**Vector manipulation by plant viruses vs. host-race specialization of insect vectors: is the preference-performance correlation of pea aphid host races altered by plant viruses?**

**Abstract**

Many plant pathogens manipulate host preference and performance of their vectors in ways that can improve their transmission. Research on these phenomena has been confined to examining intraspecific transmission of the pathogens, but most pathogens and their vectors utilize more than one plant host. For example, *Pea enation mosaic virus* (PEMV) and *Bean leafroll virus* (BLRV) modify the preference, performance, or both, of their principal vector, the pea aphid, *Acyrthosiphon pisum* on its host *Pisum sativum,* but the viruses and aphids have multiple host species. Here, we evaluate hostplant preference and performance of five pea aphid biotypes on five hostplant species after being exposed to PEMV or BLRV, or sham-inoculated. Aphid performance, measured as the rate of increase on potted plants, differed among hostplant species depending on their biotype, and their relative performance among these hostplants was altered by virus infection. Preference, measured using a free-choice bioassay in which aphids were released in an arena where they could settle on any of the five plant species, also differed among aphid biotypes but was unaffected by virus infection status. These results show that host preference and performance is deeply imprinted in aphid biotypes, and that such adaptations may become hardly reversible even in presence manipulating plant pathogens.

Keywords: insect biotypes, inter-specific transmission, Bean leafroll virus, pea aphid, Pea enation mosaic virus, virus reservoir.

# Introduction

Almost half of the emerging plant infectious diseases worldwide are caused by viruses (Anderson et al., 2004) and their success as pathogens is increasingly threatening global food security (Nicaise, 2014). Most plant viruses depend upon vectors for their transmission, typically generalist insect herbivores with pierce-sucking mouthparts (Gray and Banerjee, 1999). Evidence is accumulating that many of these plant viruses, and other vector-borne plant pathogens, alter hostplant traits or directly affect vector behavior in a manner that enhances the movement of virions from infected to healthy hostplants (Eigenbrode et al., 2018).

The “vector manipulation” hypothesis states that plant viruses alter vector-hostplant interactions as a function of transmission mode in predictable ways (Heil, 2016, Ingwell et al., 2012). Persistently (PT) or semi-persistently (SPT) transmitted viruses require relatively long vector feeding periods for an effective uptake, but they can either bind to the infectious insect foregut or circulate within its hemocoel, often making vectors competent for transmission throughout several days or their remaining lifetime. Plants infected with PT or SPT viruses often express disease symptoms associated with a “honest” syndrome that attract, arrest, and increase performance and survival of vectors to ensure effective acquisition and transmission. Non-persistently transmitted (NPT) viruses are acquired by vectors faster (long feeding periods are not required), but virions remain only in the insect mouthparts and transmission competence is lost within hours. Infections of NPT viruses express symptoms that trigger a “deceptive” syndrome which induce attraction of vectors, but neither arrestment nor performance is granted, so as to favor quick insect movement from infected to healthy hostplants after acquisition. There are three additional syndromes which involve attraction to infected hostplants depending on the infective/non-infective status of vectors (“conditional”), differential attraction of vectors across the disease progression (“dynamic”), and the induction of changes in vector feeding behavior after virus acquisition (“consumption related”).

Most of the theory and empirical work on vector manipulation has been built upon tripartite host-virus-vector pathosystems rarely considering multiple hostplants (Eigenbrode et al., 2018). However, the persistence of pathogens in host populations that exhibit temporal or spatial structure depends on pathogen’s ability to infect more than one hostplant (Ashby et al., 2014, McLeish et al., 2018). Moreover, a diverse vector diet breadth can favor virus prevalence in transient host populations by either increasing interspecific transmission rates between hosts and reservoirs (Duffus, 1971), or ensuring the maintenance of high vector populations over time even when virus hosts are scarce (Swei et al., 2011). Most plant viruses have multiple hosts, although many seem to be constrained to one or a few vector species, especially those that are PT and SPT (Power and Flecker, 2003), as are most vectors, which are generalist or oligophagous herbivores that can feed and survive on several plant species (Nault, 1997, Gilbertson et al., 2015, Gadhave et al., 2020). However, the “host-race specialization” hypothesis states that local adaptations to plant communities imply trade-off costs for herbivores that result in host breadth restrictions, host specialization, reproductive and genetic isolation and eventually sympatric speciation (Drès and Mallet, 2002, Loxdale et al., 2011). A similar pattern has been described for plant viruses, which experience host-specific selection pressures that often lead to antagonistic pleiotropies that limit their ability to overcome host defenses across plant taxa (García-Arenal and Fraile, 2013). On the one hand, both plant pathogens and insect vectors experience a selection pressure towards host specialization, but specialization could become maladaptive when host populations are transient, and especially challenging for pathogens whose transmission depends on one or a few insect vectors.

