Pathogens Manipulate the Preference of Vectors, Slowing Disease Spread in a Multi-host System
Supplementary Material

Lauren G. Shoemaker, Evelyn Hayhurst, Alexander T. Strauss, Anita Porath-Krause, Christopher P. Weiss-Lehman, Elizabeth T. Borer, Eric W. Seabloom, and Allison K. Shaw

1 Experimental Methods

1.1 Viral Detection

Total RNA extraction was carried out using a modified version of TRIzol Reagent RNA-extraction protocol (Invitrogen^{TM}). Between 50 and 100 mg of each sample tissue was weighed, recorded, and cut into 1-2 mm pieces using scissors and placed into a 2 ml plastic screw cap microcentrifuge tube containing one 4.5 mm cal. steel BB (Copperhead). After adding 500 ul of TRIzol Reagent, the tissue was ground in a Mini-Beadbeater-16 (Biospec Products) at 10 second intervals until solution was fully homogenized. After a 4 minute incubation period at room temperature, 100 ul of chloroform was added to each tube and shaken for 15 seconds by hand. Each sample was incubated for 3 minutes at room temperature, followed by cold centrifugation (4°C) at 7,000g for 15 minutes. The aqueous phase was then transferred to a new microcentrifuge tube and 100 ul of isopropanol was added to each sample. Samples were shaken by hand then incubated at room temperature for 10 minutes. Samples were cold centrifuged at 7,000g for 10 minutes. The supernatant was discarded and 1 ml of 75 percent ethanol was added. Samples were briefly vortexed and cold centrifuged again at 7,000g for 5 minutes. The supernatant was discarded and the pellet containing RNA was allowed to dry in the tube for a minimum of 30 minutes. The pelleted RNA was then dissolved in 27.5 ul of RNase-free water and allowed to incubate for 10 minutes at 58°C. RNA quantification was performed using a NanoDrop^{TM} 2000c Spectrophotometer (Thermo Fisher Scientific). Following quantification, the samples were placed on ice for at least 5 minutes and then

stored at −80°C. Reverse transcription polymerase chain reaction (RT-PCR) was performed to generate complementary DNA (cDNA). For each sample, a mixture of 0.5 ul of random hexamers (1ug/ul) and 4.5 ul of RNA were quick spun and then heated in a S1000^{TM} Thermal Cycler (Bio-Rad) at 70°C for 5 minutes. RT-PCR reactions were carried out in 20 ul reactions with 4 ul of 5x Reaction Buffer (ImProm-II^{TM} Reverse Transcriptase (Promega)), 1.2 ul of MgCl2 (25mM), 1 ul of dNTPs (10mM), 0.5 ul of Recombinant RNasin Ribonuclease Inhibitor (Promega) (40U/ul), 1 ul of ImProm-II^{TM} Reverse Transcriptase (Promega), 7.3 ul of RNase free water, 0.034 ul of T4 Gene 32 Protein (New England BioLabs), and the 5 ul of random hexamer/RNA mixture. RT-PCR samples were briefly vortexed, spun, and placed in a thermal cycler with the following conditions: 5 min at 25°C, 60 min at 45°C, and finally 15 min at 70°C. The cDNA was stored at −20°C.

The presence of BYDV-PAV in plant tissue was determined using polymerase chain reaction (PCR) with the cDNA described above. Two BYDV-PAV primers, which are specific to the P3 coat protein gene, were used to isolate and amplify a 206 base pair region. PCR was carried out in 20 ul reactions with 2 ul of 10x buffer, 2.8 ul MgCl2 (25mM), 10.4 ul nanopure water, 0.8 ul of each forward and reverse primer (10 uM), 0.8 ul dNTPs (10 mM), 0.4 ul HotStarTaq DNA Polymerase (Qiagen), and 0.068 ul T4 Gene 32 Protein (New England BioLabs). Using forward primer: 5' -CCT TAA AGC CAA CTC TTC CG - 3' (PAV_3082_F), and reverse primer: 5' - TAG CTA GCC AGG GCT GAT T - 3' (PAV_3288_R), the target region was amplified in a thermal cycler using the following procedure: initial heating to 95°C for 15 min; a step-down segment of 95°C for 30 sec, 59°C for 30 sec, and 72°C for 1 min (reducing from 59°C to 54°C in 1°C increments); 29 cycles of 95°C (30 sec), 54°C (1 min), and 72°C (1 min); and 72°C for 10 min. The amplified target DNA was loaded into a 2.0 percent UltraPure Agarose-1000 (Thermo Fisher Scientific) gel mixed with SYBR Safe DNA Gel Stain (Invitrogen) and run at 125 V for approximately 40 minutes alongside a GeneRuler 100bp DNA Ladder (Thermo Fisher Scientific). DNA was visualized using Gel Doc^{TM} EZ Imager (Bio Rad).

