Landscape composition is more important than local management for crop virus–insect vector interactions

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ABSTRACT

Managing crop viruses is difficult due to complex interactions among vectors, reservoirs, and mediating factors such as land cover. Identifying the appropriate ecological neighborhood, or the spatial area in which the most influential interactions occur affecting virus epidemiology, would therefore be beneficial in exposing which of the many explanatory variables to target in the plant-pathogen system. We constructed partial least squares path models to find the neighborhood size for vectors of stylet-borne nonpersistent viruses infecting pumpkins, and compare the relative influence of within-field and extra-field land cover. Two economically important aphid-vectored viruses in the U.S. Midwest are included in these analyses: papaya ringspot virus (PRSV) and watermelon mosaic virus (WMV). In 2010 and 2011, we used commercial pumpkin fields to quantify virus infection, vector (aphid) alightment (i.e., landing rates), and within-field weed communities, and subsequently analyzed extra-field cover from 1–5 km concentric buffer zones within the surrounding landscape. Alightment rates of total noncolonizing vectors and the top three numerically dominant species (*Aphis craccivora* Koch, *Therioaphis trifolii* (Monell), and *Rhopalosiphum padi* [L.]) were included in individual path models. Overall, we found that extra-field landscape composition had a far stronger influence on vector alightment than within-field weed cover; this pattern was consistent for seven of eight statistical models. In one exception, weed cover influenced alightment of the putative PRSV vector, *A. craccivora*, while surrounding landscape had no effect. In this case, weed coverage and vector alightment were inversely related, demonstrating that aphids were less likely to land in weed-infested fields. However, weed cover did not predict alightment of *T. trifolii* or total noncolonizers. The neighborhood size scales for total noncolonizers' tended to be larger than for individual species (4-5 km), suggesting future studies of dispersal by multi-species aphid groups may benefit from an extended gradient. On balance, our results indicate that while surrounding land cover interactions are complex, they exert greater influence over vector dispersal than within-field weed cover, calling into question whether management of local weeds is an effective method of crop virus prevention in some systems.

Keywords: ecological neighborhood, nonpersistent, land cover, papaya ringspot virus, watermelon mosaic virus, path models

INTRODUCTION

The abundance of agricultural pests and the damage they inflict on crops are shaped by some combination of management decisions made at the within-field scale (i.e., tillage regimes, irrigation, pesticide inputs) and extra-field factors (i.e., land use at a local or regional scale) that are typically beyond the control of individual farms. While this spatial delineation is implicitly recognized and many studies have tested the impacts of the two scales independently, few have integrated within-field and landscape-level factors into a single analysis (but see Kennedy *et al.*, 2013; Scheper *et al.*, 2015; Tamburini *et al.*, 2016). For example, while there is evidence that insect-vectored crop viruses are mediated by both within-field weeds (e.g., Duffus, 1971; Ali *et al.*, 2012; Smith *et al.*, 2012; Srinivasan *et al.*, 2013) and surrounding land cover (e.g., Fabre *et al.*, 2005; Margosian *et al.*, 2009; Carrière *et al.*, 2014), there have not been direct comparisons of the relative strength of these two scales. Uncovering differences between spatial scales is important for crop protection because it reveals which management approaches may be more or less effective in combating pests. In the case of crop pathosystems—the subsystems concerning crop-vector-pathogen interactions—if the impact of local weed management affects vector dispersal, then growers could alter herbicide regimes to help manage virus transmission. However, if land use beyond the farm boundary is the critical factor driving vector movement patterns, then changing within-field decisions are unlikely to affect virus prevalence. In this study, we explore landscape-level interactions in a nonpersistent crop virus system, and implement a direct comparison of within-field versus surrounding landscape scales on virus epidemiology.

Nonpersistent viruses are a large and agriculturally-important group. For example, cucumber mosaic virus (CMV) is an economically important pathogen of cucurbits, celery, lettuce, pepper, banana, legumes and ornamentals (Tomlinson, 1987; Palukaitis *et al.*, 1992; Flasiński *et al.*, 1995; Jones, 2004): afflicted crops frequently experience loss rates of 10-20%, at times reaching 100% (Zitter and Murphy, 2009). Like CMV, the vast majority of nonpersistent viruses are transmissible by aphids (Gray and Banerjee, 1999; USAID *et al.*, 2004). Nonpersistent virus epidemiology is challenging to study due to the combination of: a) rapid transmission time, b) the large number of aphid species capable of serving as vectors, and c) the broad host-plant range for many viruses, leading to numerous potential reservoirs in the landscape.

