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Research

The Effects of *Bean Leafroll Virus* on Life History Traits and Host Selection Behavior of Specialized Pea Aphid (*Acyrthosiphon pisum*, Hemiptera: Aphididae) Genotypes

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Abstract

Intraspecific specialization by insect herbivores on different host plant species contributes to the formation of genetically distinct "host races," but the effects of plant virus infection on interactions between specialized herbivores and their host plants have barely been investigated. Using three genetically and phenotypically divergent pea aphid clones (*Acyrthosiphon pisum* L.) adapted to either pea (*Pisum sativum* L.) or alfalfa (*Medicago sativa* L.), we tested how infection of these hosts by an insect-borne phytovirus (*Bean leafroll virus*; BLRV) affects aphid performance and preference. Four important findings emerged: 1) mean aphid survival rate and intrinsic rate of population growth (R_m) were increased by 15% and 14%, respectively, for aphids feeding on plants infected with BLRV; 2) 34% of variance in survival rate was attributable to clone × host plant interactions; 3) a three-way aphid clone × host plant species × virus treatment significantly affected intrinsic rates of population growth; and 4) each clone exhibited a preference for either pea or alfalfa when choosing between noninfected host plants, but for two of the three clones tested these preferences were modestly reduced when selecting among virus-infected host plants. Our studies show that colonizing BLRV-infected hosts increased *A. pisum* survival and rates of population growth, confirming that the virus benefits *A. pisum*. BLRV transmission affected aphid discrimination of host plant species in a genotype-specific fashion, and we detected three unique "virus-association phenotypes," with potential consequences for patterns of host plant use by aphid populations and crop virus epidemiology.

Key words: agricultural entomology, phenotypic plasticity, ecology & behavior, vector-borne pathogen, vector ecology

Herbivores evolve to optimize utilization of host plant resources and maximize fecundity by various means, often through adaptation to plant primary and secondary chemistry (Bernays and Chapman 1994), natural enemy communities (Bernays and Graham 1988), and local environmental conditions, among other factors. Combinations of these factors can drive reproductive isolation of herbivores on host plant genera, species, or genotypes (Jaenike 1990), leading to the formation of intraspecific "host races" (Claridge and Den Hollander 1983), with distinct host plant ecologies. Intraspecific host races typically differ in their behavior, phenology, and distribution on the landscape, and the evolution of host races is now widely considered a precursor to ecological speciation (Bush 1975, Berlocher and Feder 2002, Drès and Mallet 2002). Ecological speciation depends upon the maintenance of ecological specialization or host race structure, reinforced by pre- and postzygotic barriers to gene flow among populations. This isolation must be maintained over evolutionary time and is vulnerable to environmental factors that weaken specialization and isolation; yet, the factors that contribute to host specialization in locally adapted herbivore populations and the robustness of this specialization in response to varying environmental conditions remain poorly understood.

The pea aphid (*Acyrthosiphon pisum* L.) is an oligophagous phloem-feeding herbivore with a host range that includes many leguminous species (Blackman and Eastop 2006). Despite a large host range, populations of pea aphid are known to become reproductively isolated on their host plants (Via 1991, Via et al. 2000), leading to population genetic structure characterized by distinct sympatric populations and multiple host races that specialize on different species of legumes (Peccoud et al. 2009). Aphid clones (used here to refer to a colony originating from parthenogenesis and composed of a single genotype) colonizing the "wrong" host suffer low reproductive rates and rapid mortality, and both ecological and postzygotic barriers constrain the suitability of host plants species for pea aphid clones (Via 1991, Via et al. 2000). These barriers strongly promote fidelity of some clones to specific host plant species and discourage host switching through hybrid unfitness (Peccoud et al. 2014), with microbial symbionts playing

key mechanistic roles in host plant utilization and genetic isolation of sympatric populations (Oliver et al. 2010). This pattern of host use and genetic structure has led to the widespread adoption of pea aphid as a model system of ecologically driven sympatric speciation (Peccoud and Simon 2010).

Acyrthosiphon pisum transmits multiple plant viruses (phytoviruses), some of which have substantial effects on host plant suitability and aphid behavior (Wu et al. 2014). Specifically, *Bean leafroll virus* (BLRV) (Luteoviridae) has been shown to positively affect *A. pisum* reproduction and population growth: in previous studies, *A. pisum* feeding on pea plants infected with BLRV had shorter prereproductive periods, higher lifetime and age-specific fecundities, and were longer-lived than *A. pisum* feeding on noninfected plants (Wu et al. 2014). This effect is similar to what has been reported for other Luteoviridae (Castle and Berger 1993, Jiménez-Martinez et al. 2004), with emerging patterns that are suggestive of widespread virus × vector mutualisms (Mauck et al. 2012, Roossinck 2015), though some viruses have neutral or negative effects on their vectors (Mauck et al. 2010, 2012, Wu et al. 2014). The influence of phytoviruses on the performance of *A. pisum* clones specializing on different host plant species is unexamined. However, viruses that indirectly affect vector performance may impact patterns of host plant utilization and virus epidemiology, with consequences for the management of agricultural systems where pea aphid is a pest or virus vector.

