

Supplemental Information for:

An aphid symbiont confers protection against a specialized RNA virus, another increases vulnerability to the same pathogen

Clesson H.V. Higashi¹, William L. Nichols¹, Germaine Chevignon¹², Vilas Patel V¹, Suzanne E. Allison¹³, Kyungsun L. Kim¹, Michael R. Strand¹ & Kerry M. Oliver¹

¹Department of Entomology, University of Georgia, Athens GA 30602 USA

²Present address: Laboratory of Genetics and Pathology of Marine Mollusca, French Research Institute for Exploitation of the Sea, France

³Present address: Department of Biology, Hollins University, Roanoke, VA 24020

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Table S1: Percentage of RNAseq reads mapped to reference genome.

References	A2C	NY26	AS3	AS3AB	ZA17
<i>H. defensa</i>	0.471%	0.155%	0.167%	0.002%	0.098%
<i>B. aphidicola</i>	1.795%	1.744%	1.247%	1.126%	1.319%
APV	0.126%	0.009%	25.851%	25.913%	14.970%
<i>A. pisum</i>	86.123%	88.434%	54.201%	58.878%	71.272%
Unmapped	11.485%	9.658%	18.533%	14.080%	12.341%

Table S2A. Maternal transmission rate of APV from aphids with and without *H. defensa*

Line	Symbiont infection	Mother (or Rep)	% Offspring with APV (n=11)	% Offspring with <i>H. defensa</i> (n=10)	Within group Fisher's Exact	Overall infection rate	Between group Fisher's Exact
ND18 APV+	Ham-	1	36%	-	P = 0.450	31%	P = 0.548
		2	27%	-			
		3	45%	-			
		4	9%	-			
		5	36%	-			
ND18 APV+	Ham+	1	45%	100%	P = 0.597	38%	
		2	18%	100%			
		3	45%	100%			
		4	36%	100%			
		5	45%	100%			

Table S2B: Horizontal transmission of APV by aphids feeding on host plant.

Donor (color)	Recipient (color)	Replicate	Infection at wk 1 # infected / # tested	Infection at wk 3 # infected / # tested
ND18 APV+ (Green)	WI246-8 UI (Pink)	1	6/8	8/8
		2	4/8	8/8
		3	6/8	8/8
WI246-8APV+ (Pink)	WI576N-27 UI (Green)	1	4/8	8/8
		2	7/8	8/8
		3	5/8	8/8
WI246-8APV+ (Pink)	ND18 UI (Green)	1	7/8	8/8
		2	6/8	8/8
		3	7/8	8/8
WI246-8APV+ (Pink)	ND18+H3 (Green)	1	4/8	8/8
		2	5/8	7/8
		3	5/8	8/8
ND18APV+ (Green)	LSR1-AB (Pink)	1	4/8	8/8
		2	6/8	8/8
		3	8/8	7/8
ND18APV+ (Green)	LSR1-Ri (Pink)	1	8/8	7/8
		2	7/8	8/8
		3	4/8	8/8

Table S3: *A. ervi* can facilitate the transfer of APV between aphids

Aphid recipient line & infection status	Parasitism group	Parasitism sequence (Ct value)		
		1 st aphid	2 nd aphid	3 rd aphid
ND18 APV- / Ham-	A	Negative	Negative	Negative
	B	Negative	Negative	Negative
	C	Negative	Negative	Negative
	D	Negative	Negative	Negative
	E	POSITIVE (Ct=22.09)	Negative	Negative
	F	Negative	Negative	Negative
	G	Negative	Negative	Negative
	H	POSITIVE (Ct=21.85)	Negative	Negative
ND18 APV- / Ham+	A	Negative	Negative	Negative
	B	Negative	Negative	Negative
	C	POSITIVE (Ct=23.92)	Negative	Negative
	D	Negative	Negative	Negative
	E	Negative	Negative	Negative
	F	Negative	Negative	Negative
	G	Negative	Negative	Negative
	H	Negative	Negative	Negative

Negative – Aphid not infected with APV indicated by diagnostic qPCR

Positive – Aphid infected with APV indicated by diagnostic qPCR

Table S4: Logistic regression analysis of fungal assays. N = 10 replicates, each with 10 individual aphids for each line.

