Supplementary Information

Plant Viruses Alter Insect Behavior to Enhance their Spread

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Supplementary Methods

Determination of virus infection status of plants and aphids. Virus-infection status of plants and aphids was determined using a reverse transcription PCR procedure.

RNA extraction. An adapted Dellaporta nucleic acid extraction method[42] was used to extract total RNA from plants used in the bioassays and samples of aphids from the membrane feeding dishes. For each plant sample, approximately 0.03-0.05 g of tissue were processed. Aphids were individually processed. Samples were placed in a 1.5 mL tube and ground in 400 µL of Dellaporta I extraction buffer (containing 1 mL of 100 mM Tris, pH 8.0, 1 mL of 50 mM EDTA, 1.25 mL 500 mM NaCl, 10 µL β-mercaptoethanol, and 6.75 mL of DEPC water). After grinding, 52.8 µL of 10% SDS was added to each sample, vortexed and incubated at 18 °C for 10 min. After incubation, 128 µL of 5M potassium acetate solution was added to each sample, vortexed and centrifuged at 12000 rpm for 10 min. The supernatant from each tube was removed to a fresh tube and centrifuged for another 10 min at 12000 rpm. The supernatant was transferred to a new tube and 240 µL of cold isopropanol was added. Samples were inverted and held on ice for one hour to allow the RNA precipitation. After one hour, samples were centrifuged for 20 min at 12000 rpm and 10 °C. The supernatant was discarded, 800 µL of cold 70% ethanol added, and centrifuged again for 10 min at 12000 rpm and 10 °C. The supernatant was discarded and the pellet air dried overnight. The RNA was then re-suspended in 80 µL DEPC treated water.

Reverse transcription (RT). The reverse transcription reaction used 2.4 µL of RNA extract from either the insect or plant samples. The RNA was denatured on a Multigene Labnet thermal cycler at 70 °C for 5 min. To each reaction, 6 µL of 5X RNA extraction buffer and 0.6 µL of

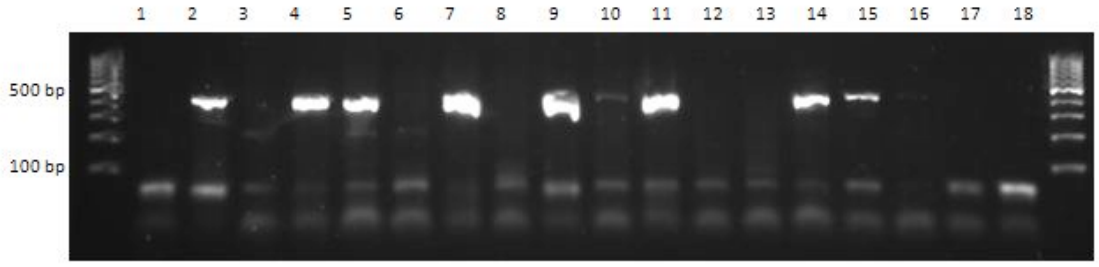
SuperScript II RNase H^- Reverse Transcriptase (Invitrogen Life Technologies, Frederick, MD, USA) was added, along with 12 µL of 2.5 mM dNTPs, 3 µM random hexamer primers, 0.3 µL RNAse Out and 7.5 µL of ddH_2O for a total reaction volume of 30 µL. The samples were then returned to the thermal cycler and ran on an RT-ST program for 90 min. The program was set up as follows: 25°C for 2 min, then increasing the temperature 1°C every 30 s up to 42 °C, hold at 42 °C for 45 min, then increasing the temperature 1 °C every 2 min up to 70 °C, finally holding the samples at 70 °C for 10 min.