Because they are noncirculatory and only temporarily adhere to an aphid's stylet—until subsequent probing and salivation occurs—transmission occurs when susceptible plants are tested during exploratory probes (Wang *et al.*, 1996; Martin *et al.*, 1997). This induces rapid acquisition and inoculation: even a single probe of an aphid's stylet can transmit a virus. As a result, insecticides are either ineffective at preventing the spread of infection among plants or must be applied at rates which are not cost effective (e.g., Thackray *et al.*, 2000). Instead, preventative measures primarily consist of growing resistant cultivars, using costly UV-reflectant mulches or row covers, and/or removing sources of virus and vectors in the farmscape (Lecoq and Katis, 2014).

Aphid-vectored viruses are also often transmissible by a large suite of species, including noncolonizers (i.e., those that do not feed and reproduce on a plant). In fact, several studies have documented the relative importance of noncolonizing aphid species' transient alightment (i.e., landing) within fields over that of colonizing species in virus epidemiology (e.g., Raccach *et al.*, 1985; Summers *et al.*, 1990; Fereres *et al.*, 1992, 1993; Webb *et al.*, 1994; Perez *et al.*, 1995; Nebreda *et al.*, 2004). Having a broad range of vectors with disparate host-plant preferences allows many types of land cover to function as potential vector population sources, and can lead to interspecific variation in vector dispersal ranges relative to spatial vegetation patterns within and near farm sites that cultivate susceptible crops.

Additionally, numerous factors simultaneously act on vector species' dispersal behavior and the likelihood of pathogen acquisition/inoculation. Within-field or surrounding land cover can mediate nonpersistent virus epidemiology in several ways. One is by provisioning vector natal habitat. Nonpersistent viruses often have large host-plant ranges: for example, over 800 species of plants are vulnerable to CMV infection (Zitter *et al.*, 1996). Thus, both vector and virus reservoirs can lurk in the surrounding landscape or within fields, affecting the probability of virus infection within crop systems. Finally, the landscape can also mediate virus infection via aphid response to visual and structural variation present through windbreaks, and species-specific attraction to color and/or contrast (e.g., Moericke, 1955; Mayse and Price, 1978; Bottenberg and Irwin 1992).

Although the effects of within-field management on crop virus-vector interactions are fairly well-studied, landscape effects remained largely uninvestigated until very recently (Alexander *et al.*, 2014; Carrière *et al.*, 2014; Cunniffe *et al.*, 2015). This is surprising because

techniques from landscape ecology have increasingly been employed to model insect responses to agricultural land use, including herbivores, predators, parasitoids, and pollinators (Ricketts *et al.*, 2008; Chaplin-Kramer *et al.*, 2011; O'Rourke *et al.*, 2011; Sivakoff *et al.*, 2013). While numerous variables embedded in the landscape can influence nonpersistent plant virus epidemiology, the spatial scales within which these variables have the largest influence, or the ecological neighborhood (Addicott *et al.*, 1987), in vector-virus dynamics is unknown. The migratory ability of vector species could play a role, as an ecological neighborhood may be largely determined by the movement of mobile organisms (reviewed in Addicott *et al.*, 1987). However, there is a large range in recorded dispersal distances among aphids. At sufficiently low wind speeds aphids are capable of sustaining short intervals of directed flight for an estimated max. 200 m, losing directional control in winds above *ca.* 0.6 m s^{-1} (Haine, 1955; Loxdale *et al.*, 1993; Parry, 2013). In contrast, long-distance migratory flights occurring via passive wind dispersal commonly cover *ca.* 20–50 km, but are thought to be much less frequent among aphid populations than localized movement (Loxdale *et al.*, 1993).

