1   *Supporting Online Material for*

2   ***Wolbachia* in a Major African Crop Pest Increases Susceptibility to Viral**

3   **Disease Rather than Protects**

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8   **This PDF file includes:**

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10   **SF TABLES (PAGES # - #)**

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20   **SF FIGURES (PAGES**

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24 **Table S1.** Determinants of overt viral deaths in *S. exempta* across outbreaks.

Term	Coef	s.e.m.	d.f.	Δ deviance	F	P-value
Season *	-2.799	0.272	1	127.27	13.01	**0.0007**
Head-capsule width (mean)	4.717	0.558	1	139.18	14.23	**0.0004**
Wolbachia prevalence †	4.195	0.436	1	117.44	12.01	**0.0011**
Excluded terms						
Larval density			1	24.63	3.49	0.0680
Outbreak number			1	6.52	0.62	0.4363
Date ‡			1	11.56	1.17	0.2847

25 * model simplification revealed two levels: 2007/08 vs 2009/10 and 2010/11; † square-root

26 transformed; ‡ days since January 1st of calendar year.

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28 The dependent variable in this analysis was the prevalence of virus-dead larvae observed across

29 each of 59 outbreaks, where a minimum of thirty standardized counts were conducted; eight

30 observations were excluded due to missing data. The proportion of dead larvae across all counts

31 was weighted by the total number of larvae counted to control for variation in sampling

32 intensity. Analysis was by generalized linear model with binomial errors and logit link function.

33 To account for over-dispersion, an empirical dispersion parameter and *F*-tests were used.

34 Parameter estimates in the minimal model are for explanatory terms when fitted last in the

35 model. All other terms were excluded with $P > 0.05$ when fitted last in the model. Final model:

36 intercept = -15.918 $\pm$ 1.244; null deviance = 848.60 (50 d.f.); residual deviance = 312.62 (47

37 d.f.). None of the interactions between the main effects were statistically significant.

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Table S2. Determinants of overt viral deaths in *S. exempta* across outbreaks.

Term	Coef	s.e.m.	d.f.	Δ deviance	*F*	*P*-value
Season *	-3.663	0.331	1	159.35	16.68	**0.0002**
Head-capsule width (mean)	5.589	0.801	1	105.52	11.04	**0.0012**
(a) Each strain included in the model separately						
wExe1 prevalence †	10.305	0.863	1	186.97	7.77	**0.0076**
wExe2 prevalence †	3.356	0.355	1	100.73	8.08	**0.0066**
wExe3 prevalence †	0.371	0.645	1	0.32	0.01	0.9056
(b) All 3 strains included in the model together						
wExe1 prevalence †	9.603	0.907	1	151.34	15.84	**0.0002**
wExe2 prevalence †	4.696	0.537	1	90.32	9.45	**0.0036**
wExe3 prevalence †	5.923	0.887	1	39.16	4.10	**0.0489**
(c) Number of strains						
Number of *Wolbachia* strains	2.160	0.161	1	289.05	60.61	**<0.0001**

* 2 levels: 2007/08 vs 2009/10 and 2010/11; † square-root transformed

The dependent variable in this analysis was the prevalence of virus-dead larvae observed across each of 59 outbreaks; eight observations were excluded due to missing data (see legend to Table S1 for further details). In contrast to Table S1, this version of the model tested the significance of individual *Wolbachia* strains. Final model with all three strains included: intercept = -15.387 $\pm$ 1.960; null deviance = 848.60 (50 d.f.); residual deviance = 152.29 (45 d.f.).

47 **Table S3.** Determinants of viral load in *S. exempta* across outbreaks.

Term	Coef	s.e.m.	d.f.	Δ deviance	*F*	*P*-value
Larval density	-0.0055	0.0024	1	8.00	6.49	**0.0138**
Outbreak number	-0.0082	0.0393	1	12.97	10.53	**0.0021**
Wolbachia prevalence *	-1.7481	1.3588	1	6.10	4.96	**0.0306**
Outbreak no : *Wolbachia*	0.3642	0.1172	1	11.89	9.66	**0.0024**
Excluded terms						
Season †			1	1.02	0.36	0.3627
Head-capsule width (mean)			1	0.39	0.41	0.5239
Date ‡			1	11.38	1.27	0.2652

48 * square-root transformed; † 2 levels: 2007/08 vs 2009/10 and 2010/11; ‡ days since January 1st.

49 The dependent variable in this analysis was the mean $\log_{10}$-transformed viral load of apparently

50 healthy larvae observed across each of 59 outbreaks; two observations were excluded due to

51 missing data. Viral load was determined using SpexNPV-specific qPCR. Analysis was by

52 general linear model (GLM with Gaussian error and identity link). Final model: intercept =

53 3.592 ± 0.522; null deviance = 102.92 (56 d.f.); residual deviance = 64.05 (52 d.f.).

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56   **Table S4.** SpexNPV bioassay mortality analysis.