All viral transmissions were successful, where the six BYDV-PAV uninfected colonies showed no sign of infection while PCR results exhibited BYDV-PAV for the six infected colonies (Fig. S1). Infection was additionally verified in the original tissue used to inoculate the six infected colonies

for the aphid preference experiments.

1.2 Optimal Time for Aphid Settlement

The optimal time for the *R. padi* dual-choice preference assays was determined over a 24 hour period to be 4 hours with moist filter paper lining the petri dish to prevent plant tissue from drying out (Figure S2).

2 Preference Effects Across Community Compositions and Vector Fecundity

We examine the effect of community composition on disease spread across observed vector preferences. For communities dominated by non-preferred hosts, with even composition, or dominated by preferred hosts, the combination of both infection status preference and host species preference (Figure S3, black dashed line; panels a, c, e) decreases pathogen spread when compared to vectors with no preference. When vectors exhibit a preference for either infection status (non-infected versus infected) or host species (species 1 or species 2), pathogen spread is intermediate between the no preference and both preference cases. Community composition has a stronger effect on vectors, where host communities dominated by the non-preferred species or with even composition have a slower pathogen spread in the vector population (Figure S3, panels b and d) compared to communities dominated by the preferred host species (Figure S3, panel f).

We additionally examine how variable vector demographic rates may alter results shown in the main text (Figure S4). Here, we incorporate variable aphid demography, where *R. padi* have been shown to have over twice the fecundity on exotic annuals compared to native perennials (Borer *et al.*, 2009). However, incorporating this variation in vector fecundity has minimal effects on disease spread in the host and vector populations (Figure S4).