Here, the effects of BLRV on *A. pisum* life history traits and clone × host plant interactions were investigated. Using a three-factor experimental design and life-table analyses, interactions between *A. pisum* clone, host plant species, and BLRV infection were tested on aphid life history traits including survival, prereproductive period, and intrinsic rate of population growth. Aphid host-selection behaviors were also evaluated within this framework, and the preference of clones for natal and nonnatal host plant species was tested in the presence and absence of BLRV. These studies are significant to understanding how plant pathogens may affect patterns of host plant use in genetically and phenotypically variable pest insect populations, and the experiments reported here provide evidence that insect-borne phytoviruses can affect the suitability of host plant species for specialized insect herbivores in a genotype-specific fashion.

Materials and Methods

Origin and Maintenance of Aphid Colonies

Viruliferous and nonviruliferous aphid colonies are maintained at the Manis Entomological Laboratory at the University of Idaho (Moscow, ID). Colonies of *A. pisum* were established from individual aphids captured by sweepnet in commercial pea and alfalfa fields near Moscow, ID (46.7325° N, 116.9992° W, 786 m a.s.l.) and are maintained in multiple 60- by 60- by 60-cm mesh tents (BugDorm 2120F; BioQuip, Rancho Dominguez, CA) in a greenhouse under the following environmental conditions: 20 ± 2 °C, photoperiod of 18:6 (L:D) h, and 50% RH. Colonies are provided with potted fava bean plants (*Vicia faba* L., Fabaceae), a universal host for pea aphid (Peccoud et al. 2014), on an ad libitum basis, and colonies were maintained on fava bean for at least 20 generations prior to use in experiments. The experiments reported below used three field-collected, phenotypically divergent clones: a green phenotype "AG" and a pink phenotype "AP" both with alfalfa as the natural host, and a green phenotype "P" with pea as the natural host. It was confirmed that colonies were genetically separate using 12 autosomal microsatellite loci (described in Eigenbrode et al. 2016), and both microsatellite genotyping, population genetic analyses, and performance assays were consistent with previous reports of "host races" (Peccoud et al. 2009, Eigenbrode et al. 2016). Altogether, the three colonies used here represent ~75% of the host race diversity found on the landscape in the Pacific Northwestern USA (Eigenbrode et al. 2016). In addition, the laboratory of H.C. Godfray (Oxford University) genotyped facultative bacterial endosymbionts of each clone using the methods described in Henry et al. (2013), and each clone was found to be associated with a different facultative endosymbiont. The "AG" clone is associated with both *Hamiltonella defense* and *Rickettsia insecticola*, the "AP" clone is associated with *H. defensa*, and the "P" clone is associated with *Serratia symbiotica*. This source of variability is not unexpected, as host-adapted pea aphid clones are typically associated with different facultative symbionts in the field (Tsuchida et al. 2002); thus, "symbiotypes" consisting of unique aphid clone × bacterial endosymbiont combinations represent pertinent units of management in agricultural systems.

Viruliferous aphid colonies were established by introducing a single viruliferous (BLRV-positive) alfalfa plant into noninfected aphid colonies maintained on fava bean, and colonies were subsequently maintained by adding noninfected fava bean plants as needed to support aphid populations. Colonies and fava bean plants were tested approximately every 2 wk for the presence (or absence) of BLRV using the enzyme-linked immunosorbent assay (ELISA; described in [Vermulapati et al. 2014]).

The Effects of BLRV on Aphid Clone × Host Plant Species Interactions

Pea (cv. 'Aragorn') and alfalfa (cv. 'Surpass') seeds were sown in pots (12 cm diameter) containing a potting mix (Sunshine Mix#1; SunGro Horticulture, Bellevue, WA) augmented with sand in the greenhouse under the same environmental conditions described above, and thinned to yield a density of one plant per pot. The methods of Wu et al. (2014) were used to inoculate experimental plants. Briefly, plants were inoculated at the three-leaf stage with BLRV by confining five aphids from the viruliferous "AP" colony in clip cages (5 cm diameter) on the apical leaflet for 3 d. Following the 3-d inoculation access period, aphids were carefully removed from plants using a soft bristled paintbrush and plants were maintained free of aphids until used in experiments. In order to control for the effects of aphid feeding, a subset of plants was treated as described above, except with aphids from the noninfected "AP" colony ("sham" inoculation). Fifteen days following inoculation treatments, plant infection status (BLRV or sham) was used as a treatment variable to test the life history of each aphid clone on infected and sham-treated host plants. The infection status of all plants was confirmed using ELISA prior to use in bioassays.