To tackle the challenges of working within a nonpersistent pathosystem, which likely involves many vector-landscape interactions occurring at multiple spatial scales, we used partial least squares path models (PLS-PM). This is a 'soft-modeling' technique which takes a prediction-oriented approach to testing theoretical concepts. PLS-PM works by first creating an outer measurement model, which uses manifest variables (MVs or indicator variables), which are quantifiable observations, to generate a latent variable (LV), which can be a complex concept that is not directly observable. An inner structural model is then designed as a network of LVs with interconnections based on theoretical prediction, allowing one to test causal relationships among complex concepts. The path coefficients explain the residual variance of LVs, meaning that PLS-PM is better suited for predictive use rather than for statistically accurate estimates (Chin, 2010). It is especially useful for conducting exploratory analyses, building complex models, and utilizing data with small sample sizes, non-normal distributions, and multicollinearity (Tenenhaus *et al.*, 2005; Chin, 2010; Bagozzi and Yi, 2012).

We studied a nonpersistent pathosystems in pumpkins, and used PLS-PM to identify the ecological neighborhood for vectors and corresponding virus infection in focal fields, a major goal of which was to determine whether interactions occurring within or surrounding fields mediate virus spread. To address the former, we quantified land cover components within

concentric buffer zones surrounding focal sites (Brennan *et al.*, 2002), and identified the spatial scales that best predict vector behavior and virus infection (Jackson and Fahrig, 2012). PLS-PM allowed us to quantify land cover to create a LV embodying the greater concept of ‘landscape composition’ within each concentric buffer zone relative to focal fields, and compare the predictive ability of surrounding landscapes at different scales with that of within-field weed cover on vector alightment and virus infection within fields (Fig. 1).

METHODS

Aphid, Virus Quantification

Pumpkins host several aphid-vectored nonpersistent viruses (Zitter *et al.*, 1996), and potential vectors and viruses in this system were recently described (Angelella *et al.*, 2015). Aphid species alightment and virus infection were quantified within Indiana pumpkin fields under varied management regimes in 2010 (n=10) and 2011 (n=15) (see Angelella *et al.*, 2015 for further details). Field locations varied between years (Figs. A.1a,b). Aphid alightment (i.e., landing within pumpkin fields) was determined by identifying individuals landing in five pan traps per field for the duration of pumpkin growing season, which were collected on a weekly basis. Counts were averaged per unit time to account for sampling inconsistencies in 2010, and summed across the season for cumulative totals in 2011. In 2010, data collection at one field began two weeks after the others. To standardize 2010 aphid alightment data, totals were divided by the number of data collection weeks. All 2011 aphid alightment totals are analyzed using raw data. Individual pumpkin leaf samples were collected from each field (n=20) at the end of the growing season, and tested for virus infection using a multiplex-RT-PCR procedure. Although pumpkins host four aphid-vectored nonpersistent viruses— watermelon mosaic virus type 2 (WMV; *Potyviridae*), papaya ringspot virus type W (PRSV; *Potyviridae*), zucchini yellow mosaic virus (ZYMV; *Potyviridae*), and cucumber mosaic virus (CMV; *Bromoviridae*) (Zitter *et al.*, 1996)—only WMV and PRSV were detected in pumpkin leaf assays collected at the end of the growing season.

Cover Quantification

Weed communities were surveyed during both years. Specimens were surveyed twice in 2010 (late July and late August) by walking ten steps on a random trajectory at three separate

locations into each field, and collecting all weeds within a two meter radius. Survey methods conducted in 2011 were augmented to provide more comprehensive cover data: a survey of weed species occurred every three weeks from mid-June through the end of September within each field. Stopping at four occurrences each along four transects between randomly-selected rows at 20 m intervals, weeds within a 0.75 x 0.75 m quadrat were identified. Weeds were identified to species except those in the genera *Amaranthus*, *Prunus*, or *Trifolium*, and grasses were identified to species or genus when possible using reference guides (Newcomb, 1977; Uva *et al.*, 1997). Cover was quantified as number of stems species⁻¹ and total number of stems in 2010; weed cover m⁻² species⁻¹ and total weed cover m⁻² in each field were generated from 2011. Any aphids found on weeds during surveys were collected in 70% ethanol for subsequent identification; however, only a single specimen was spotted (*Aphis nerii* Boyer de Fonscolombe on eastern black nightshade, *Solanum ptycanthum* Dunn). Inventoried species susceptible to WMV include the following: *Amaranthus* spp., *Chenopodium album* L., *Datura stramonium* L., *Digitaria sanguinalis* (L.), *Ipomoea hederacea* Jacq., *Mollugo verticillata* L., *Phytolacca americana* L., *S. ptycanthum*, and *Trifolium* spp. (Angelella, unpublished results; Brunt *et al.*, 1996; Zitter, 2001). Only one PRSV-susceptible species was present in cover surveys: *C. album* (Brunt *et al.*, 1996).