The pea aphid, *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae), is an oligophagous herbivore that feeds on leaves, buds, and pods of legumes (Fabaceae), whose host range may cover hundreds of plant species, and is a limiting pest and vector of viruses of several crops worldwide, including pea, lentil, and alfalfa (Eastop, 1971, Peccoud and Simon, 2010). The pea aphid is considered a model organism for several study fields, including the evolution of distinct biotypes in response to local plant communities (Brisson and Stern, 2006, Eigenbrode et al., 2016, Via, 1991, Peccoud and Simon, 2010). Distinct biotypes of pea aphid associated with local specializations to host plants have been reported in the United States and Europe, several of which include evidence of reproductive isolation, via pre- and post-zygotic barrier (Via et al., 2000, Via, 1999, Peccoud et al., 2008, Eigenbrode et al., 2016). The pea aphid is a vector for more than 30 plant viruses, including the *Pea enation mosaic virus* (PEMV) and the *Bean leafroll virus* (BLRV), two of the main causes of yield losses in peas, broad beans and lentils in the northern hemisphere (Sandhi and Reddy, 2020, Skaf et al., 1999, Makkouk et al., 2012). Both PEMV and BLRV are PT viruses that can infect numerous crop and non-crop legumes, and utilize perennial pea aphid hostplants as reservoirs when host crops are not present (Rashed et al., 2018). PEMV functions as a mutualistic association of two unrelated taxonomically, autonomously replicating viral RNAs (families Enamovirus and Umbravirus) (Demler et al., 1993, Hull and Lane, 1973), while BLRV (recently reassigned to family Tombusviridae) (Walker et al., 2021) consists of a single non-segmented RNA strand (Miller, 1999). Previous work has shown that both PEMV and BLRV infections manipulate pea aphids through a “honest” syndrome that includes both increased attraction and settling and increased/neutral performance and survival of vectors in virus-infected plants, relative to uninfected pea plants (Wu et al., 2014, Hodge and Powell, 2010, Davis et al., 2017).

In the present study, we aim to disentangle the convergence of the “vector manipulation” and the “host-race specialization” hypotheses. If viruses require interspecific transmission for long-term persistence and vector biotypes (i.e., host races) prefer and perform better on different hostplants, the “vector manipulation” hypothesis predicts that vector host preference and performance should be altered in presence of infected hostplants in a manner that enhances interspecific transmission among virus hosts. In contrast, the “host-race specialization” hypothesis predicts that specialization, as an irreversible process that implies pleiotropies associated with physiological and behavioral adaptations, will constantly reinforce preference and performance towards a single o a few hosts (Drès and Mallet, 2002, Loxdale et al., 2011). In particular, while host preference and performance seems to be deeply imprinted in pea aphid biotypes (Via, 1991, Eigenbrode et al., 2016), the presence of pathogens that rely on interspecific transmission to persist in transient host populations might cause an evolutionary conflict with significant consequences for the evolution of host-virus-vector pathosystems. In the Pacific Northwest, USA (PNW), several pea aphid host races coexist, most of them associated with either alfalfa or pea crops (Eigenbrode et al., 2016). Some perennial or winter-hardy legumes, such as hairy vetch (*Vicia villosa*), red clover (*Trifolium pratense*), and alfalfa (*Medicago sativa*), are known to both sustain early pea aphid immigrant infestations during early spring and serve as reservoirs of PEMV or BLRV (Clark et al., 2023, Rashed et al., 2018). Here we test the hypothesis that performance and preference of pea aphid biotypes across a panel of potential host plants is altered when these plants are infected with PEMV or BLRV. We evaluate the effects of virus infection on the preference performance relationships among these hosts and aphid biotypes. We interpret the results in the context of horizontal transmission of the viruses among these hosts.