Aphid life history parameters were evaluated using life table experiments similar to those described in Wu et al. (2014). A single mature, uninfected apterous aphid was placed on the tertiary leaf and enclosed in a 2-cm-diameter clip cage ventilated with mesh on one side. After 24 h, all aphids except for one nymph (foundress) were removed from each clip cage. The foundress was checked daily thereafter and any new nymphs were recorded and removed, and each foundress was allowed to larviposit for a length of time equal to their prereproductive period (d), which ranged from 5–12 d. The method of Wyatt and White (1977) was used to convert the number of progeny (M_d) produced by each foundress into estimates of intrinsic rate of population growth (R_m), where $R_m = 0.74\ (\log/M_d)/d$. Survival rate was determined for each replicate clone × host plant species × virus infection treatment combination using a parallel assay. Twenty randomly selected apterous adults were taken from each colony and placed on an opposing tertiary leaf, and enclosed in

cages constructed from dialysis tube (3 cm long by 1 cm diameter; Spectrum Lab, Inc., Rancho Dominguez, CA) stoppered at each end with foam. Each day, new offspring were removed to prevent crowding, and the number of surviving adults was recorded at the end of the assay period, which was dictated by the larviposition period of individual foundresses used for the determination of R_m.

Thus, for each replicate aphid clone × host plant species × virus infection treatment combination, data on survival, prereproductive period, total progeny, and intrinsic rate of population growth were concurrently recorded for $n = 10$ replicates ($N = 120$ experimental units in total).

The Effect of BLRV on Host Selection by Specialized *A. pisum* Clones

The influence of BLRV infection on aphid preferences for host plant species was further investigated using two bioassay tests designed to evaluate 1) aphid settling preferences in response to whole plants, and 2) aphid orientation preferences in response to volatile cues from plants.

The first test employed a choice test as described in Wu et al. (2014). Thirty mature apterous aphids were collected at random from colonies maintained on fava bean, placed in polystyrene petri dishes (150 mm diameter), and starved for 2 h prior to testing. To evaluate host settling preference, aphids were introduced into the center of a clear plastic tube (11 cm × 5 cm, L × D) with leaflets of each treatment, still attached to plants, inserted into either end. Host settling preferences were recorded by census after allowing aphids to freely access plants for an 18-h period, and environmental conditions for the duration of the bioassay were otherwise as described above for greenhouse settings. Ten simultaneous replicates ($n = 10$) of the bioassay were performed for each aphid clone ($n = 3$: "AG", "AP", "P") in response to plant infection status (i.e., aphids selected between sham-treated hosts or aphids selected between BLRV-infected hosts) for a total of $N = 60$ experimental units.

A second test evaluated the orientation preference of each clone in an arena in response to volatile cues only. Arenas were constructed from polystyrene Petri dishes (150 mm diameter) with floors constructed of nylon mesh (0.5 mm by 0.5 mm; described in Eigenbrode et al. 2002). The arena was positioned 3 mm above the surface of two intact leaflets from pea or alfalfa plants, still attached to plants and positioned under each side of the arena, so that aphids could move freely on the screen above the leaflets but were unable to contact the leaf surface. For each replicate, 20 apterous aphids, starved prior to testing for 2 h, were released onto the center of the screening. Aphid positions above odor sources (leaflets) were recorded after 60 min under red light. Simultaneous replicates were performed for each clone and aphids were allowed to choose between volatile cues from sham-treated pea and alfalfa and BLRV-infected pea or alfalfa, for a total of $N = 20$ experimental units representing each clone.