Polymerase Chain Reaction (PCR). The total reaction volume was 20 µL. Each PCR reaction included 2 µL of RT product, 2 µL 10X PCR buffer and 0.30 µL My taq (New England Biolabs Inc., Ispwich, MA, USA), 2 µL 2.5mM dNTPs, 1.6 µL of 5 µM forward primer (5'-ATG AAT TCA GTA GGY CGT AGA-3'), 1.6 µL of 5 µM reverse primer (5'-CCC ARG GCT GAT TGC TTG CA-3') and 10.50 µL ddH_20. The primers are designed to produce a band at 411 bp indicating the presence of BYDV-PAV. The samples were amplified on the Labnet Thermal Cycler with the following PCR conditions: 95 °C for 2 min, followed by 11 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 2 min 30 s, followed by 22 cycles of 95 °C for 30 s, 52 °C for 30 s and 72 °C for 2 min 30 s concluded with a hold at 72 °C for 7 min.

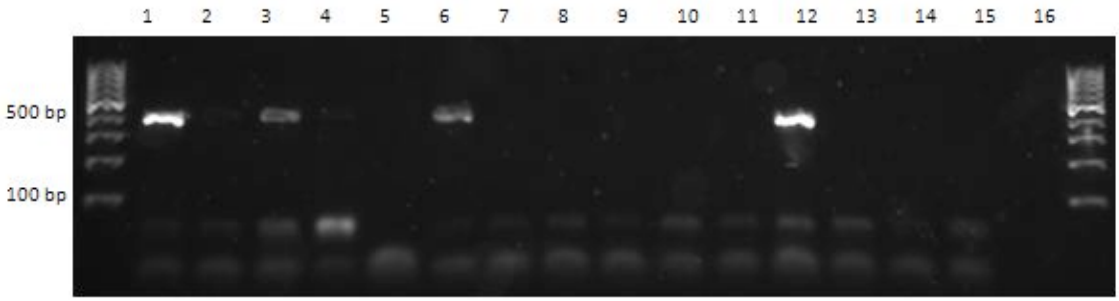
Analysis of amplified product. PCR products were analyzed using gel electrophoresis on a 1.2% agarose gel using GelStar™ nucleic acid gel stain (Lonza Group Ltd, Basel, Switzerland) in a 1X TBE buffer. The products were visualized under UV illumination using AlphaEase FC Software (Alpha Innotech Corporation, Santa Clara, CA, USA). Fragmented sizes were determined by comparison with a 100 bp DNA ladder (Fermentas Life Sciences, Glen Burnie,

MD, USA). Results demonstrated the presence of BYDV in all of the inoculated plants, and no presence of BYDV in sham-inoculated plants (Fig. S1). Samples of aphids obtained from membrane chambers, showed that aphids fed on membranes with diet containing BYDV successfully acquired the virus and that aphids fed only on the amino acid-sucrose solution did not contain BYDV (Fig. S2).

Supplementary figures and captions



Supplemental Figure S1. Agarose gel analysis of a subset of the plants used in the dual-choice bioassays. Lanes 2, 4, 5, 7, 9, 11 and 14 are from samples of plants inoculated with infective aphids showing the 411-bp BYDV-PAV band indicating successful virus inoculation. Lanes 1, 3, 6, 8, 10, 12 and 13 are from samples of plants inoculated with noninfective aphids, representing sham-inoculated plants. Lane 15 is a positive control, using plant tissue from the infective aphid colony. Lane 16 is a negative control, using plant tissue from the noninfective aphid colony. Lanes 17-18 are negative controls from the RT and PCR reactions.



Supplemental Figure S2. Agarose gel analysis of a subset of the individual aphids from membrane feeding assays. Lanes 1, 3 and 6 are from aphids fed on the membrane with amino acid and sucrose solution diet infused with purified BYDV, showing the 411-bp BYDV-PAV band indicating successful virus acquisition. Lanes 2, 4, 5, and 7 to 11 are from noninfective aphids fed on the membrane with amino acid and sucrose solution diet. Lane 12 is a positive control (an infective aphid from the virus-infected colony) showing the 411-bp BYDV-PAV band. Lane 13 is a negative control (a noninfective aphid from the noninfected aphid colony). Lanes 14 and15 are negative controls from the RT and PCR reactions. Lane 16 was left empty.