To obtain surrounding landscape composition, we took GPS coordinates at each field site, and used GRASS GIS to measure land cover and land use within 5 km of fields (GRASS Development Team, 2015). We measured these land cover classes within nested 1-km annuli (i.e., 1–5 km). Landscape data were collected from the National Agricultural Statistics Service Cropland Data Layer, which combines satellite imagery with agricultural ground truthed data (NASS, 2011). Cover data were converted to proportion of the surrounding landscape at each spatial scale from pixels, with each pixel representing a 900 m² area, and one of ca. 80 land cover classes. Field site buffers did not overlap except in fields “T3” and “T20” in 2010, with no overlap occurring in 2011 (Figs. A.1a,b).

Variable Selection

Aphid variables included in analyses were 2010 and 2011 alightment totals of the cowpea aphid (*Aphis craccivora* Koch), the spotted alfalfa aphid (*Therioaphis trifolii* [Monell] f. *maculata*), the bird-cherry oat aphid (*Rhopalosiphum padi* [L.]), and total noncolonizers (all aphids, excluding *Aphis gossypii* Glover). These aphid species were previously identified as the

best predictors of variation in PRSV- or WMV-infected pumpkins among 2010 and 2011 fields, and were three of the most abundantly trapped in fields (Angelella et al., 2015). Although *A. gossypii* was also abundant in traps, the species was frequently observed colonizing and reproducing on pumpkins within fields; therefore, alightment totals from individuals flying into fields were confounded by the number of individuals originating within fields. Because the aim of this study is to explore effects of landscape features at different spatial scales on vector alightment within fields and subsequent virus infection, *A. gossypii* was not included. It should be noted that there are few studies examining vector competence of PRSV type-W, and although only four species have been confirmed (*Myzus persicae* [Sulzer], *Aulacorthum solani* [Kaltenbach], *Aphis craccivora*, and *Macrosiphum euphorbiae* [Thomas]; Karl and Schmelzer, 1971), it is likely that many more are capable. At least 29 species are documented vectors of WMV (Edwardson and Christie, 1986). For that reason, all aphid species are included in models regardless of documentation of vector competence.

Land cover types were combined when biologically relevant, and retained when they consisted of >10% of the total landscape (Table 1). Variables included land characterized by poaceous row crops (corn/wheat); fabaceous row crop (soybeans); pasture, grass, or hay fields; woody vegetation (forest/shrubland); and 20–100% constructed materials such as buildings or asphalt (urban). These variables were selected to encompass potential aphid natal host habitat and structural diversity for the observation of reservoir or behavioral mediation, in large enough proportions as to be distinct from environmental stochasticity. *Aphis craccivora* is a generalist of multiple plant-host families, but prefers Fabaceae; *T. trifolii* is a specialist on alfalfa; and *R. padi* prefers host-plants in the Poaceae family (Blackman 2015).

Prior to analyses, the proportions of pumpkin leaf samples infected with WMV or PRSV in each field were arcsine square root transformed. Land cover data were standardized within each spatial scale (1–5 km). Initial spatial autocorrelation tests using Moran's *I* coefficients on virus and aphid alightment raw data showed no evidence of spatial autocorrelation. However, it should be noted that tests of spatial autocorrelation are at increased risk of type II error when observation size is low (Legendre and Legendre, 2012). Analyses were conducted in R version 2.12.1 software (R Core Team, 2012).