Statistical Analysis

The first experiment testing the effects of BLRV infection on aphid clone × host plant interactions was analyzed as a three-way ANOVA, treating virus infection ($n = 2$: sham treatment and BLRV treatment), aphid clone ($n = 3$: "AG", "AP", and "P"), host plant species ($n = 2$: alfalfa and pea) and all two- and three-way interactions as fixed effects on the responses of *A. pisum* survival rate (%), prereproductive period (d), and intrinsic rate of population growth (R_m). Response variables were checked for adherence to assumptions of normality and homoscedasticity, and responses conformed to assumptions. Analyses were carried out using the statistical software JMP 10.0 (SAS Institute; Cary, NC) using a Type I error rate of $\alpha = 0.05$ for establishing statistical significance. Main effects were not analyzed in the case of significant interactions. In addition, pre-planned contrast tests (conventionally denoted by Greek letter psi, ψ) were used to test three specific hypotheses for each response variable: 1) that *A. pisum* performance is enhanced on plants infected with BLRV (test pools results across clones); 2) that in the absence of virus (i.e., comparisons made among sham-treated plants), clones exhibit superior performance on their natal host species; and 3) in the presence of virus (i.e., comparisons made among BLRV-infected plants), clones do not exhibit superior performance on natal host species. Host selection experiments testing the effect of BLRV infection on aphid host selection behavior were analyzed using the chi-square (χ^2) statistic. For each experiment (access to whole plants or access to volatile cues only), the proportion of aphids choosing either pea or alfalfa was compared between aphids selecting among sham-treated plants and aphids selecting among BLRV-infected plants to test the null hypothesis that host settling and orientation preferences of *A. pisum* clones do not differ relative to host infection status.

Table 1. ANOVA table summarizing the effects of aphid clone ("AG," "AP," and "P"), host plant species (alfalfa or pea), virus treatment (BLRV-infected or sham-treated), and all two- and three-way interactions on three *Acyrthosiphon pisum* life history traits

Source	df	SS	*F*	*P*
(a) Survival rate				
Whole model (R^2=0.51)	11	2.119	10.203	<0.0001
Clone	2	0.242	6.427	0.0023
Host plant	1	0.526	27.886	<0.0001
Infection status	1	0.581	30.763	<0.0001
Clone × Host plant	2	0.729	19.302	<0.0001
Clone × Infection status	2	0.023	0.629	0.5346
Host plant × Infection status	1	0.015	0.804	0.3719
Clone × Host plant × Infection status	2	0.001	0.029	0.9707
Error	108	4.159	–	–
(b) Prereproductive period				
Whole model (R^2=0.27)	11	85.067	3.563	0.0003
Clone	2	45.016	10.370	<0.0001
Host plant	1	16.133	7.433	0.0075
Infection status	1	1.200	0.553	0.4587
Clone × Host plant	2	12.616	2.906	0.0590
Clone × Infection status	2	1.350	0.311	0.7334
Host plant × Infection status	1	4.800	2.211	0.1399
Clone × Host plant × Infection status	2	3.950	0.910	0.4056
Error	108	234.400	–	–
(c) Intrinsic growth rate, R_m				
Whole model (R^2=0.50)	11	0.534	9.984	<0.0001
Clone	2	0.086	8.897	0.0003
Host plant	1	0.174	35.812	<0.0001
Infection status	1	0.033	6.905	0.0098
Clone × Host plant	2	0.197	20.215	<0.0001
Clone × Infection status	2	0.005	0.606	0.5473
Host plant × Infection status	1	0.005	1.043	0.3093
Clone × Host plant × Infection status	2	0.032	3.314	0.0401
Error	108	0.526	–	–

Results

The Effects of BLRV on Aphid Clone × Host Plant Species Interactions

There was significant variation in *A. pisum* survival rate due to the main effect of virus infection and a host plant × aphid clone interaction, and virus infection treatment did not interact with other main effects (Table 1a). Pooling results from all three clones, mean

Table 2. The effect of *Bean leafroll virus* (BLRV) on *Acyrthosiphon pisum* clone × host plant interactions on aphid life history traits including (a) survival rate (%), (b) prereproductive period (d), and (c) intrinsic rate of population growth (R_m)

Variable	Clone	Host plant	Sham	BLRV
(a) Survival rate (%)	"AG"	Alfalfa	28% ± 3%	46% ± 3%
		Pea	33% ± 2%	48% ± 5%
	"AP"	Alfalfa	42% ± 3%	55% ± 6%
		Pea	46% ± 2%	53% ± 5%
	"P"	Alfalfa	14% ± 1%	32% ± 4%
		Pea	52% ± 4%	65% ± 5%
(b) Prereproductive period	"AG"	Alfalfa	8.8 ±0.4	9.0 ± 0.4
		Pea	8.8 ± 0.5	8.5 ± 0.4
	"AP"	Alfalfa	9.7 ± 0.6	10.1 ± 0.5
		Pea	10.3 ± 0.2	8.9 ± 0.5
	"P"	Alfalfa	9.1 ± 0.5	9.1 ± 0.5
		Pea	7.5 ± 0.3	7.4 ± 0.3
(c) Intrinsic rate of growth, R_m	"AG"	Alfalfa	0.195 ± 0.018	0.226 ± 0.013
		Pea	0.247 ± 0.023	0.270 ± 0.034
	"AP"	Alfalfa	0.208 ± 0.006	0.203 ± 0.025
		Pea	0.144 ± 0.014	0.255 ± 0.027
	"P"	Alfalfa	0.157 ± 0.024	0.193 ± 0.030
		Pea	0.359 ± 0.016	0.364 ± 0.011