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Term	Coef	s.e.m.	d.f.	Δ deviance (X^2)	*P*-value
Bioassay *	1.117	0.238	1	24.22	**<0.0001**
Viral dose †	1.918	0.238	1	133.34	**<0.0001**
Wolbachia status ‡	1.711	0.239	1	58.81	**<0.0001**
Excluded terms					
Bioassay : Virus dose			1	1.01	0.3134
Bioassay : *Wolbachia* status			1	1.37	0.5053
Virus dose : *Wolbachia* status			1	0.13	0.7210

58   * 3 separate bioassays, two (bioassays 1 and 3) comparing mortality of *wExe1* infected larvae

59   with larvae made *Wolbachia*-free via tetracycline treatment (*wExe1$_{tet}$*) and one (bioassay 2)

60   comparing with *wExe1*-infected larvae with larvae from a line naturally free of *Wolbachia*;

61   model simplification revealed that bioassays 1 and 3 could be combined without reducing the

62   amount of variation explained. † log$_{10}$-transformed; ‡ *Wolbachia*-free or *Wolbachia*-infected.

63   The dependent variable in this analysis was the binary indicator of mortality or otherwise of

64   individual 4th instar larvae inoculated with one of a range doses of SpexNPV (0, 100, 500, 1000

65   and 5000 OB). Analysis was by generalized linear model with binomial errors and logit link

66   function. Parameter estimates in the minimal model are for explanatory terms when fitted last in

67   the model. All other terms were excluded with p > 0.05 when fitted last in the model. Final

68   model: intercept = -7.216 $\pm$ 0.715; null deviance = 717.85 (574 d.f.); residual deviance = 492.24

69   (574 d.f.).

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71 **Table S5.** SpexNPV bioassay summary data.

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Bioassay	Comparison *	LD_{50} (OB/larva) Wol+ †	LD_{50} (OB/larva) Wol- ‡	Virus yield (OB/larva) Wol+ §	Virus yield (OB/larva) Wol- §
1	*wExe1* (line 1) *vs* *wExe1$_{tet}$* (gen 2)	659	9,370	7.14×10^7	9.78×10^7
2	*wExe1* (line 2) *vs* *Wolbachia-free* stocks (gen 2)	196	1,539	7.38×10^7	8.72×10^7
3	*wExe1* (line 1) *vs* *wExe1$_{tet}$* (gen 3)	1,130	6,702	8.28×10^7	10.57×10^7

73 * 3 separate bioassays, two (bioassays 1 and 3) comparing mortality of *wExe1* infected larvae

74 with larvae made *Wolbachia*-free via tetracycline treatment (*wExe1$_{tet}$*) and one (bioassay 2)

75 comparing with *wExe1*-infected larvae with larvae from a line naturally free of *Wolbachia*; †

76 *Wolbachia*-infected; ‡ *Wolbachia*-free; § mean number of virus occlusion bodies (OB)

77 produced per infected cadaver.

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81 **Table S6.** SpexNPV bioassay survival analysis.

Term	Coef	s.e.	d.f.	Δ deviance (X^2)	*P*-value
Bioassay *	0.842	0.165	1	26.01	**<0.0001**
Viral dose †	1.267	0.157	1	71.67	**<0.0001**
Wolbachia status ‡	1.222	0.164	1	61.31	**<0.0001**
Excluded terms					
Bioassay : Virus dose			1	0.02	0.8865
Bioassay : *Wolbachia* status			1	0.06	0.7973
Virus dose : *Wolbachia* status			1	0.72	0.3977

82 * 3 separate bioassays, two (bioassays 1 and 3) comparing mortality of *wExe1* infected larvae

83 with larvae made *Wolbachia*-free via tetracycline treatment (*wExe1$_{tet}$*) and one (bioassay 2)

84 comparing with *wExe1*-infected larvae with larvae from a line naturally free of *Wolbachia*;

85 model simplification revealed that bioassays 1 and 3 could be combined without reducing the

86 amount of variation explained. † log$_{10}$-transformed; ‡ *Wolbachia*-free or *Wolbachia*-infected.

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88 The dependent variable in this analysis is the censored survival time from the bioassay

89 experiment. Thus, the response is the time to death where larvae that survival were censored at

90 day 11 post-infection (by which time all survivors had pupated). Analysis was by Cox's

91 proportional hazards model. Parameter estimates in the minimal model are for explanatory

92 terms when fitted last in the model. All other terms were excluded with *P* > 0.05 when fitted last

93 in the model. Final model: Likelihood ratio test: X^2_3 = 173, *P* <0.0001, r^2 = 0.293.

94 **Table S7.** Determinants of viral loads in bioassay experiments.

Term	Coef	s.e.	d.f.	Δ deviance	F	P-value
Speed of kill *	-4.769	0.5684	1	71.41	16.16	**<0.0001**
Wolbachia status †	-0.193	0.0319	1	35.79	8.10	**0.0050**
Excluded terms						
Virus dose ‡			1	1.73	0.39	0.5330
Bioassay			2	10.61	1.19	0.3068
Wolbachia status : Speed			1	5.03	1.14	0.2867

95   * speed of kill = 1 / number of days taken to die post infection; † *Wolbachia*-free or *Wolbachia*-

96   infected; ‡ $\log_{10}$-transformed.