Data Analyses

To compare vector alightment and PRSV or WMV infection variation within fields by landscape cover at each spatial scale and within-field weed cover, partial least squares path model (PLS-PM) analyses were conducted using the “semPLS” package (Monecke and Leisch, 2012). Path model graphics were generated with Graphviz – Graph Visualization Software (Gansner and North, 2000). The PLS-PM analyzes linear relationships between latent variables, in which each latent variable summarizes related groups of observed and quantified manifest variables (Sanchez, 2013). Latent variables are considered reflective or formative depending on their relationship to manifest variables. Reflective variables measure the same underlying concept, while formative variables do not necessarily and may be uncorrelated (Sanchez, 2013). In our models, the landscape at each spatial scale surrounding pumpkin fields (“land cover [1 km],” “land cover [2 km],” “land cover [3 km],” “land cover [4 km],” or “land cover [5 km]”) was considered a formative latent variable, with land cover variables “corn/wheat,” “soy,” “forest/shrubland,” “pasture/hay/grassland,” and “urban,” which were assigned weights dependent on relative contribution to the latent variable. PLS-PM estimates partial relationships among LVs using ordinary least squares (OLS) regressions (Monecke and Leisch, 2012), which is robust to multicollinearity, allowing it to limit bias caused by spatially autocorrelated variables (e.g., Puech *et al.*, 2015). The remaining LVs were measured with a single MV and considered reflective: the aphid alightment variables are comprised of alightment totals of *A. craccivora*, *T. trifolii*, *R. padi*, or all species excluding *A. gossypii* (“total noncolonizers”); both “PRSV” and “WMV” occurrence were measured by the proportion of PRSV- or WMV-infected pumpkin tissue samples per field, respectively; and LV “weed cover” was measured by either total stems (2010), or total cover $\text{m}^{-2} \text{ field}^{-1}$ (2011). Path coefficients are generated by OLS regressions, and all input variables are scaled prior to analysis. Because all of the LVs with multiple contributing measures are formative and there are no prior informative models, bootstrap resampling was used to validate the predictive abilities of the path models (Chin, 2010). Specifically, bootstrap resampling evaluated the significance of direct path coefficients and the weights of formative MVs (but see caveat below). Indirect path coefficients between cover variables and virus were calculated as the product of cover $\rightarrow$ aphid alightment and aphid alightment $\rightarrow$ virus coefficients (Sanchez, 2013). The predictive capability, or the variance a LV explains in a dependent variable, is the absolute value of the variable’s R^2 Pearson’s correlation coefficient multiplied by the path coefficient between the LV and the dependent variable (Falk and Miller, 1992). Thus,

the larger absolute value path coefficient to a dependent variable indicates a greater contribution by that LV to dependent variable variation.

We included all five land cover variables within each landscape buffer within the path model despite the likelihood of multicollinearity among measured values, because we felt each variable contributed a specific value potentially imparting biological significance. Removing variables from formative constructs, such as the concept of landscape within each buffer zone, can decrease model validity (Jarvis *et al.*, 2003). Moreover, although such multicollinearity can make interpretation of the importance of individual MVs in a path model difficult due to inaccurately low or nonsignificant path weights and unreliable estimate signs, it does not affect the accuracy of the inner/structural model (Chin 1998). Thus, the predictive capacity of the path model remains intact, allowing it to compare variance explained by the overall landscape at different spatial scales, with effects of specific land cover variables assessed in separate regression analyses. Path model reliability and validity were assessed according to Chin (2010). The extent to which an MV relates to its assigned LV relative to others is assessed with cross loadings, which indicated that measurements for WMV, PRSV, and vector alightment obtained loadings of 1.0 under their respective LVs and <0.7 under all other variables, suggesting they are reliable measurements. Formative measures (surrounding land cover) were assessed based on the predictive ability of the LV they contributed to.

Lastly, individual cover effects on vector alightment and PRSV or WMV infection were assessed with Poisson or linear regressions, respectively. These include assessing reservoir weed and crop species as virus predictors, and surrounding cover types within the best predicting land cover scales as alightment predictors. Although alfalfa was <10% of surrounding landscapes and thus combined with pasture, hay, and grassland in PLS-path models, it is a natal habitat for both *A. craccivora* and *T. trifolii* and the predictive ability of proportion of alfalfa on aphid alightment was independently assessed with regressions.

RESULTS

Cover as a Mediator of Vector Dispersal

Final PLS-path models have not been amended, but are shown including weed cover and the best-fit surrounding landscape scale for each year for brevity (Figs. 2, 3). Best fit was determined by relative coefficient size and bootstrap significance, with significance indicated by

the coefficient falling outside of the bootstrap-generated 5% and 95% confidence interval range. Full summaries of outer model (Table A.1) and inner model (Table A.2) coefficients and bootstrap values are available in the Appendix. All but 8 of 200 manifest variables fall within the range of bootstrap-predicted values, suggesting a fairly good overall predictive accuracy of the path models. Path models indicated land cover within and surrounding fields contributed moderately-to-substantially to vector alightment, with correlation coefficients ranging from 0.63 to 0.95 (Table A.2). Vector contributions to virus variance ranged from weak to moderate, with correlation coefficients ranging from 0.00018 to 0.34 (Table A.2).