aphid survival was 49.6 ± 2.4% on plants infected with BLRV and 35.7 ± 2.0% on sham-treated plants ($\Psi = 30.763$; df = 1, 108; $P < 0.0001$).

Neither the "AG" ($\Psi = 0.661$; df = 1, 108; $P = 0.417$) nor the "AP" ($\Psi = 0.423$; df = 1, 108; $P = 0.516$) clone exhibited greater survival on sham-treated alfalfa, the host species from which they were collected in the field, as compared with pea. However, mean survival rate of the "P" clone was 37.5% higher on sham-treated pea than alfalfa ($\Psi = 37.228$; df = 1, 108; $P < 0.0001$), indicating superior survival by this clone on its natural host. Contrasts comparing survival among different host species on BLRV-treated plants indicated that survival of the "AG" and "AP" clones did not differ between pea or alfalfa ($\Psi = 0.105$; df = 1, 108; $P = 0.745$). However, mean survival of the "P" clone on BLRV-infected pea was 77% higher on pea plants infected with BLRV in comparison with BLRV-infected alfalfa ($\Psi = 28.830$; df = 1, 108; $P < 0.0001$; Table 2a).

There was also significant variation in the response of *A. pisum* pre-reproductive period due to the main effects of aphid clone and host plant species (Table 1b). No statistically significant interactions between factors were detected. Mean time (in days) until first reproduction was the shortest for the "P" clone at 8.2 ± 0.3 d, intermediate for the "AG" clone at 8.8 ± 0.2 d, and longest for the "AP" clone at 9.8 ± 0.3 d. Aphid clones tended to achieve first reproduction most rapidly on pea at 8.5 ± 0.21 d, while this was delayed on average when colonizing alfalfa, at 9.3 ± 0.20 d. Prereproductive period was not affected by BLRV infection ($\Psi = 0.522$; df = 1, 108; $P < 0.458$; Table 2b). Prereproductive period on sham-treated plants did not differ between pea and alfalfa for the "AG" (Ψ _ 0.000; df = 1, 108; $P = 1.000$) or "AP" clones ($\Psi = 0.829$; df = 1, 108; $P = 0.364$). In contrast, prereproductive period of the "P" clone was on average 17.6% shorter on sham-treated pea than sham-treated alfalfa ($\Psi = 5.897$; df = 1, 108; $P = 0.016$), suggesting superior performance of this clone on its natal host species. Similar to the case for sham-treated plants, prereproductive period of the "AG" ($\Psi = 0.575$; df = 1, 108; $P = 0.449$) or "AP" ($\Psi = 3.317$; df = 1, 108; $P < 0.071$) clone did not differ between BLRV-infected pea and BLRV-infected alfalfa. For the "P" clone, there was a statistically significant 19% shorter prereproductive period on BLRV-infected pea compared with BLRV-infected alfalfa ($\Psi = 6.657$; df = 1, 108; $P < 0.011$).

There was significant variation in *A. pisum* intrinsic rate of population growth (R_m) due to a three-way aphid clone × host plant species × plant infection status interaction, and each clone was affected uniquely ($P = 0.040$; Table 1c). Contrast tests revealed that aphids colonizing BLRV-infected plants exhibited 14% greater intrinsic rates of growth on average than aphids colonizing sham-treated plants ($\Psi = 6.905$; df = 1, 108; $P = 0.009$). However, population growth rates of the "AG" clone did not significantly differ between pea and alfalfa under the sham-treatment ($\Psi = 2.734$; df = 1, 108; P = 0.101) or BLRV infection treatment ($\Psi = 1.951$; df = 1, 108; $P = 0.165$). Conversely, mean population growth rates of the "P" clone was 56.3% greater on sham-treated pea compared with sham-treated alfalfa ($\Psi = 42.096$; df = 1, 108; $P < 0.0001$), and 47.0% greater on average when colonizing BLRV-infected pea than BLRV-infected alfalfa ($\Psi = 29.948$; df = 1, 108; $P < 0.0001$). Mean intrinsic population growth rates of the "AP" clone was 30.8% greater on alfalfa than pea when colonizing sham-treated plants ($\Psi = 4.257$; df = 1, 108; $P = 0.041$); however, when colonizing BLRV-infected hosts, there was not a significant difference between mean intrinsic rates of population growth between pea and alfalfa ($\Psi = 2.927$; df = 1, 108; $P = 0.090$, Table 2c).

