	29.8
	Percent natural habitat within 1000 m	2	0.004255	0.852	29.8
	Percent natural habitat within 1500 m	2	0.01015	0.715	29.7
2013 PVY prevalence	Percent cropland within 500m	2	-0.02118	0.355	29.8
	Percent cropland within 1000 m	2	-0.02716	0.318	29.6
	Percent cropland within 1500 m	2	-0.04586	0.204	28.7
2013 PVY prevalence	Percent natural habitat within 500 m	2	0.01511	0.465	30.2
	Percent natural habitat within 1000 m	2	0.016447	0.482	30.2
	Percent natural habitat within 1500 m	2	0.03374	0.281	29.4

Supplementary Table 6: Results from linear poisson-family models evaluating the effect of landscape composition on aphid species richness (counts of different species captured at each site). Significant p-values are in bold. Analysis is of the full dataset, and bold type shows critical distances, given by model with lowest AIC value, for each year and landscape type (agricultural or natural).

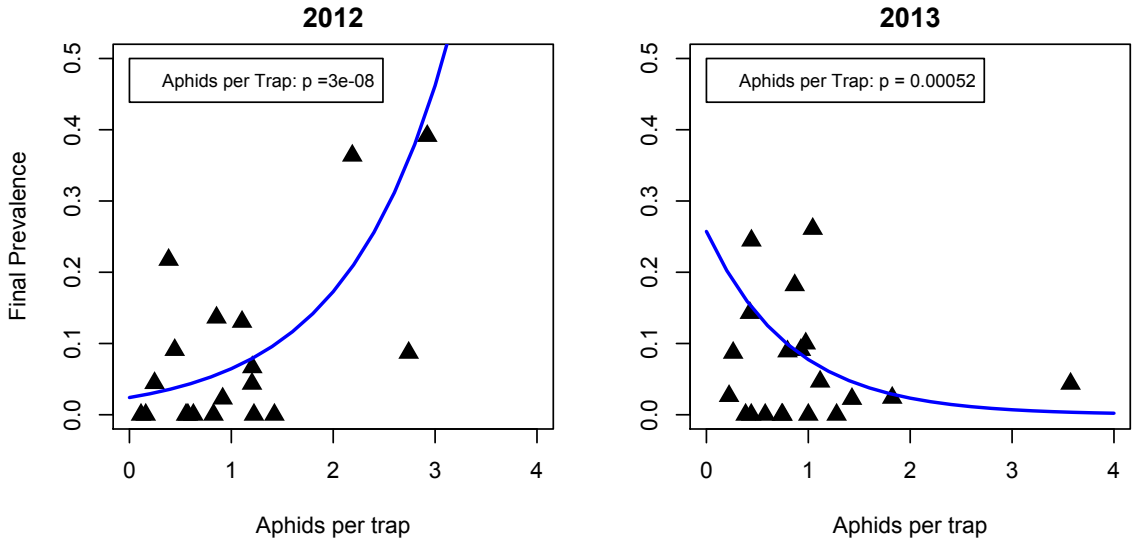
Response variable	**Explanatory variable**	**df**	**estimate**	**p**	**AIC**
2012 Aphid species richness	Percent cropland within 500 m	2	-0.006107	0.0245	164.6
	Percent cropland within 1000 m	**2**	**-0.010722**	**0.000898**	**158.9**
	Percent cropland within 1500 m	2	-0.011841	0.00282	161.0
2012 Aphid species richness	Percent natural habitat within 500 m	2	0.005371	0.0295	164.9
	Percent natural habitat within 1000 m	**2**	**0.009092**	**0.00132**	**159.6**
	Percent natural habitat within 1500 m	2	0.009939	0.00329	161.3
2013 Aphid species richness	**Percent cropland within 500m**	**2**	**-0.006394**	**0.0105**	**160.7**
	Percent cropland within 1000 m	2	-0.006394	0.0157	161.5
	Percent cropland within 1500 m	2	-0.004955	0.125	164.9
2013 Aphid species richness	Percent natural habitat within 500 m	2	0.005155	0.0227	162.0164
	Percent natural habitat within 1000 m	**2**	**0.005651**	**0.0193**	**161.8332**
	Percent natural habitat within 1500 m	2	0.005307	0.0651	163.8566



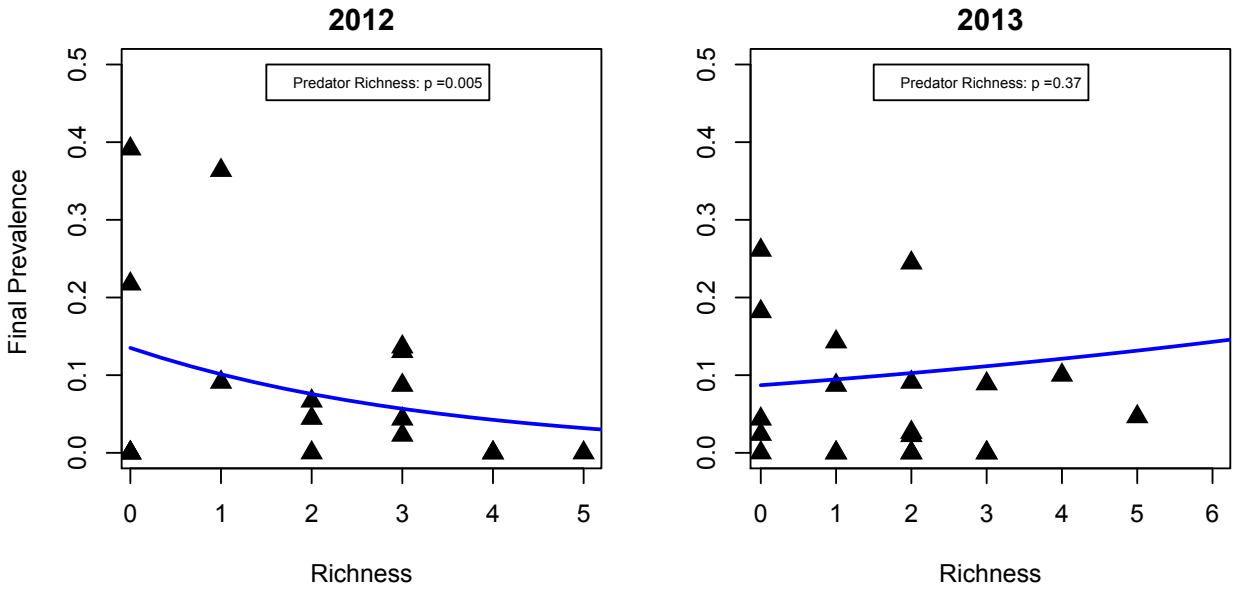
Supplementary Figure 1: Results from binomial success/failure modeling of count data, including infected and uninfected farms (full dataset). *Top two panels:* PVY prevalence increases with increasing percentage of agricultural land, years 2012 in left panel and 2013 in right panel. Percent cropland at 500 m, black symbol and line, at 1000 m (the most predictive scale in 2012 and 2013), red symbol and line, and at 1500 m, green symbol and line. *Bottom two panels:* PVY prevalence decreases with increasing percentage of natural habitat in the landscape, years 2012 in left panel and 2013 in right panel. Percent natural at 500, blue symbol and line, percent natural at 1000 m (the most predictive scale in 2012 and 2013), green symbol and line and percent natural at 1500 m, purple symbol and line.