Comparison	Line	Contrast	Variable	LRT (L-R ChiSq; Prob>ChiSq)
A	LSR01.LSR	Regi-/APV- vs Regi-/APV+	Survival	$F_{\text{Regi-APV+}} = 29.595, P < 0.0001$
			Sporulation	$F_{\text{Regi-APV+}} = 2.455, P = 0.117$
			Dual Mortality	$F_{\text{Regi-APV+}} = 7.521, P = 0.006$
B	LSR01.LSR	Regi-/APV- vs Regi+/APV+	Survival	$F_{\text{Regi+APV-}} = 19.594, P < 0.001$
			Sporulation	$F_{\text{Regi+APV-}} = 27.108, P < 0.0001$
			Dual Mortality	$F_{\text{Regi+APV-}} = 0.287, P = 0.592$
C	LSR01.LSR	Regi+/APV+ vs Regi+/APV-	Survival	$F_{\text{Regi+APV+}} = 0.085, P = 0.771$
			Sporulation	$F_{\text{Regi+APV+}} = 6.736, P = 0.009$
			Dual Mortality	$F_{\text{Regi+APV+}} = 4.269, P = 0.039$
D	LSR01.LSR	Regi+/APV+ vs Regi-/APV+	Survival	$F_{\text{Regi-APV+}} = 84.894, P < 0.0001$
			Sporulation	$F_{\text{Regi-APV+}} = 80.219, P < 0.0001$
			Dual Mortality	$F_{\text{Regi-APV+}} = 0.022, P = 0.882$

Table S5: Logistic regression analysis of parasitism assays. N = 8 replicates, each with 20 individual aphids for each line.

Comparison	Line	Contrast	Variable	LRT (L-R ChiSq; Prob>ChiSq)
A	ND18	Ham-/APV- vs Ham-/APV+	Survival	$F_{\text{Ham- APV+}} = 0.0003, P = 0.985$
			Mummification	$F_{\text{Ham- APV+}} = 2.028, P = 0.154$
			Dual Mortality	$F_{\text{Ham- APV+}} = 4.390, P = 0.036$
B	ND18	Ham-/APV- vs Ham+/APV-	Survival	$F_{\text{HAM- APV-}} = 169.615, P < 0.001$
			Mummification	$F_{\text{HAM- APV-}} = 304.468, P < 0.001$
			Dual Mortality	$F_{\text{HAM- APV-}} = 10.309, P = 0.001$
C	ND18	Ham+/APV+ vs Ham+/APV-	Survival	$F_{\text{Ham- APV+}} = 2.189, P = 0.139$
			Mummification	$F_{\text{Ham- APV+}} = 1.058, P = 0.304$
			Dual Mortality	$F_{\text{Ham- APV+}} = 3.373, P = 0.066$
D	ND18	Ham+/APV+ vs Ham-/APV-INFECTED	Survival	$F_{\text{HAM- APV+}} = 206.265, P < 0.001$
			Mummification	$F_{\text{HAM- APV+}} = 307.421, P < 0.001$
			Dual Mortality	$F_{\text{HAM- APV+}} = 7.517, P = 0.0006$
E	576N-27	APV- vs APV+	Survival	$F_{\text{APV- APV+}} = 0.416, P = 0.519$
			Mummification	$F_{\text{APV- APV+}} = 0.326, P = 0.568$
			Dual Mortality	$F_{\text{APV- APV+}} = 3.320, P = 0.068$
F	246-8	APV- vs APV+	Survival	$F_{\text{APV- APV+}} = 0.230, P = 0.632$
			Mummification	$F_{\text{APV- APV+}} = 12.733, P = 0.0004$
			Dual Mortality	$F_{\text{APV- APV+}} = 717.323, P < 0.0001$
G	5D-AB	Ham-/APV- vs Ham-/APV+	Survival	$F_{\text{Ham- APV+}} = 0.848, P < 0.357$
			Mummification	$F_{\text{Ham- APV+}} = 0.059, P = 0.808$
			Dual Mortality	$F_{\text{Ham- APV+}} = 0.465, P = 0.496$
H	5D-AB	Ham-/APV- vs Ham+/APV-	Survival	$F_{\text{HAM- APV-}} = 169.615, P < 0.001$
			Mummification	$F_{\text{HAM- APV-}} = 7.482, P < 0.006$
			Dual Mortality	$F_{\text{HAM- APV-}} = 0.056, P = 0.813$
I	5D-AB	Ham+/APV+ vs Ham+/APV-	Survival	$F_{\text{Ham+ APV+}} = 1.966, P = 0.161$
			Mummification	$F_{\text{Ham+ APV+}} = 0.238, P = 0.626$
			Dual Mortality	$F_{\text{Ham+ APV+}} = 1.638, P = 0.201$
J	5D-AB	Ham+/APV+ vs Ham-/APV+	Survival	$F_{\text{HAM- APV+}} = 7.636, P < 0.006$
			Mummification	$F_{\text{HAM- APV+}} = 6.171, P < 0.013$
			Dual Mortality	$F_{\text{HAM- APV+}} = 0.130, P = 0.718$