The Effect of BLRV on Host Selection by Specialized *A. pisum* Clones

Host settling preferences of aphid clone "AG" were unaffected by plant infection status, and on average 77% of aphids preferentially settled on pea when presented with sham-treated pea or alfalfa and BLRV-infected pea or alfalfa ($\chi^2 = 0.588$, df = 1, $P = 0.443$, $N = 469$). In contrast, the response of host settling preferences of aphid clone "AP" to host infection status was statistically borderline ($\chi^2 = 3.784$, df = 1, $P = 0.051$, $N = 522$). On average, 58% of "AP" aphids preferentially settled on alfalfa when presented with sham-treated alfalfa or pea, but only 48% of aphids settled on alfalfa when presented with BLRV-infected pea or alfalfa, indicating that host preferences became slightly more general in the presence of BLRV. Clone "P" exhibited a significant reduction in host fidelity due to the effect of plant infection status: on average 90% of aphids settled on pea when presented with sham-treated pea or alfalfa, but when choosing between BLRV-infected pea or alfalfa this preference was reduced to 80% ($\chi^2 = 7.992$, df = 1, $P = 0.004$, $N = 465$, Fig. 1).

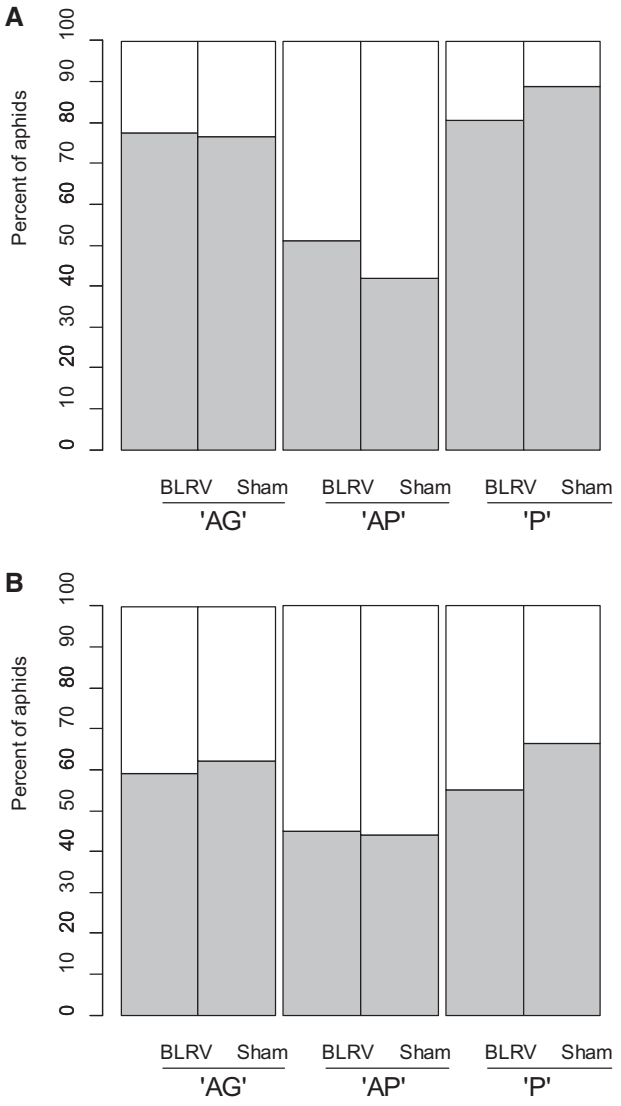

Fig. 1. Relative proportion of aphids (*Acyrthosiphon pisum*) of each test clone orienting to pea (gray bars) or alfalfa (white bars) when (**A**) allowed unrestricted access to whole plants, or (**B**) when allowed access to volatile cues only.