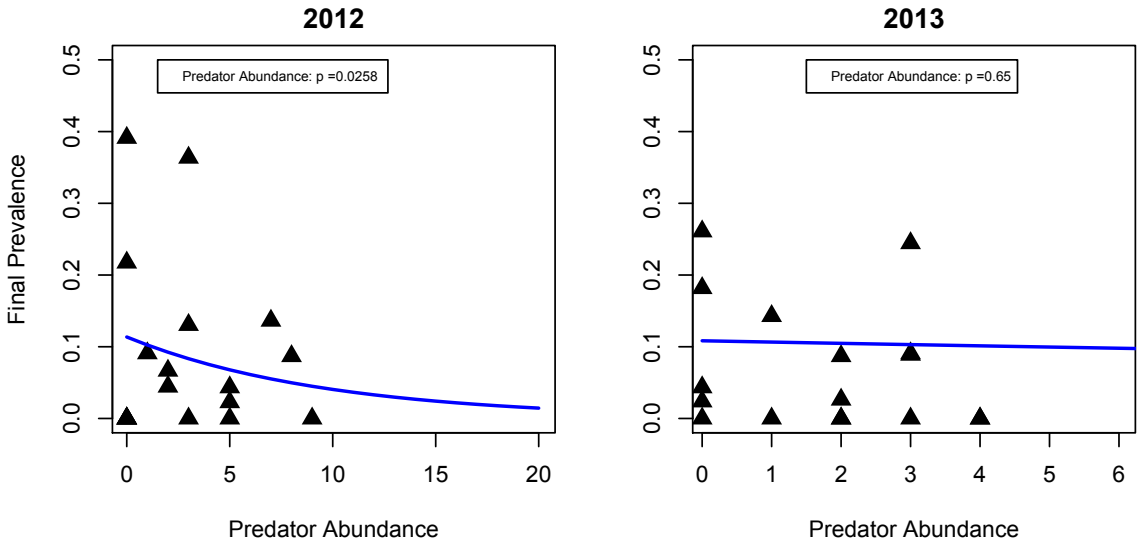
Supplementary Figure 2a: Aphid species richness (interpolated using rarefaction) and final PVY prevalence in 2012 and 2013. Analysis is of full dataset.



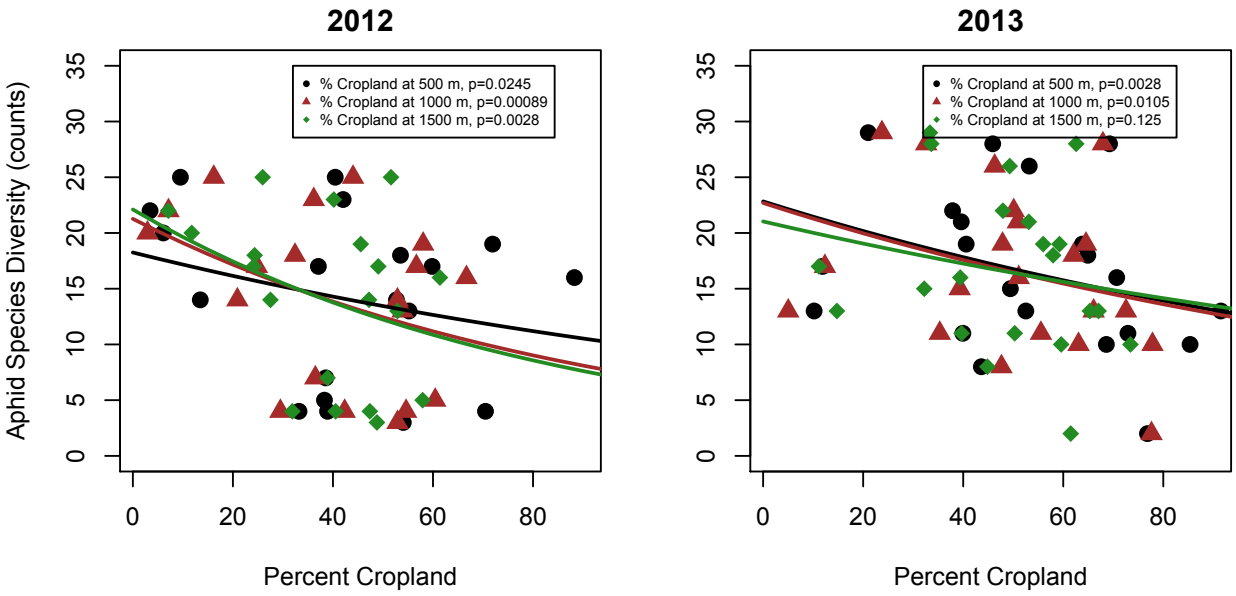
Supplementary Figure 2b: Aphid abundance (aphids per trap) and final PVY prevalence in 2012 and 2013. Analysis is of full dataset.



Supplementary Figure 3a: Predator species richness and final prevalence, analysis of the full dataset.



Supplementary Figure 3b: Predator Abundance and final prevalence, analysis of the full dataset.