Table S6: Statistical analysis using t-test to compare *Regiella insecticola* titers in APV- and APV+ LSR1 lines in developing aphids.

Aphid Age (Days Old)	Aphid Lines Compared (APV- vs APV+)		t - ratio	df	P value
	LSR1-Ri Mean \pm SE (n)	LSR1.RiAPV+ Mean \pm SE (n)			
2	1.02 \pm 0.06 (8)	0.58 \pm 0.09 (8)	3.979	14	0.001
4	1.23 \pm 0.06 (8)	0.88 \pm 0.06 (8)	4.249	14	0.001
8	1.59 \pm 0.13 (8)	1.09 \pm 0.10 (8)	2.989	14	0.009
16	2.43 \pm 0.09 (8)	2.44 \pm 0.11 (8)	-0.106	14	0.917

Table S7: Statistical analysis using t-test to compare *Hamiltonella defensa* and APSE3 titers in APV- and APV+ ND18 lines developing aphids.

Target	Aphid Age (Days Old)	Aphid Lines Compared (APV- vs APV+)		t - ratio	df	P value
		ND18.H3 Mean \pm SE (n)	ND18.H3APV+ Mean \pm SE (n)			
A) <i>H. defensa</i>	2	0.31 \pm 0.36 (6)	0.29 \pm 0.19 (6)	0.111	10	0.914
	4	0.38 \pm 0.10 (6)	0.16 \pm 0.10 (6)	1.494	10	0.166
	8	0.69 \pm 0.18 (6)	0.55 \pm 0.15 (6)	0.620	10	0.549
	16	2.49 \pm 0.13 (6)	2.23 \pm 0.09 (6)	1.646	10	0.131
B) APSE3	2	0.87 \pm 0.15 (6)	0.60 \pm 0.12 (6)	1.448	10	0.178
	4	0.86 \pm 0.13 (6)	0.79 \pm 0.09 (6)	0.397	10	0.699
	8	1.35 \pm 0.27 (6)	1.74 \pm 0.18 (6)	-1.202	10	0.257
	16	1.26 \pm 0.09 (6)	1.44 \pm 0.18 (6)	-0.940	10	0.369

Table S8: Statistical analysis using t-test to compare *Hamiltonella defensa* and APSE2 titers in APV- and APV+ 5D-AB lines in developing aphids.