Surrounding landscape composition explained aphid alightment variation to a greater extent than within-field weed cover in seven of eight path models. Surrounding land cover was a significant contributor to aphid alightment in seven of eight path models, and within-field cover in five of eight (Figs. 2,3). Four path models indicated both surrounding and within-field cover significantly predicated aphid alightment, but in these cases surrounding cover coefficients were uniformly greater than within-field. In one 2010 path model, increasing weed cover predicted decreases in *A. craccivora* alightment while variation in land cover had no effect on alightment rates (Fig. 2c). Weed cover significantly predicted decreasing alightment in 2010 *T. trifolii* and *R. padi* as well (Figs. 2b,d); however, surrounding landscape coefficients of effect on alightment were larger, indicating stronger explanatory ability. Likewise, although increased weed cover predicted an increase in *R. padi* alightment in 2011, the coefficient of effect is much smaller than that of surrounding landscape composition (Fig. 3d). The remaining four path models did not predict an influence of weed cover on alightment rates (Figs. 2a; 3a,b,c). The landscape neighborhoods that best predicted alightment varied across aphid types and years, and are as follows: 5 km and 4 km for total noncolonizers 2010 and 2011, 1 km and 3 km for *T. trifolii* 2010 and 2011, within-field and 3 km for *A. craccivora* 2010 and 2011, and 1 km and 4 km for *R. padi* 2010 and 2011, respectively.

Individual regressions suggest several surrounding land cover types predicted increases or decreases in aphid alightment. Corn/wheat and soy were positively associated with *R. padi* alightment in 2010 (Figs. A.2a,b). *Aphis craccivora* and total noncolonizer alightment were also both positively associated with corn/wheat in 2011 (Figs. A.3a, A.4a), and total noncolonizers were negatively associated with forest/shrubland and pasture/hay/grassland (Figs. A.4b,c). An increase in *T. trifolii* alightment was predicted by increased alfalfa cover as well (Fig. A.3b).

Cover as a Virus Reservoir

Results of linear regressions testing the response of virus infection to cover indicated variation in overall weed cover did not predict corresponding changes in the extent of PRSV or WMV infection within fields. Cover of specific weed and landscape virus reservoirs similarly had no effect on infections. Last, no interactive effects occurred between total aphid alates and virus reservoirs to predict either PRSV or WMV infection.

DISCUSSION

Extra-field landscape composition exerted a stronger influence on vector alightment in pumpkin than did within-field weed cover. Additionally, we found some evidence of possible vector natal habitat provisioning in the surrounding landscape, and cover mediation of vector behavior through visual and/or structural variation both within and surrounding fields. We did not, however, find evidence of either within- or extra-field land cover serving as virus reservoirs. The relative strength of extra-field landscape composition was indicated both by the PLS-path models and individual regressions between aphid species alightment and cover within or surrounding fields. With one exception, surrounding landscape composition explained alightment variation to a greater degree than weed cover in path models. Perhaps more importantly for virus management, when weed cover was a significant predictor of PRSV vector alightment, they were inversely related. This suggests the weed community was not functioning as a significant source of virus or vector, as would have been indicated by a predicted increase in PRSV with increasing weed cover. We should note that this study does not examine cover effects on colonizing vector species, which originate from within the cropscape and may respond differently to weed cover within fields. However, significant declines in melon aphid alightment rates were likewise predicted with increases in weed cover in both years (Angelella, unpublished results). Reinforcing this notion, within-field surveys of weedy virus-susceptible species provided no evidence for reservoir effects. Cover of virus-susceptible species within fields did not predict an increase in crop virus infection. However, the influential effect of surrounding landscapes was not explained by coverage of potential reservoirs, as it did not predict infection among fields either. There are limited data documenting PRSV—and to a lesser extent, WMV—

susceptibility among plant species and it is likely that additional reservoirs in within-field weed communities (or surrounding landscapes) have not yet been identified.