Supplementary Figure 4: Aphid species diversity (counts) decreases with increasing agriculture in a landscape. Percent cropland at 500 m (the most predictive scale in 2013), black symbol and line, at 1000 m (the most predictive scale in 2012), red symbol and line, and at 1500 m, green symbol and line. See *Supplementary Table 2* for parameter estimates, p-values, and AIC values, and for results from analysis of the effects of natural landscape on aphid species diversity. Analysis was completed on the full dataset.

Chapter 2 Supplementary Material

Supplemental Table 1: Landscape composition (measured as percent agriculture and percent natural habitat) at sampling sites in both NY and WI, at the three sampled scales (m).

			500m		1000m		1500m	
State	**Year**	**Site**	**Natural Habitat (%)**	**Agriculture (%)**	**Natural Habitat (%)**	**Agriculture (%)**	**Natural Habitat (%)**	**Agriculture (%)**
NY	2012	BT	56.9	40.5	53.6	44.0	44.9	51.6
NY	2012	CS	33.2	55.2	36.2	53.9	40.8	52.9
NY	2012	EG	81.4	9.5	74.8	16.2	67.2	25.9
NY	2012	FF	7.8	88.3	26.8	66.7	27.5	61.4
NY	2012	FI	63.5	33.2	65.7	29.5	62.5	31.9
NY	2012	FM	43.6	52.7	42.9	52.9	47.5	47.2
NY	2012	GT	8.5	70.5	24.1	54.6	31.1	47.4
NY	2012	HH	94.4	3.4	91.1	7.1	91.5	7.1
NY	2012	HT	33.3	59.8	37.3	56.7	43.6	49.1
NY	2012	KB	91.6	6.1	95.4	2.9	85.9	11.8
NY	2012	MA	23.5	71.9	32.5	58.0	46.2	45.6
NY	2012	MC	31.3	37.1	34.1	24.8	32.9	24.2
NY	2012	MF	54.3	42.0	61.2	36.2	54.6	40.2
NY	2012	RE	44.3	54.1	39.9	52.9	41.6	48.8
NY	2012	RV	84.0	13.5	75.3	20.9	68.2	27.5
NY	2012	SF	56.5	38.9	53.3	42.3	54.8	40.5
NY	2012	SQ	59.7	38.3	32.5	60.4	36.1	57.9
NY	2012	TG	54.8	38.6	59.8	36.5	56.5	38.9
NY	2012	WH	41.9	53.5	54.7	32.4	63.3	24.3
NY	2013	BT	56.8	40.6	50.2	47.9	40.4	56.0
NY	2013	CS	22.7	69.3	22.2	67.9	31.2	62.6
NY	2013	DL	5.7	85.4	15.0	77.8	20.2	73.4
NY	2013	EG	66.7	21.0	66.8	23.8	59.0	33.3
NY	2013	FF	4.7	91.5	19.6	72.5	22.1	67.1
NY	2013	FI	56.4	39.9	60.0	35.3	54.8	39.7
NY	2013	FM	27.6	68.6	32.3	63.1	35.6	59.6
NY	2013	GT	5.0	72.9	16.5	55.5	24.4	50.3
NY	2013	HH	85.5	11.9	85.5	12.3	87.4	11.2
NY	2013	KB	87.4	10.2	93.3	5.0	83.0	14.8
NY	2013	MA	19.2	76.8	17.3	77.6	28.7	61.5
NY	2013	MB	41.5	49.4	48.3	39.3	55.3	32.2
NY	2013	MC	49.7	43.6	48.9	47.6	49.3	44.8
NY	2013	MF	57.3	39.6	45.8	50.9	42.2	53.1
NY	2013	PW	25.3	64.9	31.0	62.1	28.4	58.0
NY	2013	RE	34.7	63.8	28.9	64.5	31.7	59.2
NY	2013	RF	47.3	45.9	62.0	32.6	59.6	33.6
NY	2013	SF	45.4	52.5	27.5	66.1	28.9	65.3
NY	2013	SQ	58.7	37.9	45.7	50.1	47.6	47.9
NY	2013	TG	39.9	53.2	49.9	46.3	46.1	49.3

NY	2013	WH	21.5	70.7	39.5	51.1	49.0	39.4
WI	2010	15	42.7	52.8	31.5	58.2		
WI	2010	28	4.3	94.1	25.8	65.3	34.7	57.0
WI	2010	29	25.0	63.9	62.1	22.7		
WI	2010	2nd_Dr	3.8	94.4				
WI	2010	47	2.8	81.4	14.7	57.8	29.6	37.2
WI	2010	Czech	16.6	76.6	25.9	66.3	32.8	61.7
WI	2010	CR_I	0.2	95.6	8.2	86.2		
WI	2010	Pioneer	0.2	95.1	0.7	94.6	7.1	89.2
WI	2011	1st_Ave	3.8	93.0				
WI	2011	25	0.3	41.5	7.6	69.2	28.0	66.4
WI	2011	39	0.0	46.6				
WI	2011	59	2.2	82.5	2.4	89.5	7.4	86.5
WI	2011	Cree	15.2	79.5	16.6	78.3	10.0	86.5
WI	2011	CRV_CS	1.9	93.0				
WI	2011	CRV_LC	28.2	55.9	40.7	47.6		
WI	2011	Price Neva	3.7	89.4	2.8	93.7	5.0	91.7
WI	2011	Polar	8.8	88.0	8.1	86.1	20.1	74.2

Supplemental Table 2: The results of Mantel tests evaluating the relationship between the Euclidean distance of the NY sites (determined by their latitudes and longitudes) and the percent agricultural land surrounding them within the measured scales.

Year	Scale (m)	r	Significance
All farms			
2012	500	0.031	0.348
2012	1000	0.003	0.444
2013	500	0.009	0.46
2013	1000	0.041	0.318
2013	1500	0.023	0.382
Infected farms only			
2012	500	-0.051	0.562
2012	1000	0.056	0.324
2013	500	-0.077	0.562
2013	1000	-0.042	0.525
2013	1500	-0.084	0.643

Supplemental Table 3: The results of Mantel tests evaluating the relationship between the Euclidean distance of the WI sites (determined by their latitudes and longitudes) and the percent agricultural land surrounding them within the measured scales.

Scale (m)	r	Significance
500	0.160	0.065
1000	0.0723	0.194
1500	-0.138	0.859

Supplemental Table 4: Spearman correlations of the percent agriculture and percent natural habitat surrounding sampling sites in NY and WI. Significant p-values are bolded.