# Materials and methods

## Aphid and Virus Colonies and Treatments

Five clonal colonies of pea aphid, *Acyrthosiphon pisum*, were established from individual apterous reproductive aphids field-collected from pea, vetch, clover, or alfalfa near Moscow ID (46.7325° N, 116.9992° W, 786 m.a.s.l.) or near McMinnville OR (45.2108° N, 123.1945° W, 40 m.a.s.l.) in 2017. The aphid genotypes were as follows: Two collected from alfalfa and exhibiting better performance on alfalfa, AL-PK-ID and AL-GN-OR, hereafter referred to as “alfalfa” biotypes, two collected from pea, PA-GN-ID and PA-GN-OR and one collected from clover, CL-PK-OR and exhibiting better performance on pea, hereafter referred to as “pea” biotypes. Clones with designation PK are pink and those with designation GN are green; clones with designation OR were collected in Oregon those with designation ID were collected in Idaho. These colonies were maintained in a greenhouse at the Manis Entomological Laboratory at the University of Idaho in individual 60- by 60- by 60-cm mesh tents (BugDorm 2120F; BioQuip, Rancho Dominguez, CA) under the following environmental conditions: 20 ± 2°C, photoperiod of 18:6 (L:D) h, and 50% RH. Colonies were reared on potted fava bean plants (*Vicia faba* L.), a universal host for pea aphid (Peccoud et al., 2014) for at least 20 generations prior to use in experiments. It was confirmed that colonies were genetically separate using 12 autosomal microsatellite loci [described in Eigenbrode et al. (2016)].

Plant species used in the study were pea (*Pisum sativum* L.), red clover (*Trifolilium pratense* L.), common vetch (*Vicia sativa* L.), alfalfa (*Medicago sativa* L.), lentil (*Lens culinaris* Medik.), and faba bean (*Vicia faba*). All were grown from commercial seed in a greenhouse with conditions identical to those for maintaining insect colonies. As required for experiments, plants were infected with either *Pea enation mosaic virus* (PEMV) or *Bean leaf roll virus* (BLRV). Infectious colonies of pea aphid on *V. faba* infected with either virus were maintained for this purpose. The virus isolates were collected from commercial fields of alfalfa (BLRV) or pea (PEMV) near Moscow ID. Plants for bioassays were grown in 15-cm diameter pots filled with commercial soil mix (Sunshine Mix #1; SunGro Horticulture, Bellevue, WA, USA). Fifteen days after emergence they were inoculated by placing five aphids from an infectious colony of either virus in a clip cage (5 cm. diam) onto a leaf from the top node of the test plant. Following a 3-day inoculation access period, aphids were carefully removed from plants using a soft bristled paintbrush and the plants were maintained aphid-free until they were used in experiments. To control for the effects of aphid feeding on the test plants, noninfected controls were treated as above using aphids from a virus free colony (‘sham’ inoculation).

## Host settling bioassay

Host settling was evaluated using a multiple-choice test. The bioassay was conducted using a circular arena approximately 30 cm in diameter with a flat foam core floor and enclosed with a clear plastic wall coated with Fluon (PTFE-30) to prevent aphids escaping. Notches cut into this wall and the floor, evenly spaced around the perimeter, held the stems of the test plants. Plants were germinated at different times to control for the different growth rates of the test species so that all were approximately the same size at the time of the test. For each trial, 50 apterous aphids were released into the center of the arena. After 24 hours, the number of aphids on each test plant was counted and converted to a proportion settling based on the number of aphids living at the end of the 24 hours (mortality was 1.6% of aphids tested across all trials). There were 5 replicate trials for each aphid biotype × virus treatment (PEMV, BLRV, sham). The experiment was conducted in two temporal sets.