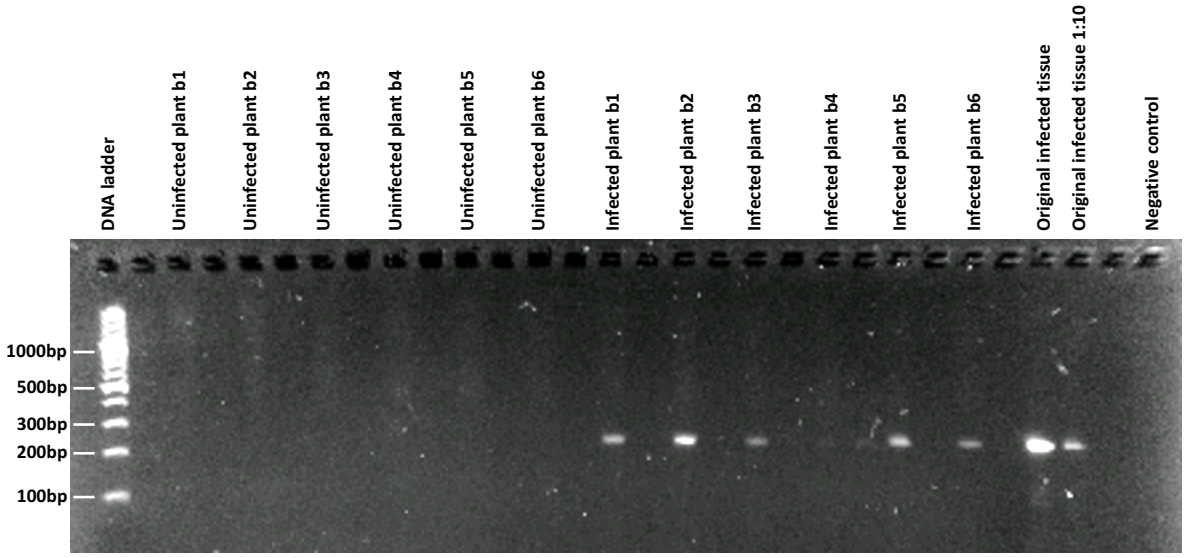

Figure S1: Confirmation of BYDV-PAV infection status in *Avena sativa.*

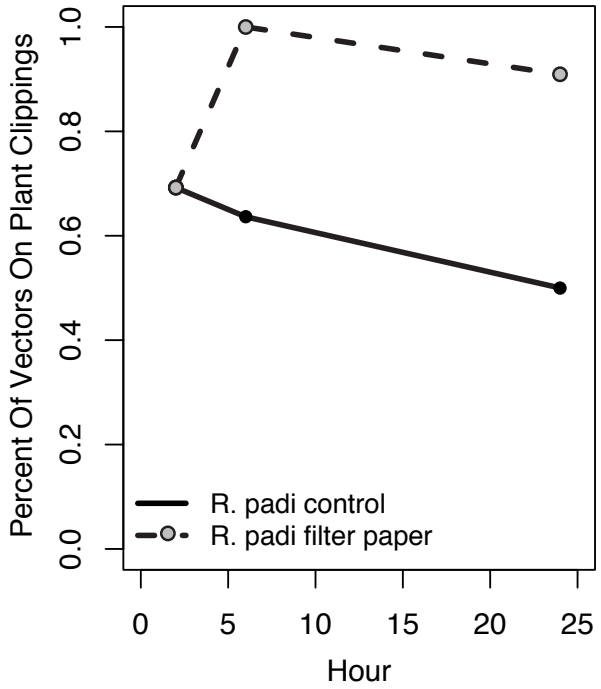

Figure S2: Optimal time for aphid settlement on plant clippings with and without moist filter paper lining the petri dish.