State	**Scale (m)**	**p-value**	**Direction**
NY	500	**<0.0001**	Negative
NY	1000	**<0.0001**	Negative
NY	1500	**<0.0001**	Negative
WI	500	0.0641	Negative
WI	1000	**<0.0001**	Negative
WI	1500	**0.0003**	Negative

Supplemental Table 5: Results of a generalized linear mixed effects model with the total number of aphids per trap collected in NY as a response variable and the number of traps as an offset variable, and of generalized linear mixed effects models with aphid species richness in both regions and the number of aphids collected in WI as response variables. Significant p values are in bold.

Response Variable	Explanatory Scale (m)	Predictor Variable	Estimate	Std. Error	z value	p value	R^2
NY							
Number of aphids per trap	500	Week	-0.093	0.023	-4.071	**4.69E-05**	0.52
Number of aphids per trap	500	Percent natural habitat	-0.0031	0.0049	-0.647	0.517	
Number of aphids per trap	500	Week:Percent natural habitat	-0.0031	0.00047	-6.65	**2.93E-11**	
Richness	500	Week	-0.065	0.0297	-2.188	**0.0287**	0.46
Richness	500	Percent natural habitat	0.0045	0.0037	1.23	0.2186	
Richness	500	Week:Percent natural habitat	-0.0012	0.00057	-2.225	**0.0261**	
WI							
Number of aphids	1500	Week	-5.13E-01	2.60E-02	-19.7	**<2E-16**	0.67
Number of aphids	1500	Percent natural habitat	-2.81E-02	2.02E-03	-14	**<2E-16**	0.061
Number of aphids	1500	Week:Percent natural habitat	5.07E-03	3.47E-04	14.6	**<2E-16**	
Richness	1500	Week	-0.206	0.0399	-5.17	**2.32E-07**	0.51
Richness	1500	Percent natural habitat	-0.00722	0.00339	-2.13	**0.0331**	0.15
Richness	1500	Week:Percent natural habitat	0.00134	0.000543	2.46	0.139	

Supplemental Table 6: Model results of a generalized linear mixed effects model with the raw aphid species richness collected (excluding unidentified specimen) in NY without the number of traps as an offset variable. Significant p-values are in bold.

Explanatory Scale (m)	Predictor Variable	Estimate	Std. Error	z value	p value	R^2
500	Week	-0.0647	0.0291	-2.221	**0.0263**	0.47
500	Percent natural habitat	0.00481	0.0037	1.298	0.1941	
500	Week:Percent natural habitat	-0.00124	0.000568	-2.18	**0.0293**	
500	Week	-0.19	0.0316	-5.99	**2.04E-09**	0.47
500	Percent agriculture	-0.0061	0.00391	-1.56	0.1188	
500	Week:Percent agriculture	0.00145	0.0006049	2.39	**0.0167**	

Supplemental Table 7: Results of a generalized linear mixed effects model with the proportion of aphids that were colonizers as the response variable. In the NY model, the number of traps was included as an offset variable. Significant p values are in bold.

Response Variable	Explanatory Scale (m)	Predictor Variable	Estimate	Std. Error	z value	p value	R^2
NY	**NY**						
Proportion colonizing	1500	Week	-0.74	0.9	-0.82	0.41	
Proportion colonizing	1500	Percent natural habitat	-0.02	0.06	-0.28	0.78	
Proportion colonizing	1500	Week:Percent natural habitat	0.0007	0.02	0.04	0.97	
Proportion colonizing	1500	Week	-1.08	0.75	-1.44	0.15	
Proportion colonizing	1500	Percent agriculture	-0.07	0.07	-1.07	0.29	
Proportion colonizing	1500	Week:Percent agriculture	0.01	0.02	0.59	0.55	
WI	**WI**						
Proportion colonizing	1500	Week	0.299	0.144	2.078	**0.0377**	0.16
Proportion colonizing	1500	Percent natural habitat	0.0239	0.0133	1.79	0.0734	
Proportion colonizing	1500	Week:Percent natural habitat	-0.00427	0.00216	-1.98	**0.0482**	
Proportion colonizing	1500	Week	-0.119	0.0873	-1.367	0.172	
Proportion colonizing	1500	Percent agriculture	-0.0227	0.0184	-1.232	0.218	
Proportion colonizing	1500	Week:Percent agriculture	0.00556	0.00277	2.005	**0.0449**	

Supplemental Table 8: Results of a generalized linear mixed effects model with the proportion of aphids that were PVY vectors as the response variable. In the NY model, the number of traps was included as an offset variable. Significant p values are in bold.

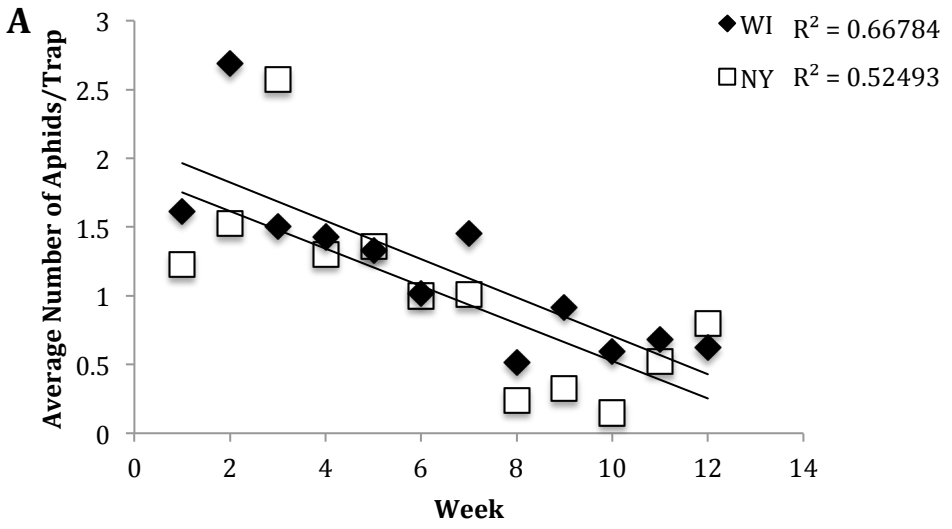
Response Variable	Explanatory Scale (m)	Predictor Variable	Estimate	Std. Error	z value	p value	R^2
NY	**NY**						
Proportion vectors	1500	Week	-0.46	0.22	-2.08	**0.03766**	0.32
Proportion vectors	1500	Percent natural habitat	-0.05	0.02	-2.28	**0.02282**	0.084
Proportion vectors	1500	Week:Percent natural habitat	0.009	0.004	2.12	**0.03421**	
Proportion vectors	1500	Week	0.4	0.2	2.002	**0.0452**	0.32
Proportion vectors	1500	Percent agriculture	0.05	0.02	1.98	**0.0476**	0.067
Proportion vectors	1500	Week:Percent agriculture	-0.0099	0.005	-2.195	**0.0281**	
WI	**WI**						
Proportion vectors	1500	Week	-0.00738	0.0612	-0.121	0.904	
Proportion vectors	1500	Ag_1500	-0.00198	0.00485	-0.409	0.682	
Proportion vectors	1500	Week:Ag_1500	0.000599	0.00085	0.704	0.481	
Proportion vectors	1500	Week	0.0661	0.0304	2.18	**0.0296**	0.23
Proportion vectors	1500	Natural_1500	0.00707	0.00664	1.064	0.287	
Proportion vectors	1500	Week:Natural_1500	-0.00137	0.00113	-1.212	0.226	