Target	Aphid Age (Days Old)	Aphid Lines Compared (APV- vs APV+)		t - ratio	df	P value
		5D-AB.H2 Mean \pm SE (<i>n</i>)	5D-AB.H2APV+ Mean \pm SE (<i>n</i>)			
A) <i>H. defensa</i>	2	0.66 \pm 0.19 (8)	0.52 \pm 0.19 (8)	0.875	14	0.397
	4	0.88 \pm 0.09 (8)	0.74 \pm 0.07 (8)	1.705	14	0.110
	8	1.13 \pm 0.09 (8)	1.45 \pm 0.06 (8)	-2.087	14	0.056
	16	2.61 \pm 0.04 (8)	2.44 \pm 0.08 (8)	0.606	14	0.554
B) APSE2	2	1.25 \pm 0.12 (8)	0.97 \pm 0.09 (8)	1.774	14	0.098
	4	1.25 \pm 0.16 (8)	1.16 \pm 0.06 (8)	0.495	14	0.629
	8	1.37 \pm 0.13 (8)	1.32 \pm 0.15 (8)	0.238	14	0.815
	16	1.70 \pm 0.06 (8)	1.28 \pm 0.06 (8)	4.62	14	0.0004

Table S9: Statistical analysis using t-test to compare APV RNA abundance in LSR1 aphids with and without *R. insecticola*.

Aphid Age (Days Old)	Aphid Lines Compared (Ri- vs Ri+)		t - ratio	df	P
	LSR1-AB Mean \pm SE (<i>n</i>)	LSR1.Ri Mean \pm SE (<i>n</i>)			
2	1.85 \pm 0.45 (8)	1.20 \pm 0.69 (8)	-0.796	12.067	0.442
4	4.10 \pm 0.57 (8)	3.09 \pm 0.63 (8)	-1.197	13.839	0.252
8	6.55 \pm 0.26 (8)	5.30 \pm 0.43 (8)	-2.487	11.480	0.029
16	6.96 \pm 0.44 (8)	4.85 \pm 0.26 (8)	-4.087	11.326	0.002

Table S10: Statistical analysis using t-test to compare APV RNA abundance in ND18 aphids with and without *H. defensa*/APSE2.

Aphid Age (Days Old)	Aphid Lines Compared (APV- vs APV+)		t - ratio	df	P
	ND18APV+ Mean \pm SE (<i>n</i>)	ND18.H3APV+ Mean \pm SE (<i>n</i>)			
2	2.93 \pm 0.41 (6)	3.55 \pm 0.24 (6)	1.287	8.154	0.233
4	6.55 \pm 0.35 (6)	6.70 \pm 0.11 (6)	-1.184	6.062	0.280
8	7.47 \pm 0.10 (6)	7.61 \pm 0.08 (6)	-0.913	9.430	0.383
16	6.99 \pm 0.18 (6)	6.43 \pm 0.19 (6)	2.091	9.899	0.063

Table S11: Statistical analysis using t-test to compare APV RNA abundance in 5D-AB aphid with and without *H. defensa*/APSE2.

Aphid Age (Days Old)	Aphid Lines Compared (APV- vs APV+)		t - ratio	df	P
	5D-AB.H2 Mean \pm SE (<i>n</i>)	5D- AB.H3APV+ Mean \pm SE (<i>n</i>)			
2	2.45 \pm 0.50 (8)	5.40 \pm 0.25 (8)	-5.324	10.455	0.0003
4	5.88 \pm 0.31 (8)	6.70 \pm 0.22 (8)	-2.163	12.461	0.051
8	6.40 \pm 0.18 (8)	6.84 \pm 0.16 (8)	-1.797	13.795	0.094
16	5.67 \pm 0.43 (8)	6.47 \pm 0.41 (8)	-1.337	13.998	0.201