This lack of reservoir effect could be explained by either a very low infection frequency among reservoir species within and surrounding fields, or by limited interaction with vectors. In support of the former, an assay of weed samples collected from the study field sites detected WMV at a very low rate and did not detect PRSV at all (Angelella, unpublished results). One would expect higher rates of infection among susceptible perennials such as alfalfa in the surrounding landscape, leading to a “spillover effect” of high infection rates within nearby crops (e.g., Seabloom *et al.*, 2009; Borer *et al.*, 2010), but our results do not corroborate this. It is possible that our snapshot of cumulative virus and vector occurrence within fields lacks the resolution needed to pinpoint virus reservoir relationships. Contact with vectors among weed communities may have been limited if they did not support vector colonies. On the other hand, there is possible support for surrounding cover serving as vector natal habitats. For example, our data indicate local alfalfa as a possible source of *T. trifolii* in 2011. *Rhopalosiphum padi* alightment was also predicted by corn/wheat, which are potential hosts. However, some cover–vector relationships cannot be explained by the provisioning of natal habitat. Surprisingly, *A. craccivora*, a legume specialist known to colonize both soy and alfalfa, showed no relationship with either in independent regressions, but was positively associated with corn/wheat, neither of which are hosts; *R. padi* was also associated with the non-host, soy.

Relationships between vector alightment and surrounding land cover may also reflect their structural effects on the dispersal behavior of aphids in flight, such as color contrast or wind-breaks. The negative impact of forest/shrubland on total noncolonizing aphid counts may reflect a wind-break effect blocking alightment within fields, as greater concentrations of wind-dispersed herbivores are often found leeward of windbreaks (Mayse and Price, 1978). Further, ground cover appears to be one of the most important factors in large-scale plant architecture affecting rates of aphid alightment (Bottenberg and Irwin, 1992; Ogenga-Latigo *et al.*, 1992; Döring *et al.*, 2004; Hooks and Fereres, 2006; George *et al.*, 2012), and this preferential orientation toward high or low degrees of ground cover and contrast is likely species-specific (e.g., Favret and Voegtlin, 2001). The positive association of row crops (corn/wheat and/or soy) we observed on *A. craccivora* and *R. padi* alightment rates may reflect a preference for contrast serving to collect alates in proximity to pumpkin fields. *Rhopalosiphum padi* alightment was also

negatively associated with weed cover within fields in one year, further suggesting a preference for contrast. A previous study likewise found a greater number of *R. padi* and total aphid counts in the air above open-canopy crops relative to a closed-canopy prairie (Favret and Voegtlin 2001).

Ecological neighborhoods of vector activity, indicated by the best significant predictive power by land cover scale, varied by species and year but were overwhelmingly extra-field in scale. In most models the best-predicting spatial scales fell along the middle of the distance continuum, but total noncolonizers fell toward the far end at 4 and 5 km. This could be an indication that variables were quantified within an optimal distance gradient in most cases, but that the gradient might have been too small to capture the region containing landscape interactions with greatest influence on total noncolonizers in 2010 (Jackson and Fahrig 2015). This inconsistency may be explained in part by wide variation in dispersal capabilities among species, which is well-documented by flight mill studies (e.g., *Aphis fabae* [Scop.], Nottingham and Hardie, 1989; *Rhopalosiphum maidis* [Fitch], Bottenberg and Irwin, 1992; *R. padi*, Cheng *et al.*, 1997; *Sitobion avenae* [F.], Cheng *et al.*, 2002; *Aphis gossypii*, Liu *et al.*, 2003). Species may be considered migratory or non-migratory based on these abilities, with migratory aphids predictably engaging in longer flights (Kring, 1972). Pooling aphid alightment data across species may have an averaging effect as well.

Although certain aphid morphologies are primed for longer flights than others (e.g., David and Hardie, 1988; Nottingham *et al.*, 1991), our results support previous arguments that shorter flights characteristic of summer morphs are more likely biologically relevant than longer flights concerning population or genotype distributions and virus epidemiology (Loxdale and Hardie, 1993). Shorter flights occur with greater frequency than longer migratory flights, and many economically important crops are vulnerable to virus damage during the spring and summer after migratory flights occur. A preponderance of shorter flights by these summer morphs would thus be expected to pull median aphid dispersal distances closer to focal pumpkin fields, as we observed from 1–4 km ecological neighborhoods indicated for *R. padi*, *T. trifolii*, and *A. craccivora*. In contrast, migratory dispersal can occur at much greater distance scales: for example, *R. padi* regularly migrates from Denmark to Sweden, ca. 500 km (Wikteliuss, 1984).