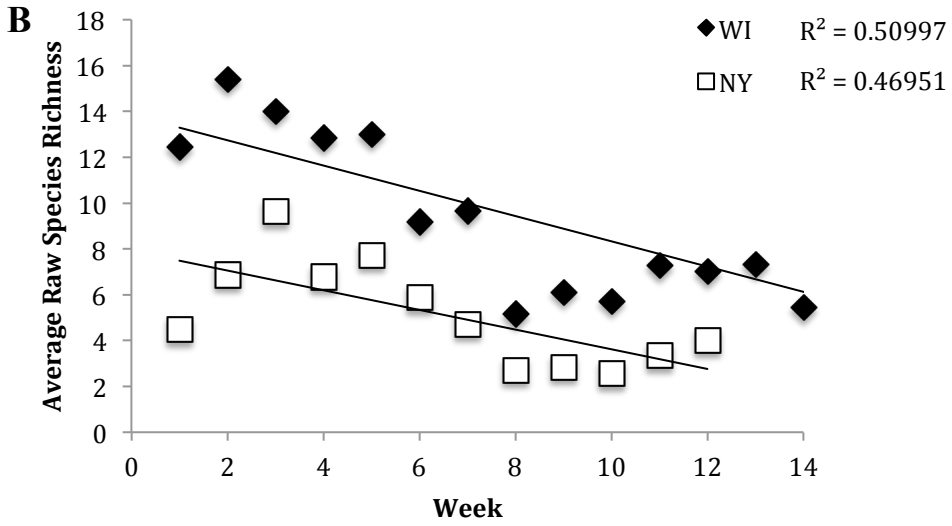
Supplemental Table 9: Results of a generalized linear mixed effects model with the proportion of aphids that were unestimated, low-transmitting, and high-transmitting PVY vectors as the response variable. In the NY model, the number of traps was included as an offset variable. Significant p values are in bold.

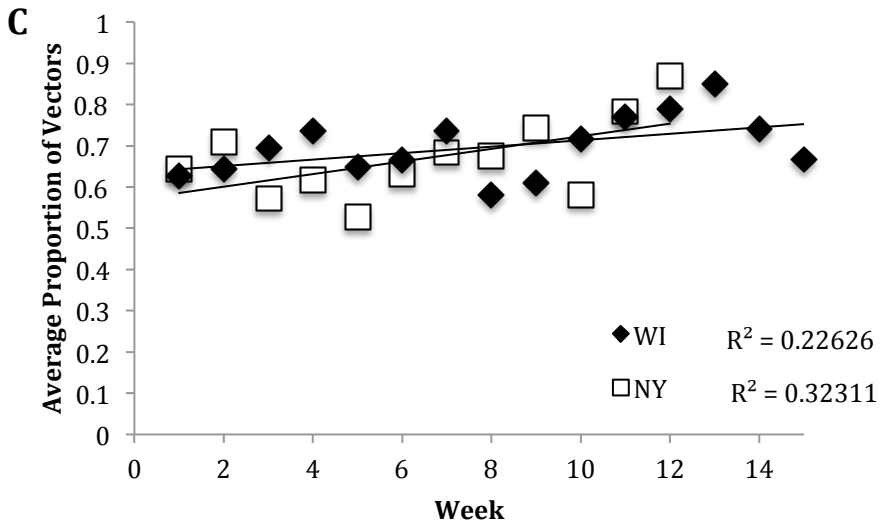
Response Variable	Explanatory Scale (m)	Predictor Variable	Estimate	Std. Error	df	z value	p value	R^2
NY								
Proportion unestimated vectors	1500	Week	4.49E-02	1.68E-02	1.53E+02	2.67	**0.0084**	0.79
Proportion unestimated vectors	1500	Percent natural habitat	1.72E-05	1.94E-03	1.07E+02	0.01	0.9900	
Proportion unestimated vectors	1500	Week:Percent natural habitat	1.24E-04	3.20E-04	1.50E+02	0.39	0.6990	
Proportion unestimated vectors	1500	Week	6.58E-02	1.45E-02	1.49E+02	4.55	**1.12E-05**	0.79
Proportion unestimated vectors	1500	Percent agriculture	1.19E-03	2.07E-03	1.02E+02	0.58	0.5700	
Proportion unestimated vectors	1500	Week:Percent agriculture	-3.66E-04	3.37E-04	1.51E+02	-1.09	0.2800	
Proportion low transmitting vectors	1500	Week	-6.11E-02	1.85E-02	1.53E+02	-3.30	**0.0012**	0.61
Proportion low transmitting vectors	1500	Percent natural habitat	-3.61E-03	2.20E-03	8.47E+01	-1.64	0.1053	
Proportion low transmitting vectors	1500	Week:Percent natural habitat	5.49E-04	3.52E-04	1.53E+02	1.56	0.1210	
Proportion low transmitting vectors	1500	Week	-7.01E-03	1.60E-02	1.53E+02	-0.44	0.6600	
Proportion low transmitting vectors	1500	Percent agriculture	4.12E-03	2.40E-03	8.08E+01	1.72	0.0899	
Proportion low transmitting vectors	1500	Week:Percent agriculture	-6.78E-04	3.72E-04	1.53E+02	-1.82	***0.0701***	
Proportion high transmitting vectors	500	Week	-7.63E-03	8.72E-03	1.54E+02	-0.88	0.3800	
Proportion high transmitting vectors	500	Percent natural habitat	5.21E-05	1.08E-03	8.75E+01	0.05	0.9600	
Proportion high transmitting vectors	500	Week:Percent natural habitat	-7.49E-05	1.69E-04	1.54E+02	-0.44	0.6600	