Supplementary Table

Supplemental Table S1: Output from the model examining the effects of virus acquisition on host plant selection behavior by aphid vectors at the first observation point, 12 h after aphid release. (PROC GENMOD, binomial distribution, logit link transformation, assuming compound symmetry). Panel (a) displays indirect effects of virus acquisition and responses to the BYDV-infected plant treatment. Panel (b) displays indirect effects of virus acquisition and responses to the sham-inoculated plant treatment. Panel (c) displays direct effects of virus acquisition and responses to the BYDV-infected plant treatment. Panel (d) displays direct effects of virus acquisition and responses to the sham-inoculated plant treatment.

Score Statistics for Type 3 GEE Analysis

a

Source	DF	Chi-Square	Pr > ChiSq
Rep	11	18.41	0.0726
Aphid	1	3.12	0.0774

b

Source	DF	Chi-Square	Pr > ChiSq
Rep	11	18.41	0.0726
Aphid	1	3.12	0.0774

c

Source	DF	Chi-Square	Pr > ChiSq
Rep	11	20.66	0.0371
Aphid	1	4.24	0.0394

d

Source	DF	Chi-Square	Pr > ChiSq
Rep	11	22.75	0.0192
Aphid	1	5.64	0.0176

The aphid responses at the first observation point in the bioassay reflect the overall response of the insect treatments. The aphid responses are significant [marginally significant in panels (a) and (b], indicating a difference in host plant preference between infective and noninfective aphids as early as 12 h after release. The replicate factor is significant [marginally significant in panels (a) and (b)], indicating some variation in the response of aphid treatments among the 12 replicates performed.

Supplemental Table S2: Output from the full model examining the effects of virus acquisition on host plant selection behavior by aphid vectors, pooling all observations made throughout the 72 h period. (PROC GENMOD, binomial distribution, logit link transformation, assuming compound symmetry). Panel (a) displays indirect effects of virus acquisition and responses to the BYDV-infected plant treatment. Panel (b) displays indirect effects of virus acquisition and responses to the sham-inoculated plant treatment. Panel (c) displays direct effects of virus acquisition and responses to the BYDV-infected plant treatment. Panel (d) displays direct effects of virus acquisition and responses to the sham-inoculated plant treatment.

Score Statistics for Type 3 GEE Analysis

a

Source	DF	Chi-Square	Pr > ChiSq
Rep	11	22.14	0.0233
Aphid	1	19.33	<0.0001
Rep*Aphid	11	22.49	0.0208
Time	5	4.96	0.4203
Aphid*Time	5	1.90	0.8626

b

Source	DF	Chi-Square	Pr > ChiSq
Rep	11	21.35	0.0299
Aphid	1	20.14	<0.0001
Rep*Aphid	11	22.36	0.0217
Time	5	2.15	0.8282
Aphid*Time	5	2.26	0.8123

c

Source	DF	Chi-Square	Pr > ChiSq
Rep	11	20.04	0.0448
Aphid	1	16.18	<0.0001
Rep*Aphid	11	21.65	0.0272
Time	5	2.41	0.7906
Aphid*Time	5	4.04	0.5444

d

Source	DF	Chi-Square	Pr > ChiSq
Rep	11	19.56	0.0517
Aphid	1	16.32	<0.0001
Rep*Aphid	11	21.03	0.0331
Time	5	3.66	0.5995
Aphid*Time	5	5.72	0.3349

The majority of the variation in all the models is described by the main effect of the aphid treatment. The replicate and replicate by aphid interactions are significant [marginally significant in panel (d)], indicating some variation in the response of aphid treatments among the 12 replicates performed. There were no effects of the time at which observations were made during

either of the experiments. Light and dark observations were examined with the model separately and no significant interactions were observed, thus results were pooled in the overall analysis.

Supplementary References

42 Dellaporta, S., Wood, J. & Hicks, J. A plant DNA minipreparation: Version II. *Plant Mol. Biol. Rep.* **1**, 19–21 (1983).