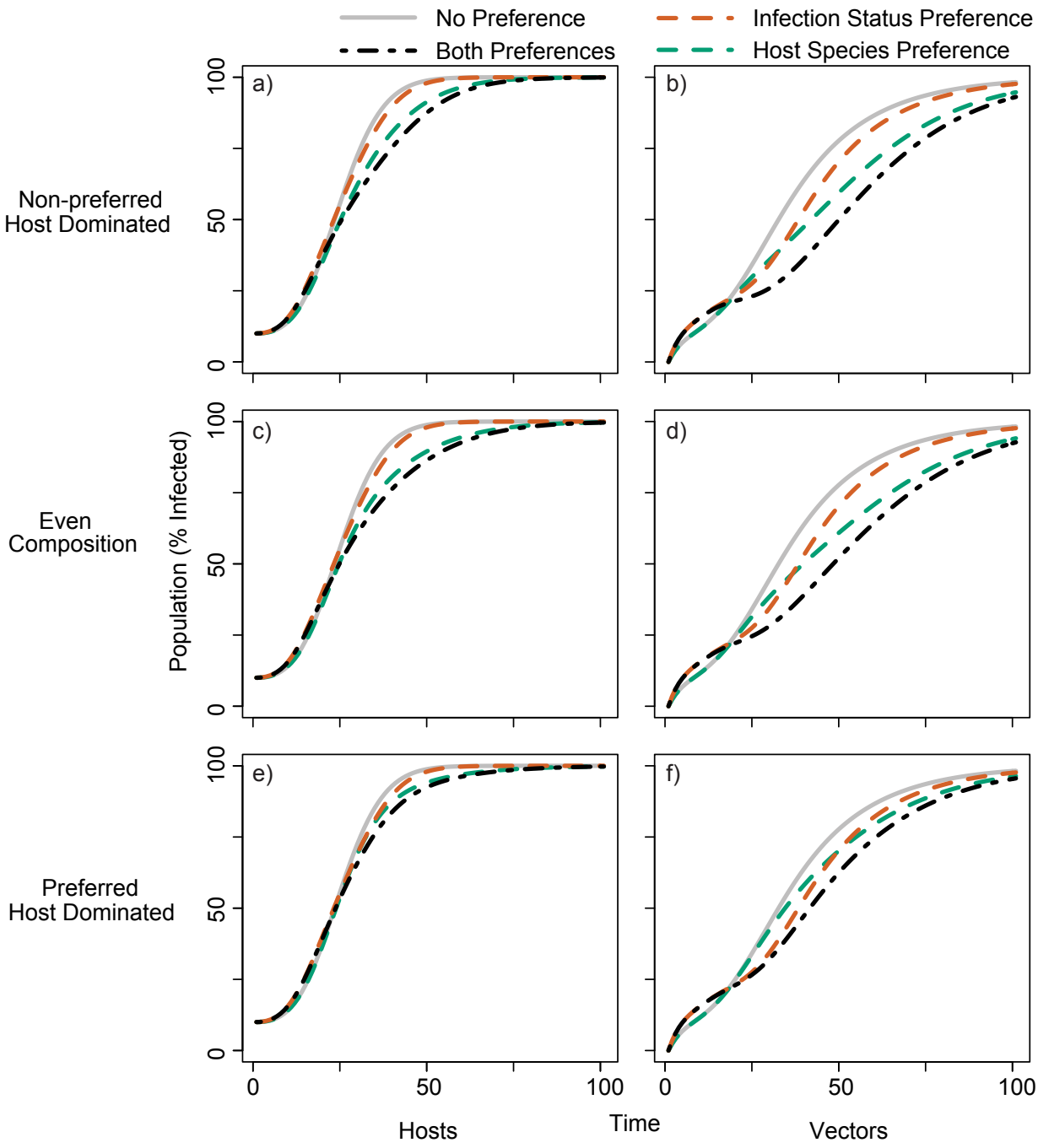

Figure S3: Pathogen spread through time in a two-host system comparing both types of pathogen-induced behavioral changes and their combined effects. Pathogen spread is shown for hosts (left column) and vectors (right column) for communities dominated by the non-preferred host (panels a and b), even community composition (panels c and d), and for communities dominated by the preferred host (panels e and f). The baseline case with no-pathogen induced behavioral changes is shown in grey, with pathogen preference for host infection status (non-infected versus infected hosts) shown in orange, pathogen preference for host species shown in green, and the combined effect of both preferences shown in black.

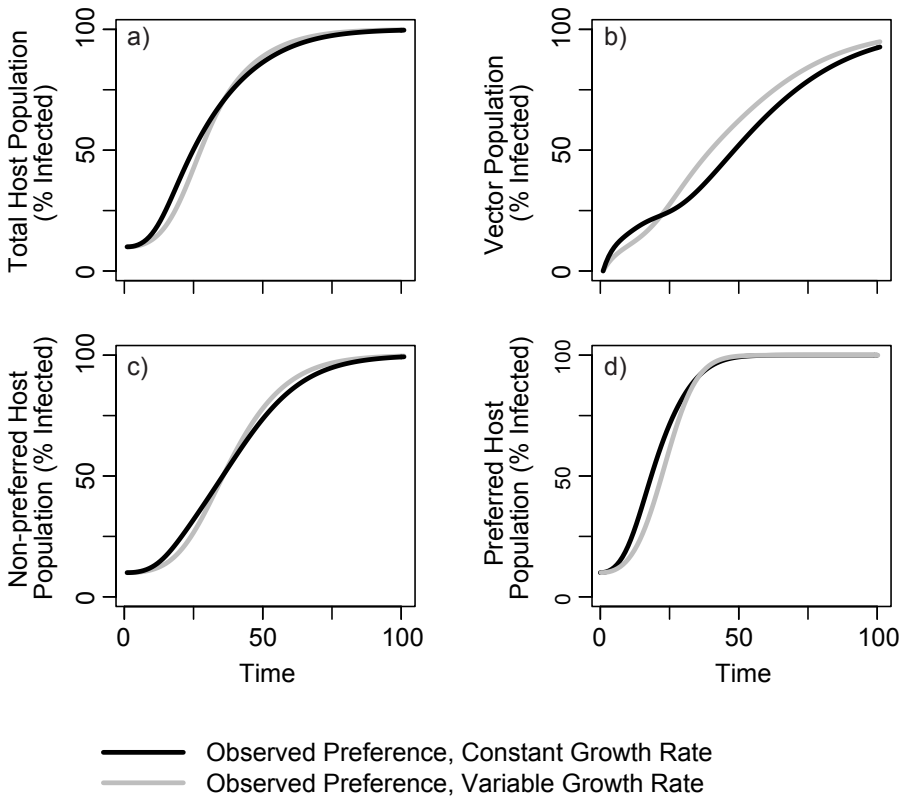

Figure S4: The effect of variable vector birth rates on annuals versus perennials. The same vector birth rate on annuals and perennials is shown in black, and a doubling of birth rate on annuals compared to perennials—as experimentally observed in (Borer *et al.*, 2009)—is shown in grey. All results are shown for a community composed of 50% preferred and 50% non-preferred hosts. Pathogen spread through time is shown for the entire host community (a), the vector population (b), the non-preferred host species (c), and the preferred host (d).

References

Borer, E. T., Adams, V. T., Engler, G. A., Adams, A. L., Schumann, C. B. & Seabloom, E. W. (2009). Aphid fecundity and grassland invasion: invader life history is the key. *Ecological Applications*, 19, 1187–1196.