Proportion high transmitting vectors	500	Week	-2.03E-02	9.12E-03	1.54E+02	-2.23	**0.0273**	0.02
Proportion high transmitting vectors	500	Percent agriculture	-1.27E-03	1.16E-03	7.93E+01	-1.10	0.2760	
Proportion high transmitting vectors	500	Week:Percent agriculture	2.02E-04	1.79E-04	1.52E+02	1.13	0.2610	
WI								
Response Variable	**Explanatory Scale (m)**	**Predictor Variable**	**Estimate**	**Std. Error**	**z value**	**p value**	**R^2**	
Proportion unestimated vectors	1000	Week	9.83E-03	8.41E-03	1.17	0.2443		
Proportion unestimated vectors	1000	Percent natural habitat	-3.05E-03	1.71E-03	-1.78	0.0777		
Proportion unestimated vectors	1000	Year	2.22E-02	3.56E-02	0.62	0.5342		
Proportion unestimated vectors	1000	Week:Percent natural habitat	3.78E-05	2.89E-04	0.13	0.8961		
Proportion unestimated vectors	1000	Week	2.68E-02	1.75E-02	1.53	0.1284		
Proportion unestimated vectors	1000	Percent agriculture	3.98E-03	1.54E-03	2.59	**0.0106**	0.00476	
Proportion unestimated vectors	1000	Year	1.73E-02	3.55E-02	0.49	0.6279		
Proportion unestimated vectors	1000	Week:Percent agriculture	-2.58E-04	2.53E-04	-1.021	0.3093		
Proportion low transmitting vectors	1000	Week	1.79E-03	9.64E-03	0.186	0.8528		
Proportion low transmitting vectors	1000	Percent natural habitat	4.43E-03	1.97E-03	2.25	**0.026**	0.00165	
Proportion low transmitting vectors	1000	Year	-8.58E-02	4.08E-02	-2.1	**0.0375**		
Proportion low transmitting vectors	1000	Week:Percent natural habitat	-2.88E-04	3.31E-04	-0.87	0.3859		
Proportion low transmitting vectors	1000	Week	-3.19E-02	2.01E-02	-1.59	0.11476		
Proportion low transmitting vectors	1000	Percent agriculture	-5.05E-03	1.76E-03	-2.87	**0.00475**	5.60E-05	

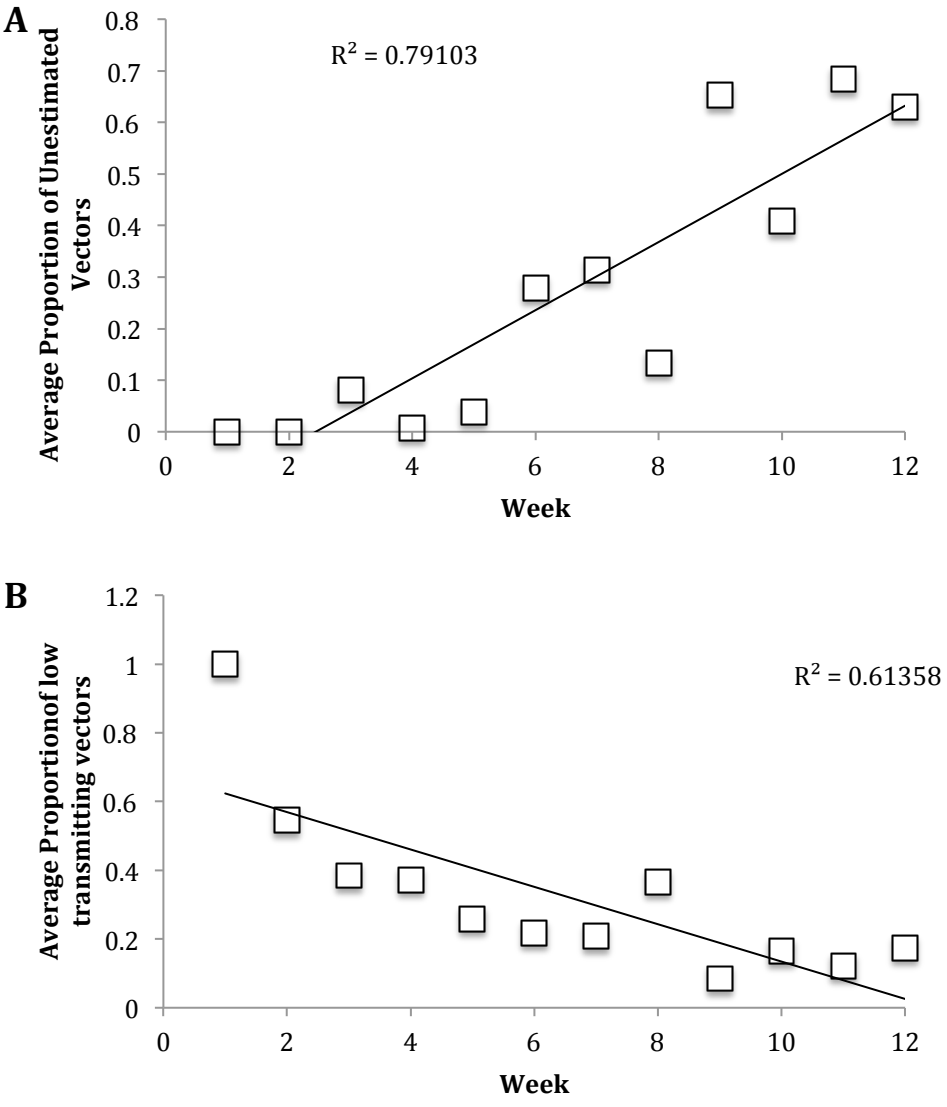
Proportion low transmitting vectors	1000	Year	-7.79E-02	4.06E-02	-1.92	0.05737	
Proportion low transmitting vectors	1000	Week:Percent agriculture	4.26E-04	2.89E-04	1.47	0.14319	
Proportion high transmitting vectors	1000	Week	-1.70E-03	3.43E-03	-0.495	0.6218	
Proportion high transmitting vectors	1000	Percent natural habitat	-3.85E-04	6.95E-04	-0.56	0.5737	
Proportion unestimated vectors	1000	Year	-3.65E-02	1.45E-02	-2.51	**0.0133**	
Proportion unestimated vectors	1000	Week:Percent natural habitat	6.58E-06	1.18E-04	0.056	0.9556	
Proportion unestimated vectors	1000	Week	-1.77E-03	7.20E-03	-0.245	0.8069	
Proportion unestimated vectors	1000	Percent agriculture	4.00E-04	6.30E-04	0.634	0.5269	
Proportion unestimated vectors	1000	Year	-3.79E-02	1.46E-02	-2.601	**0.0104**	
Proportion unestimated vectors	1000	Week:Percent agriculture	7.03E-07	1.04E-04	0.007	0.9946	