MOLECULAR ECOLOGY

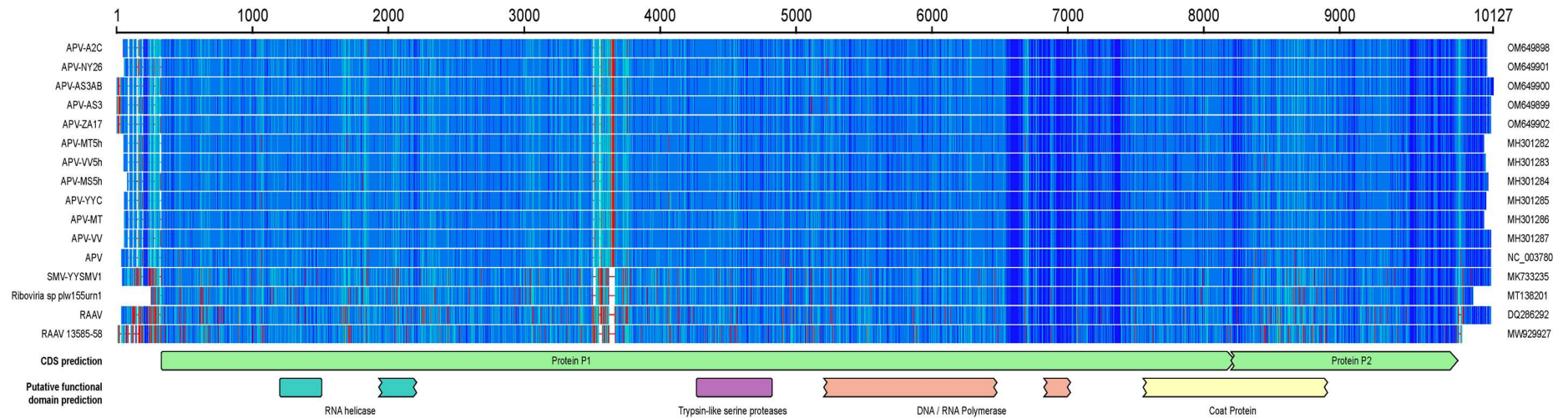


Figure S1: MAFFT whole genome alignment of APV isolates.

MOLECULAR ECOLOGY

Strain	APV USA					APV China						APV	SMV	Riboviria sp	RAAV	
	A2C	NY26	AS3AB	AS3	ZA17	MT5h	VV5h	M55h	YYC	MT	VV	/	YYSMV1	plw155um1	/	13585-58
APV USA	A2C	99,990	99,990	99,980	99,830	92,520	92,527	92,476	92,508	92,497	92,494	93,589	79,908	79,992	77,769	77,319
	NY26	99,990	100,000	99,990	99,840	92,518	92,525	92,486	92,508	92,497	92,504	93,595	79,906	79,992	77,766	77,305
	AS3AB	99,990	100,000	99,732	99,801	92,520	92,527	92,491	92,508	92,497	92,494	93,592	79,940	79,992	77,816	77,161
	AS3	99,980	99,990	99,732	99,632	92,530	92,537	92,501	92,518	92,507	92,534	93,632	79,990	80,002	77,865	77,101
	ZA17	99,830	99,840	99,801	99,632	92,520	92,527	92,491	92,508	92,497	92,524	93,622	79,990	80,002	77,885	77,191
APV China	MT5h	92,520	92,518	92,520	92,530	92,520	100,000	99,950	99,920	99,900	99,900	94,004	80,316	80,506	77,944	77,493
	VV5h	92,527	92,525	92,527	92,537	92,527	100,000	99,950	99,920	99,900	99,900	94,009	80,314	80,506	77,964	77,493
	M55h	92,476	92,486	92,491	92,501	92,491	99,950	99,950	99,970	99,950	99,950	94,005	80,330	80,516	77,928	77,594
	YYC	92,508	92,508	92,508	92,518	92,508	99,920	99,920	99,970	99,980	99,980	94,020	80,318	80,516	77,968	77,494
	MT	92,497	92,497	92,497	92,507	92,497	99,900	99,900	99,950	99,980	100,000	94,011	80,308	80,516	77,965	77,498
	VV	92,494	92,504	92,494	92,534	92,524	99,900	99,900	99,950	99,980	100,000	94,031	80,377	80,516	78,045	77,506
APV	/	93,589	93,595	93,592	93,632	94,004	94,009	94,005	94,020	94,011	94,031	80,056	80,218	77,970	77,578	
SMV	YYSMV1	79,908	79,906	79,940	79,990	79,990	80,316	80,314	80,330	80,318	80,308	80,377	80,056	98,292	78,799	78,191
Riboviria sp	plw155um1	79,992	79,992	79,992	80,002	80,002	80,506	80,506	80,516	80,516	80,516	80,516	80,218	98,292	78,596	78,940
RAAV	/	77,769	77,766	77,816	77,865	77,885	77,944	77,964	77,928	77,968	77,965	78,045	77,970	78,799	78,596	81,746
	13585-58	77,319	77,305	77,161	77,101	77,191	77,493	77,493	77,594	77,494	77,496	77,506	77,578	78,191	78,940	81,746

Figure S2: Amino acid similarity matrix among all APV (grouped by region) and related viruses compared in this study.