The relative composition of land cover across scales may shape the direction of its interaction with aphids. For example, land cover at 4 km predicted a decrease in total

noncolonizer alightment in 2010, while that at 5 km in 2011 predicted an increase. Given that corn/wheat was a strong predictor of noncolonizer alightment, it could be that the greater degree to which corn/wheat contributed to overall land cover in the latter case explains this discrepancy. Additionally, the effect of weed cover on *R. padi* alightment changed from negative to positive across years. It may be that in addition to a preference for contrast, response to differential weed community composition generates varied *R. padi* alightment totals.

Conclusions

Although evidence suggests aphid dispersal is mediated by both within-field and extra-field land cover, the predictive ability of extra-field landscape composition was stronger than that of within-field weed cover in seven of eight cases. Furthermore, rather than suggesting weeds facilitated transmission by providing virus or vector reservoirs, our path models convey either a relatively minimal effect or an inverse relationship between weed cover and abundance of PRSV vectors. This suggests that growers cannot effectively control viral epidemics via the weed community in most cases, and if so they would need to tolerate higher weed densities, which could in turn make crops less apparent to vectors alighting in their fields. While neighborhood sizes ranged across years and species, they were overwhelmingly extra-field in scale. Ecological neighborhood sizes for *R. padi*, *T. trifolii*, and *A. craccivora* alightment fell within the middle range of the 0–5 km distance gradient; however, total noncolonizers' most influential ecological neighborhood fell at the far end of the distance gradient in one year, suggesting future studies of dispersal by multi-species aphid groups may benefit from an extended gradient. Lastly, while no evidence of virus reservoirs within weed communities or the surrounding landscape were found, studies with greater temporal resolution may increase the likelihood of detecting a reservoir-infection link. Overall, the weak and/or inverse influence of weed cover on vector alightment in this study implies weed management may not be an effective method of preventing nonpersistent virus infections in some pathosystems. Rather, management approaches should focus on optimal field placement relative to surrounding land use, which is likely to show greater success (albeit more complicated than changing local within-field inputs). Interestingly, anecdotal reports from growers in our region indicate that some pumpkin producers already rent fields in other counties (10+ km distance) because of persistent virus infection at certain sites. This lends further support

to the notion that particular “spots” in the landscape are inherently virus-prone and that selective field placement is a viable option for farmers to manage viruses.

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Table 1. Land cover variables included in analyses, and cover composition by National Agricultural Statistics Service (NASS) category.

Land Cover Variables	NASS Categories Included
Corn/Wheat	Corn, Sweet Corn, Popcorn, Winter Wheat
Soy	Soybeans
Pasture/Hay/Grassland	Alfalfa, Pasture Hay, Other Hay Non-Alfalfa, Sod Grass Seed, Grassland, Developed Open Space
Forest/Shrubland	Deciduous Forest, Evergreen Forest, Mixed Forest, Woody Wetlands, Shrubland
Urban	Developed Low Intensity, Developed Medium Intensity, Developed High Intensity

Figure 1. Conceptual diagram of path model latent variables (LVs, generated from measured observations), in which landscape composition within fields and surrounding fields within 1–5 km increasing buffer zones differentially impact crop virus vector alightment within fields, in turn affecting PRSV and WMV infection.

Figure 2. PLS-path models of 2010 aphid alightment within pumpkin fields (n=10) and predicted relationship with extra-field landscape (best-fit scale shown), within-field weed cover, and virus infection in crops: a) total noncolonizers, b) *Therioaphis trifolii*, c) *Aphis craccivora*, d) *Rhopalosiphum padi*. Bolded arrow and * indicate bootstrap significance ($\alpha=0.05$).

Figure 3. PLS-path models of 2011 aphid alightment within pumpkin fields (n=15) and predicted relationship with extra-field landscape (best-fit scale shown), within-field weed cover, and virus infection in crops: a) total noncolonizers, b) *T. trifolii*, c) *A. craccivora*, d) *R. padi*. Bolded arrow and * indicate bootstrap significance ($\alpha=0.05$).

Figure 1

Figure 2a

Figure 2b

Figure 2c

Figure 2d

Figure 3a

Figure 3b

Figure 3c

Figure 3d