A
WI R² = 0.66784
NY R² = 0.52493
Average Number of Aphids/Trap
Week
0
0.5
1
1.5
2
2.5
3
0
2
4
6
8
10
12
14

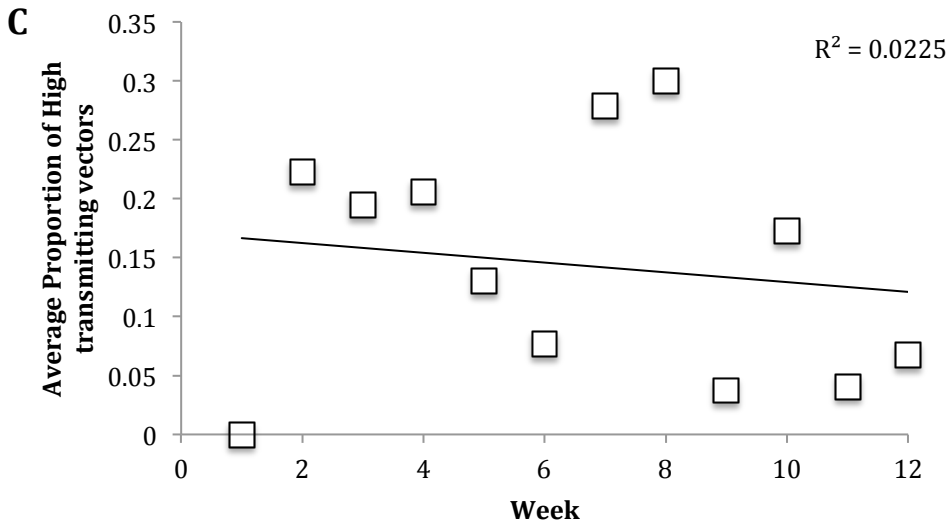

B
WI R² = 0.50997
NY R² = 0.46951
Average Raw Species Richness
Week
0
2
4
6
8
10
12
14
16
18
0
2
4
6
8
10
12
14



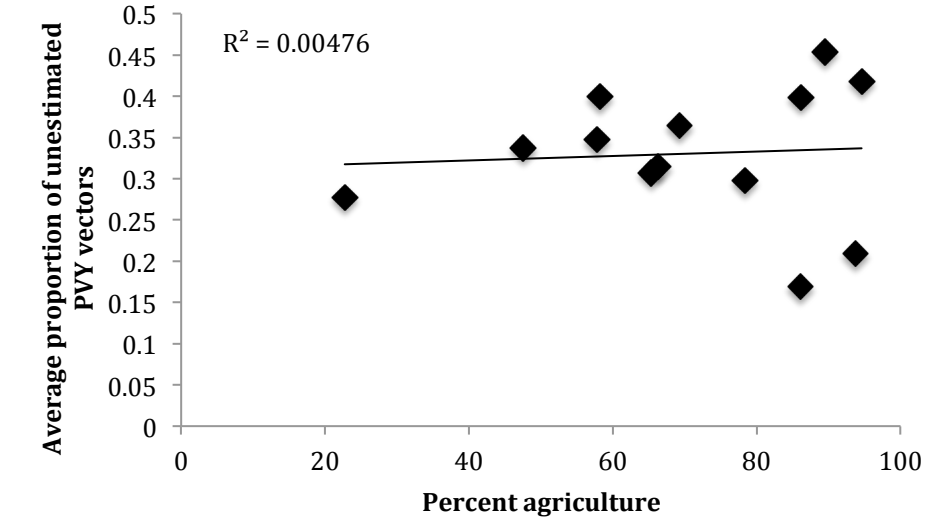
Supplemental Figure 1: The A) average total number of aphids/trap, B) average species richness, and C) average proportion of aphids collected in potato fields throughout the growing season in 2012 and 2013 in NY and 2010 and 2011 in WI.


A
Average Proportion of Unestimated Vectors
R² = 0.79103
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
Week

B
Average Proportionof low transmitting vectors
R² = 0.61358
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
Week



Supplemental Figure 2: The average proportion of aphids collected that were A) unestimated PVY vectors, B) low-transmitting (transmission efficiency <0.1) PVY vectors, and C) high-transmitting (transmission efficiency >0.1) PVY vectors in potato fields in NY throughout the growing season in 2012 and 2013.



Supplemental Figure 3: The average proportion of aphids collected that were unestimated PVY vectors in potato fields throughout the growing season in 2010 and 2011 in WI.

Chapter 3 Supplementary Material

Supplemental Table 1: The results of a generalized linear mixed effects model on the number of aphids occupying plants. Significant p values are in bold, and the * indicates the treatment held as the intercept for the model.