In the second experiment testing aphid orientation to host plant volatile cues 80.7% ± 2.5% SE of aphids oriented to an odor source on average, and the proportion of responding aphids was treated as the unit of analysis. Each clone exhibited different preferences for pea or alfalfa volatile cues, but preference did not differ relative to plant infection status. Aphid clone "AG" oriented preferentially to volatiles from pea, with 60% of aphids orienting to pea volatiles and 40% of aphids orienting to alfalfa volatiles on average, but this pattern of orientation did not differ significantly when aphids were selecting between sham-treated or BLRV-infected hosts ($\chi^2 = 0.229$, df = 1, $P = 0.632$, $N = 138$). Aphid clone "AP" exhibited no discrimination between plant volatile sources, with 50% of aphids orienting to pea volatiles and 50% of aphids orienting to alfalfa volatiles on average. This pattern of orientation did not differ when aphids were selecting between volatile cues from sham-treated or BLRV-infected hosts ($\chi^2 = 0.552$, df = 1, $P = 0.457$, $N = 145$). Aphid clone "P" oriented preferentially to volatiles from pea, with 63% of aphids orienting to pea volatiles and 37% of aphids orienting to alfalfa volatiles on average. As with the other clones, this pattern of orientation did not differ when aphids were selecting between sham-treated or BLRV-infected hosts ($\chi^2 = 0.985$, df = 1, $P = 0.320$, $N = 159$).

Discussion

Our results show that BLRV-infected plants are on average associated with increased survival and population growth rates of *A. pisum* (Table 2), which has been demonstrated for other luteoviruses (Bosque-Pérez and Eigenbrode 2011, Eigenbrode and Bosque-Pérez 2016) and for BLRV (Wu et al. 2014), confirming that *A. pisum* benefits from colonizing infected plants. However, the effect of BLRV on clone × host plant interactions was variable, and each *A. pisum* genotype responded uniquely. The suitability of host plant species switched for the "AP" clone when test plants were infected with BLRV: pea was a poorer host than alfalfa when plants were sham-treated, but BLRV-infected pea and alfalfa were equivalent as hosts, and infected pea plants were superior to sham-treated alfalfa (Table 2). This indicates that BLRV can improve the suitability of an otherwise suboptimal host beyond that of noninfected natal hosts in some cases. In contrast, BLRV did not alter the relative suitability of hosts for the "P" clone, which consistently performed best on pea regardless of plant infection status, and the "AG" clone showed no significant performance difference across host species or plant infection status.

Thus, in our study testing three *A. pisum* clones, three distinct "virus-association phenotypes" were detected: 1) clones which switch their ability to utilize host plant species when virus in present ("flexible specialists"), 2) clones for which virus has no effect on host plant suitability ("fixed specialists"), and 3) clones which exhibit no fitness differences across host plant species irrespective of virus presence or absence (generalists). A broader survey of *A. pisum* clones is needed to assess the frequency of these phenotypes within and between populations, and to evaluate whether "virus-association phenotype" is correlated with the identity of endosymbiotic bacteria. Quantifying regional variation in virus-association phenotypes of insect vectors will benefit the development of precision pest management strategies and accurate crop virus forecast models, but may also inform ecological speciation theory.

Although effect sizes were modest, the specificity of aphid host plant preference for two of the three tested clones was reduced when choosing among BLRV-infected hosts, consistent with a small (~10% difference) but statistically significant increase in the likelihood of at least some clones settling on host plant species to which they may be ecologically mismatched (Fig. 1). Since this effect was not detected in bioassays isolating aphid responses to volatile cues from the test plants, by elimination it can be concluded that visual or gustatory cues were likely responsible for the changes in discrimination occurring with BLRV infection. Nonetheless, trends in responses to volatile cues from the two host plants resembled settling responses, suggesting that volatile cues contribute to some extent to host discrimination by the aphids. The "P" and "AG" clones preferentially settled on pea and tended to prefer pea volatiles (the "P" clone significantly so), while the "AP" clone showed no preference for either host in settling preferences or response to volatile cues. Volatile profiles from alfalfa and pea differ markedly, but are little affected by BLRV infection (data not shown).

Acyrthosiphon pisum is an established model for genetically-linked host plant specialization or host race formation by phytophagous insects (Hawthorne and Via 2001, Peccoud et al. 2009), which is maintained by pre- and postzygotic reproductive isolation

(Via et al. 2000), habitat specialization due to differences in behavioral ecology (Ferrari et al. 2006, 2008), assortative mating (Caillaud and Via 2000), and hybrid inviability (Peccoud et al. 2014). Our experiments suggest that differences in habitat specialization or behavioral ecology which sustain host specialization in *A. pisum* may be weakened for some clones when host plants are infected with a phytovirus. *Acyrthosiphon pisum* is a competent vector for multiple viruses including *Pea enation mosaic virus* (PEMV), *Fava bean necrotic virus*, and others. The effects of these phytoviruses or multiple concurrent infections on the performance, host plant preferences, and ecological specialization of *A. pisum* clones remain to be studied but may also be significant in determining fitness on natal and non-natal host plant species.