## Aphid performance bioassay

Aphid performance was evaluated using a no-choice bioassay. Individual plants infected with PEMV, BLRV, or sham inoculated as described above were infested with 8 individual mature pea aphids from each biotype. The total number of aphids present (minus the 8 initially infesting the plant) was counted after 10 days and converted to a growth rate (aphids/aphid/day). There were 4 replications for each combination of aphid biotype × plant species × virus infection status (PEMV, BLRV, sham).

## Statistical analyses

All analyses were completed in R version 4.2.2 (R Development Core Team, 2022). Three models were used to evaluate the outcomes of the experiment. First, we modeled the effects of aphid biotype, plant species, and virus status on the total number of aphids counted on a host plant after one week (“performance”). All models fitted to these count data followed a negative binomial distribution and were run using the glm.nb function in the MASS package in R (Venables and Ripley, 2002). Second, we modeled the effects of aphid biotype, plant species, and viral status on the number of aphids that moved towards a host plant (“preference). Third, we completed a linear model examining the relationship between preference and performance with an interaction term of virus status. In this third model, the predictor variable aphid counts were log-transformed to meet linear regression assumptions. Both the second and third models were normally distributed and used the glm function in base R.

For the first two models, we employed a stepwise model selection approach. We started with a fully specified generalized linear model (GLM) including the third-order interaction term between aphid biotype, plant species, and virus status, as well as all underlying second and first-order terms. Given the relatively small size of the dataset to this large model, we sought to reduce model complexity and avoid overfitting via stepwise regression. After running these fully specified models, the stepAIC function in the MASS package was applied to each model (Venables and Ripley, 2002). When applied to a GLM, this function sequentially reduces the number of parameters to produce a model with the lowest AIC. Such an approach allowed for consistent, unbiased strategy to avoid overfitting across both types of aphid count data. Both full and reduced models significance tests (P-values and critical values) were completed using the car package (Fox and Weisberg, 2018), while estimated marginal means and post-hoc tests *via* Tukey HSD were calculated using the emmeans package (Lenth, 2023).

# Results

## Biotype, Plant, and Virus effects on aphid performance

Aphid counts on each host plant after one week were significantly different among host-plant species following patterns expected from prior research on pea aphid performance. The effect of host-plant species was highly significant (Table S1, P < 0.001, 𝛘2 = 23.75, DF = 5), with aphid performance being relatively lower on red clover and relatively higher on hosts like faba bean and lentil (Fig 1). Virus status of host plant also significantly impacted aphid performance, with non-additive affects based on host-plant species (Tables S1, Plant:Virus interaction, P < 0.001, 𝛘2 = 32.16, DF = 9). For example, aphids performed better on alfalfa plants exposed to BLRV compared to a sham (Fig. 2, Tukey HSD). Conversely, aphids performed more poorly on hairy vetch exposed to BLRV compared to PEMV and sham plants (Fig. 2, Tukey HSD). The remaining host-plant species did not have significant differences in aphid performances across virus exposure status (Fig. 2, Tukey HSD).

Aphid performance differed depending on host-plant species identity (Table S1, Biotype:Plant interaction, P < 0.001, 𝛘2 = 57.31, DF = 5). As predicted, the “alfalfa” biotypes performed better on alfalfa compared to pea (Fig 1, Tukey HSD). Conversely, the “pea” biotypes performed better on hairy vetch and pea compared to the “alfalfa” biotypes (Fig 1, Tukey HSD). We observed no differences in aphid performance among the remaining host plants, red clover, fava bean, and lentil (Fig 1. Tukey HSD). Given that analysis of deviance tables were calculated using Type II Wald-𝛘2 tests (Fox and Weisberg, 2018), results suggest the Biotype:Plant interaction term statistically accounts for the effect of biotype on aphid performance (Table S1, P = 0.5, 𝛘2 = 0.45, DF = 1).

## Biotype, Plant, and Virus effects on aphid preference

Aphid preference for host plants was determined using total counts of aphids settling on a respective host plant in a behavioral assay. Aphid preference differed significantly among target hosts (Table S2, P < 0.001, 𝛘2 = 111.77, DF = 5), and this preference varied according to aphid biotype grouping (Tables S2, P < 0.001, 𝛘2 = 51.78, DF = 5). Matching results from aphid performance, the "alfalfa” biotypes were more attracted to alfalfa hosts compared to the “pea” biotypes (Fig 3, Tukey HSD). Likewise, the "pea” biotypes preferred hairy vetch and red clover (Fig 3, Tukey HSD). Paradoxically, we did not see a preference for pea plants by the “pea” biotypes (Fig 3, Tukey HSD).