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98   The dependent variable in this analysis was the count of virus occlusion bodies (OB) from

99   SpexNPV-killed larvae in the bioassays. Analysis was by generalised linear model (GLM with

100   Poisson errors and log link). To account for over-dispersion, an empirical dispersion parameter

101   and F-tests were used. Final model: intercept = 4.032 $\pm$ 0.088; null deviance = 858.84 (179 d.f.);

102   residual deviance = 745.89 (177 d.f.). Using these lab-generated data for virus yield, we can

103   calculate a lab-based proxy for the basic reproductive rate of the virus (R_0), based on the

104   production of occluded virus only. This was done by multiplying the mortality rate of the larvae

105   for a given dose of virus by the average yield of virus occlusion bodies for larvae infected with

106   that dose across each of the three bioassays. The comparison of R_0 is made simply by dividing

107   the estimate for Wol+ larvae by that for Wol- larvae for each dose. Averaged across the three

108   bioassays, R_0 ratios (Wol+/Wol-) for the four viral doses are as follows: 100 OB = 2.93 (range =

109   1.16 – 4.10), 500 OB = 2.96 (2.00 – 4.40), 1000 OB = 1.60 (1.05 – 1.87), 5000 OB = 1.27

110   (Bioassay 2 only).

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112   **Table S8.** Microbial species infecting *Spodoptera exempta* in outbreak populations.

113   A list of the microbial species identified in African armyworm samples using PCR-

114   DGGE.

| Isolate no.	BLASTN match	Bacteria genus	Nucleotide % identity	Habitat isolated
1	FJ390735.1	Uncultured bacterium	91%	soil
2	GQ489023.1	*Enterococcus faecium*	100%	environment
3	GQ453520.1	*Enterococcus faecium*	100%	environment
4	GQ299825	Uncultured bacterium	100%	environment
5	GQ383919.1	*Bacillus* sp.	100%	environment
6	FJ854700.1	Uncultured *beta proteobacterium*	100%	environment
7	GQ426315.1	*Pantoea sp.*	98%	rice plant
8	GQ383917.1	*Paenibacillus* sp.	98%	soil
9	AB522100.1	*Kineococcus radiotolerans*	100%	soil
10	GQ495647.1	*Curtobacterium* sp.	100%	soil

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119 **Figure S1.** Survival of *S. exempta* larvae from *Wolbachia*-infected (Wol+) and

120 *Wolbachia*-free (Wol–) lines following inoculation with SpexNPV.

Expected survival (specified covariates)
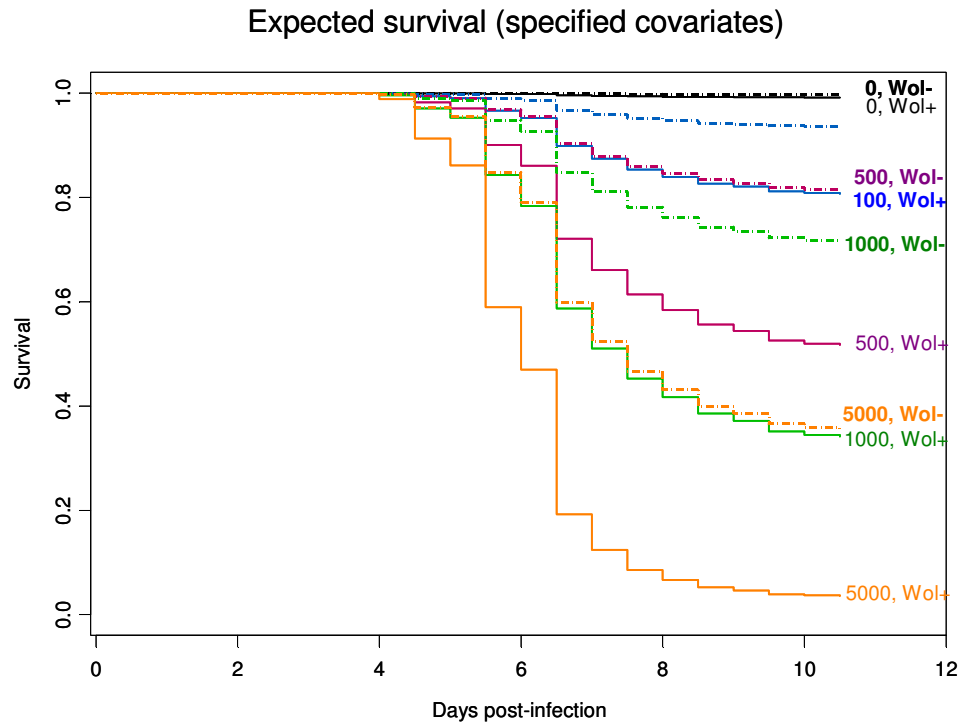
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123 Solid lines are the survival curves for *Wolbachia*-infected lines; dashed lines are for *Wolbachia*-

124 free lines. Different colours reflect different infecting doses (numbers next to lines).

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