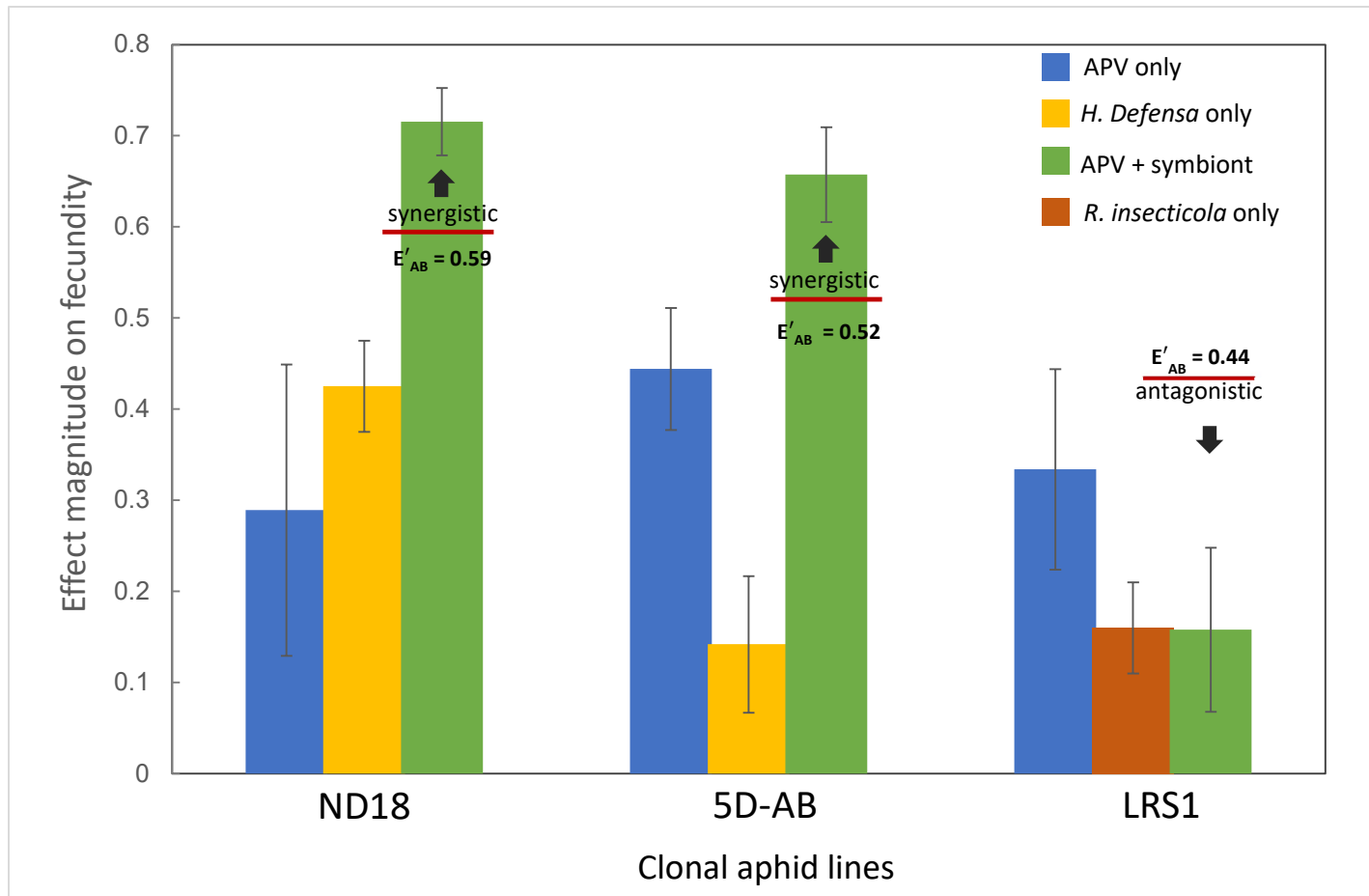


Figure S3: Effect magnitudes with 95% confidence intervals of carrying APV, facultative symbionts (*H. defensa* or *R. insecticola*) or both APV and symbiont on aphid fecundity. Values above the horizontal red line (E'_{AB}) indicate super-additive effects (i.e. synergism), while those below indicate sub-additive effects (i.e. antagonism) for aphid lines housing both microbes (E_{AB}).