Movement Experiment				
Treatment	Estimate	Std. Error	z-value	p-value
ME+ME*	1.201	0.559	2.15	0.0315
ME+AP	-1.088	0.371	-2.93	**3.35E-03**
ME	-2.19	0.437	-5.01	**5.42E-07**
AP	-1.49	0.432	-3.44	**5.87E-04**
RP	-2.5	0.518	-4.82	**1.42E-05**
ME+RP	-1.68	0.392	-4.28	**1.86E-05**
THREE	-1.26	0.384	-3.28	**1.06E-03**
Replicate	0.161	0.325	0.495	0.621
Time	0.00428	0.00763	0.561	0.574
Time:ME+AP	-0.0184	0.0158	-1.17	0.243
Time:ME	-0.0113	0.0242	-0.464	0.642
Time:AP	-0.0256	0.0401	-0.638	0.524
Time:RP	-0.0305	0.0525	-0.581	0.561
Time:ME+RP	0.0272	0.0167	1.63	0.104
Time:THREE	-0.0464	0.022	-2.11	**0.0352**
Movement/Transmission Experiment				
Treatment	Estimate	Std. Error	z-value	p-value
ME+ME*	-1.34	0.981	-1.36	0.173
ME+AP	-0.706	0.695	-1.017	0.309
ME	-3.004	0.916	-3.279	**0.00104**
ME+RP	-0.95	0.711	-1.34	0.182
Time	0.0277	0.00772	3.59	**0.000336**
Block	0.494	0.548	0.902	0.367

Supplemental Table 2: The results of a generalized linear mixed effects model on the number of *M. euphorbiae* occupying plants. Significant p values are in bold, and the * indicates the treatment held as the intercept for the model.

Movement Experiment				
Treatment	Estimate	Std. Error	z-value	p-value
ME+ME*	0.63	0.00184	342.2	<2E-16
ME+AP	-1.67	0.00184	-905.7	**<2E-16**
ME	-2.16	0.00184	-1173.1	**<2E-16**
ME+RP	-2.1	0.00184	-1139.4	**<2E-16**
THREE	-1.98	0.00191	-1039.2	**<2E-16**
Replicate	0.543	0.0019	285	**<2E-16**
Time	0.00467	0.00192	2.4	**0.0175**
Time:ME+AP	0.00531	0.00182	2.9	**0.00357**
Time:ME	-0.011	0.00183	-6	**1.97E-09**
Time:ME+RP	0.0289	0.00202	14.3	**<2E-16**
Time:THREE	-0.02	0.0019	-10.5	**<2E-16**
Movement/Transmission Experiment				
Treatment	Estimate	Std. Error	z-value	p-value
ME+ME*	-0.951	0.985	-0.965	0.334
ME+AP	-0.914	0.695	-1.32	0.188
ME	-2.97	0.903	-3.28	**0.00102**
ME+RP	-1.72	0.751	-2.29	**0.0222**
Time	0.0321	0.00809	3.97	**7.29E-05**
Block	0.212	0.558	0.38	0.704

Supplemental Table 3: The results of a generalized linear mixed effects model on the proportion of aphids moving. Significant p values are in bold, and the * indicates the treatment held as the intercept for the model.

Movement Experiment				
Treatment	Estimate	Std. Error	z-value	p-value
ME+ME*	-2.29	0.73	-3.15	0.002
ME	-1.23	0.51	-2.41	**0.02**
ME+AP	-0.48	0.45	-1.06	0.29
ME+RP	-1.29	0.47	-2.76	**0.006**
THREE	-0.72	0.46	-1.55	0.12
AP	0.19	0.54	0.35	0.73
RP	-0.72	0.59	-1.21	0.23
Time	0.01	0.009	1.69	0.09
Replicate	0.4	0.43	0.94	0.35
Time:ME	-0.01	0.03	-0.48	0.63
Time:ME+AP	-0.01	0.02	-0.69	0.49
Time:ME+RP	0.03	0.02	1.6	0.11
Time:THREE	-0.01	0.02	-0.47	0.64
Time:AP	0.11	0.05	2.06	**0.04**
Time:RP	-0.02	0.05	-0.28	0.78
Movement/Transmission Experiment				
Treatment	Estimate	Std. Error	z-value	p-value
ME+ME*	-5	1.09	-4.6	4.30E-06
ME	-2.41	1.06	-2.26	**0.02**
ME+AP	-0.18	0.78	-0.23	0.82
ME+RP	-0.64	0.8	-0.79	0.43
Time	0.05	0.01	4.5	**6.66E-06**
Block	0.72	0.62	1.16	0.25

Supplemental Table 4: The results of a generalized linear mixed effects model on the proportion of *M. euphorbiae* moving. Significant p values are in bold, and the * indicates the treatment held as the intercept for the model.

Movement Experiment				
Treatment	Estimate	Std. Error	z-value	p-value
ME+ME*	-3.25	0.66	-4.92	8.86E-07
ME	-1.16	0.44	-2.63	**0.009**
ME+AP	-0.81	0.4	-2.01	**0.04**
ME+RP	-1.48	0.43	-3.45	**0.0006**
THREE	-1.07	0.43	-2.49	**0.01**
Time	0.01	0.009	1.7	0.09
Replicate	1.05	0.4	2.65	**0.008**
Time:ME	-0.01	0.03	-0.5	0.62
Time: ME+AP	0.003	0.02	0.18	0.86
Time:ME+RP	0.03	0.02	1.21	0.23
Time: THREE	0.002	0.03	0.08	0.94
Movement/Transmission Experiment				
Treatment	Estimate	Std. Error	z-value	p-value
ME+ME*	-4.52	1.11	-4.08	4.51E-05
ME	-2.37	1.05	-2.26	**0.02**
ME+AP	-0.29	0.78	-0.37	0.71
ME+RP	-1.23	0.85	-1.44	0.15
Time	0.05	0.01	4.47	**7.80E-06**
Block	0.38	0.64	0.59	0.55

Supplemental Table 5: The results of a generalized linear mixed effects model on the average transmission efficiency of the vector assemblage in the movement/transmission experiment. Significant p values are in bold, and the * indicates the treatment held as the intercept for the model.

Movement/Transmission Experiment				
Treatment	Estimate	Std. Error	z-value	p-value
ME+ME*	2.07E+00	3.50E-02	59.07	**<2E-16**
ME	2.66E-04	3.23E-02	0.01	0.99
ME+AP	-1.75E-01	3.14E-02	-5.58	**2.45E-08**
ME+RP	-2.97E-01	3.11E-02	-9.56	**<2E-16**
Time	-1.13E-05	1.22E-03	-0.01	0.99
Block	9.04E-03	1.94E-02	0.47	0.64
Time:ME	-7.78E-06	2.17E-03	0	0.997
Time:ME+AP	7.44E-03	2.07E-03	3.59	**0.000325**
Time:ME+RP	8.20E+00	2.12E-03	3.89	**0.000101**