Our study employed naturally occurring clones of *A. pisum* that differed genetically, but also in their secondary bacterial endosymbionts. Host-adapted *A. pisum* populations often differ in their associations with primary and secondary endosymbiotic bacteria, with sympatric clones each exhibiting unique facultative bacterial communities (Tsuchida et al. 2002, Simon et al. 2003). Although microbial symbioses can strongly influence *A. pisum*–host plant interactions, their roles in host specialization are unclear, with bacterial symbionts driving host plant specialization in some instances (Leonardo and Muiru 2003, Tsuchida et al. 2004) but not in others (Leonardo 2000, Ferrari et al. 2007). Nonetheless, when specialization occurs in nature it is manifested by populations that differ both genetically and in their endosymbiont identities, as do the clones in our study. Thus, potential disruption of clone × host plant interactions within sympatric populations by phytovirus infection of the hosts is ecologically relevant. The different response of each tested clone to BLRV infection of hosts is potentially attributable to aphid genotype, endosymbiont association, or both. Further work is needed to elucidate the basis of these effects.

The effects of BLRV infection on the performance of specialized *A. pisum* clones provide a potential pathway for plant viruses to influence vector ecology in a manner that enhances virus transmission (Ingwell et al. 2012, Mauck et al. 2012, Moreno-Delafuente et al. 2013). If virus infection improves fitness of vector clones on certain host plant species, this could promote interspecific transmission of the virus to new hosts that would otherwise be inaccessible due to vector specialization. BLRV infects most of the leguminous hosts of *A. pisum*, so selection should favor effects that improve vector fitness across all hosts and reduce barriers to interspecific colonization. Vector–pathogen mutualism facilitates herbivore colonization of plants that are otherwise poor hosts through various mechanisms including improving nutritional quality or altering turgor pressure of infected hosts (Ajay 1986, Davis et al. 2015a) and by impacting host defensive or hormonal responses to herbivory (Davis et al. 2015b). Our experiments demonstrate that suitability and fidelity of some, but not all, pea aphid clones can be altered in the presence of a plant pathogen, but the specific mechanisms of this effect are unexplored. To further inform crop selection and management of crop disease cycles, it will be important to determine which physiological mechanisms drive beneficial interactions between insect vectors and viruses (Roossinck 2015).

The two most important viruses affecting pulse crops, including pea, in northern Idaho and eastern Washington are BLRV and PEMV. Of these two, only BLRV infects alfalfa (Larsen et al. 1996) and so is capable of infecting both pea and alfalfa to influence host fidelity by pea aphids for these two hosts as documented here. Our study focused on BLRV because it can infect both hosts, allowing a factorial comparison (host plant x infection status). Nonetheless, effects of PEMV on pea aphid host specialization are possible. Pea plants 4 wk after infection with PEMV are preferable to sham-inoculated plants for settling by the "P" clone tested here, but life history of the aphid is unaffected (Wu et al. 2014). Hodge and Powell (2010) report preferential settling of pea aphids on PEMV-infected pea, and greater reproduction on PEMV-infected plants, at later stages of infection (past 4 wk). It is therefore possible that PEMV-infected pea plants become more attractive for settling and better hosts for alfalfa-adapted pea aphid clones, disrupting host specialization by these clones. Furthermore, dual infection of pea by PEMV and BLRV is feasible, but the effects of dual infection on aphids have not been examined. Together, these different effects of virus infection could influence host fidelity and disease spread in complex ways, so their study is merited.

Host plant specialization is a driving force in population genetic divergence in several systems (Simon et al. 2015), and although viruses are not typically considered as symbionts that alter patterns of host plant use in herbivores, incorporating virus-association phenotype into the framework of integrated pest management theory could promote greater precision in practice. Here, we confirm that BLRV-infection of host plants increases the survival and intrinsic rates of growth of *A. pisum* on its hosts, and further show that infection can impact host discrimination in a clone-specific fashion. Further work in the *A. pisum* system is needed to determine the relative contributions of aphid genotype and endosymbiont associations to patterns of host plant utilization in the presence and absence of plant pathogens, and to evaluate the frequency of virus-association phenotypes across ecosystems. Examination of these effects in *A. pisum*, a widely studied model system for understanding host plant specialization, can reveal how pathogens contribute to vector fitness in similar plant–insect–virus pathosystems, and is important to developing new applied ecology methods for the control of pest herbivores and virus vectors.

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