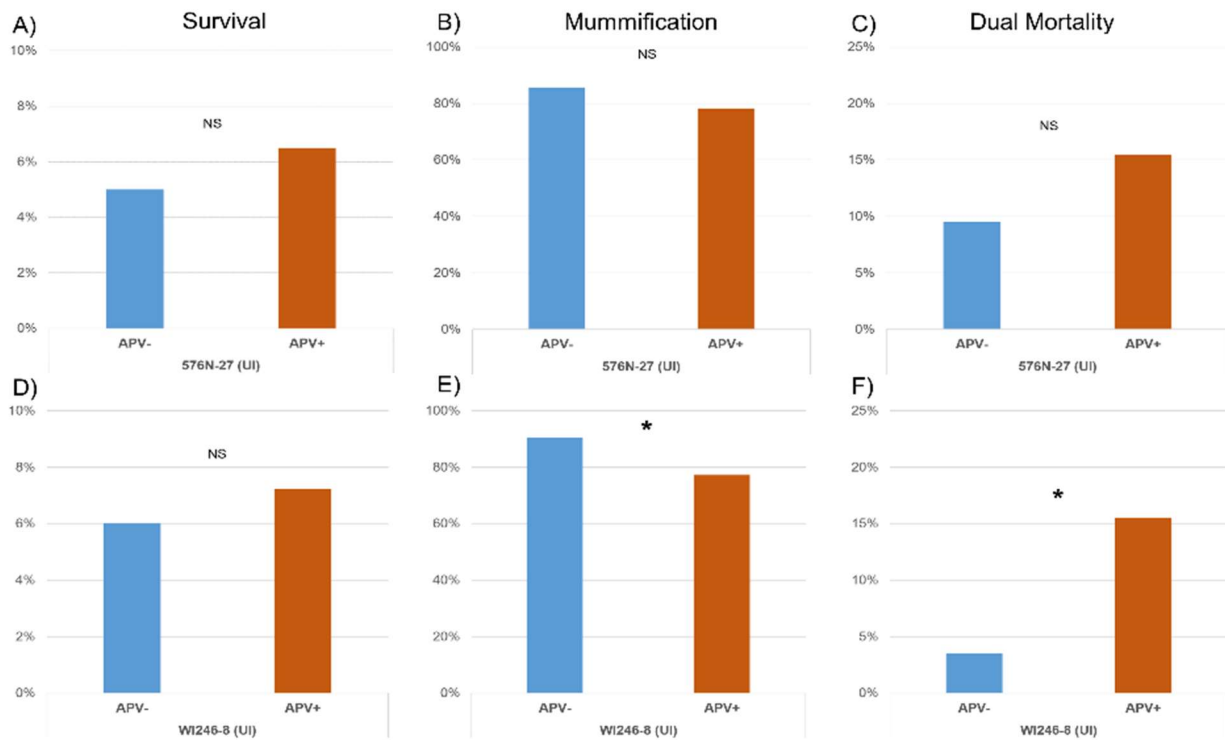


Figure S4: Aphid survival, mummification and dual-mortality of uninfected (UI) aphid lines 576N-27 and WI246-8 following parasitism by the wasp *A. ervi*. Parasitism outcomes were contrasted between aphid with and without APV. Asterisk(s) above bars indicate significant differences (NS = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; **** = $P \leq 0.0001$).