All terms including virus exposure status were dropped from final model using our stepwise AIC approach. Consequently, there was no statistical evidence for the virus exposure status of these target host plants altering aphid preference (Table S2), nor did we observe any evidence that virus status influenced the effects of aphid biotype group or plant species identity (Table S2).

## Preference by performance relationship

We examined the relationship between the number of aphids settling on host plants (metric of preference) and the total number of aphids found on infested host plants after one week (metric of performance). We observed a positive relationship between aphid preference and aphid performance (Table S3, P < 0.001, 𝛘2 = 21.71, DF =1). As such, aphid movement to host plants for a given trial was higher under similar conditions by which aphid performance was higher (Fig 4). However, we did not observe any evidence that this relationship was modified for plants exposed to BLRV or PEMV (Table S3, Log Aphid Count:Virus interaction, P = 0.57, 𝛘2 = 1.13, DF =2). Across all infection statuses, aphid preference increased in trials where aphid performance was higher (Fig 4).

# Discussion

Host specialization and manipulation by plant viruses affect simultaneously pea aphid-plant interactions in the PNW (Eigenbrode et al., 2016, Davis et al., 2017, Wu et al., 2014). On the one hand, host specialization implies performance trade-offs that often match hostplant preference and reduce the diversity of plants that insect herbivores can effectively exploit (Berlocher and Feder, 2002). But vector-borne plant viruses that rely on transmission among alternate hosts, especially when hosts are annual or transient (Elena et al., 2009, Wilke et al., 2006) may manipulate vectors to facilitate this horizontal transmission. These results show that this apparent evolutionary conflict seems to be reduced towards host specialization (in detriment of vector manipulation) presumably due to the difficulty of altering the complex network of genetic correlations between pea aphid’s host performance and preference and reproductive isolation (Via et al., 2000, Hawthorne and Via, 2001, Queller and Strassmann, 2018).

We found that pea aphid performance largely matched host preference and varied as expected as a function of hostplant, with some exceptions. Pea aphid biotypes grouped as “pea” performed better on pea and hairy vetch plants, which matches the results of previous work in the Palouse region (PNW) (Clark et al., 2023). However, their increased performance in pea plants did not match their preference for this plant, but it did for hairy vetch. This result supports the hypothesis of “fundatrix specialization”, which states that evolution of aphids is constrained by the selective pressures exerted on the morph that hatches after the winter. These morphs are heavily adapted to prefer and perform better in hostplants that are available in early spring (Moran, 1988, Moran, 1994). Hairy vetch emerges early, is widely dispersed across the Palouse region, and has been proposed as a “stopover” host for aphid alates before they build up dense populations that will eventually infest pea crops (Clark et al., 2023). Thus, we suggest that pea plants are alternate hosts for “pea” pea aphids, which are adapted to prefer and perform best in hairy vetch, the plant that fundatrix individuals encounter when they hatch after the winter. Subsequent morphs may have to switch to pea plants as hairy vetch populations senesce and become scarce. Pea aphid biotypes grouped as “alfalfa” do not need a “stopover” host because alfalfa is a winter-hardy plant, which grows in both crop and non-crop areas and is fairly available through the growing season.

Another exemption to the performance-preference correlation rule was “alfalfa” pea aphids, which preferred pea plants to a similar extent as “pea” pea aphids, despite their performance being significantly lower in these plants. We also found that pea plants were not as poor hosts for “alfalfa” pea aphid biotypes as alfalfa plants were to “pea” pea aphids, which matches Eigenbrode et al. (2016) observation that “pea” and “alfalfa” clones may reduce their performance by 93% and 17-52% when feeding on alfalfa or pea plants, respectively. Thus, it looks like pea aphid “alfalfa” biotypes are better adapted to use pea plants as hosts, than “pea” biotypes are to shift to alfalfa. We can only speculate that the preference for and ability to exploit host pea plants by “alfalfa” biotypes is a plesiomorphy shared among pea aphid biotypes, and that the host shift to alfalfa is a more recent apomorphy only evolved in the “alfalfa” biotypes. To our knowledge, there are no studies investigating the phylogenetics of pea aphid biotypes in the PNW, but Moran (1988) notes that related species of aphids tend to share hosts used by sexual generations and their immediate descendants (i.e., primary hosts), and quite different secondary hosts which have been recently acquired.

One of the most important findings of this study is that vector manipulation by plant viruses appears to be restricted to maximizing intraspecific transmission, and to have little influence on interspecific transmission and patterns of vector’s hostplant specialization. These results differ from those described by Lee et al. (2022) with BCTV-carrying beet leafhoppers [*Circulifer tenellus* (Baker)], which seem to increase their probing behavior on different hostplant species, compared to their non-viruliferous conspecifics, to apparently maximize interspecific transmission. Similarly, Shoemaker et al. (2019) found that BYDV-PAV-carrying bird cherry-oat aphids (*Rhopalosiphum padi* L.) exhibited a stronger preference for perennial grasses, compared to non-viruliferous aphids, which showed no preference for any of the offered hostplants. Phytophagous insects use specific ratios of relatively common plant volatiles to recognize and locate suitable hostplants (REF). Furthermore, the complexity associated with the ability to detect a wide range of such compounds provides the required plasticity to adapt to a greater range of potential hostplants (Bruce et al., 2005). Soluble proteins, known as odorant binding proteins (OBP), have been identified as key carriers of odorants through the insect body and responsible of olfactory recognition of and behavioral responses to semiochemicals in insects (Pelosi et al., 2006). Pea aphids are equipped with efficient mechanisms to recognize and respond to a wide range of plant volatiles, which includes at least 18 OBPs, most of which presumably related with hostplant recognition and location (Shih et al., 2023, Robertson et al., 2019). Moreover, there is evidence that indicates that OBPs are differentially expressed as a function of hostplant-biotype interaction in pea aphids, and that such profiles are hardwired and heritable (Eyres et al., 2016). In contrast, both leafhoppers and bird cherry-oat aphids seem to exhibit much simpler mechanisms of hostplant location and recognition. To date, there are no specific characterizations of the beet leafhopper OBPs, but related species may exhibit between three and 16 OBPs (He et al., 2019, He et al., 2011, He and He, 2014) [one exception with 40 OBPs (Bian et al., 2018)], of which only a few seem to be involved in hostplant recognition and location (Hu et al., 2019, He et al., 2018). Bird cherry-oat aphids also have a simpler OBP complex, compared to that exhibited by pea aphids, although the function of most of them remain to be characterized (Kang et al., 2018, Wang et al., 2019). Furthermore, neither beet leafhoppers nor cherry-oat aphids appear to be under a host-driven speciation process such as that widely documented for pea aphids (Via et al., 2000, Via, 1999, Peccoud et al., 2008, Eigenbrode et al., 2016). Although some genetic differences have been reported for different populations of both beet leafhoppers (Young and Frazier, 1954) and bird cherry-oat aphids (Simon and Hebert, 1995), they either seem to be caused by allopatry rather than by sympatric host specialization (Hudson et al., 2010, Morales-Hojas et al., 2020). We hypothesize that the complexity behind the hostplant recognition and location by pea aphids and the reproductive isolation derived from hostplant specialization prevents parasites from triggering “consumption related” syndromes that alter the preference and performance of viruliferous pea aphids for different hostplant species.

Our findings have some implications for management. Shoemaker et al. (2019) shows that host-preference shifts through vector manipulation may slow viral epidemics within crops, via dispersal of viruliferous vectors to non-crop plants. These results show that control of BLRV and PEMV should be focused on timely sprays to reduce aphid vectors within pea crops, and that interspecific transmission between pea plants and weed reservoirs, such as red clover of hairy vetch, seem to occur in very specific times (not continuously), and guided solely by the availability of hostplants for pea aphids throughout the growing season.

**Acknowledgments**

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