Supplementary Materials: Parameters for Successful Parental RNAi as An Insect Pest Management Tool in Western Corn Rootworm, Diabrotica virgifera virgifera

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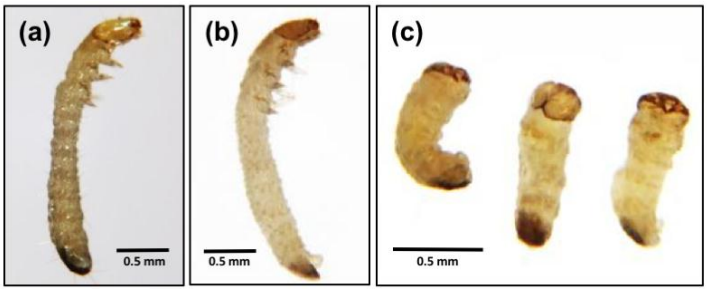

Figure S1. Parental *hunchback* dsRNA phenotypes in *D. v. virgifera*. *D. v. virgifera* females were fed with diet treated six times with 2 µg *hunchback* dsRNA. (**a**) Developed larva from a female fed with untreated diet; (**b**) Developed larva from an *GFP* dsRNA-fed female. (**c**) Larvae dissected from eggs of a *hb* dsRNA-fed female. The larvae exhibit a "hunchback" phenotype that includes fewer segments, missing limbs and deformed mouth parts.

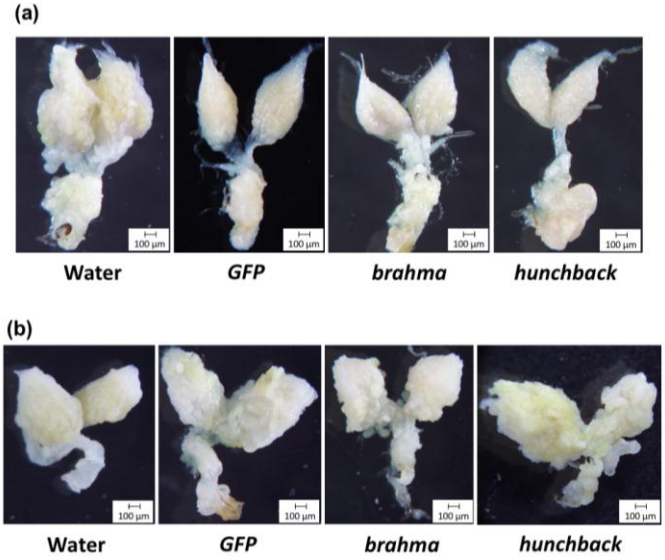

Figure S2. Ovaries of *hunchback* and *brahma* dsRNA-fed *D. v. virgifera*. Ovaries of females fed diet treated with water, 2 µg of GFP, *brahma* or *hunchback* dsRNA six times. Diet provided every other day for 12 days. Dissections performed one day after receiving the last dsRNA treatment. (**a**) Ovaries of females fed dsRNA before mating; (**b**) Ovaries of females fed dsRNA after four days of mating.

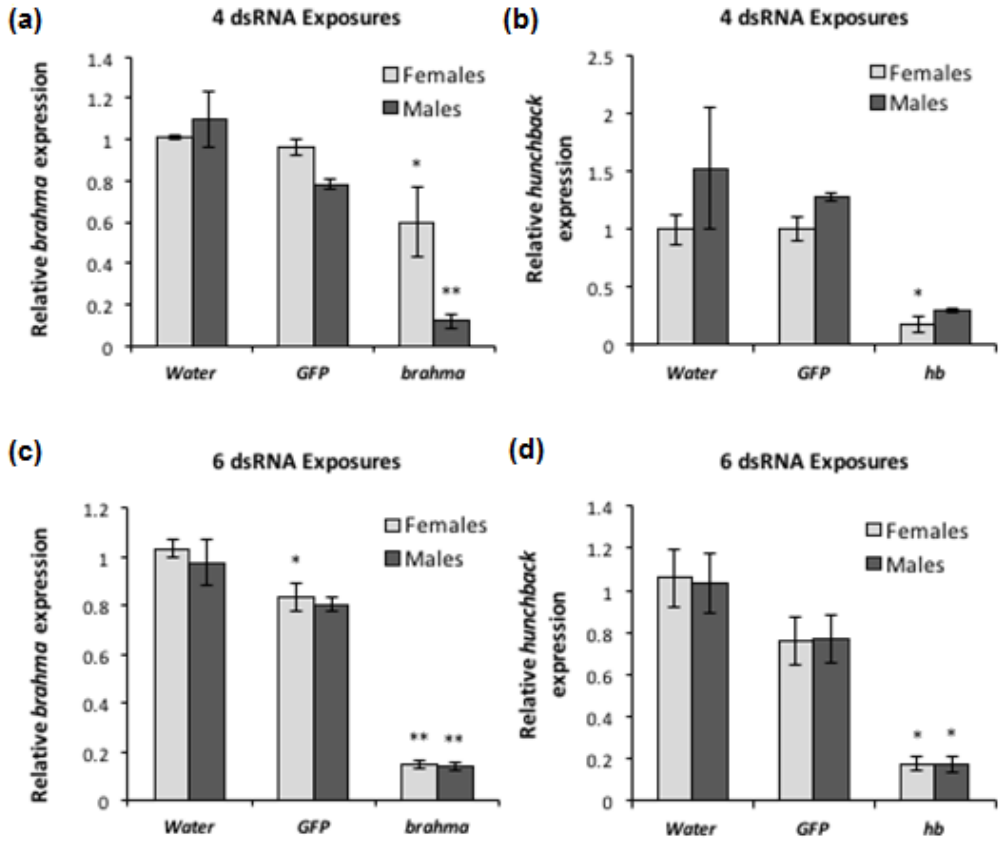

Figure S3. Comparison of relative transcript level for *brahma* (*brm*) and *hunchback* (*hb*) between *D. v. virgifera* females and males. (**a**) Relative *brahma* transcript expression for females and males exposed four times to *brm* dsRNA; (**b**) Relative *hunchback* transcript expression for females and males exposed four times to *hb* dsRNA; (**c**) Relative *brahma* transcript expression for females and males exposed six times to *brm* dsRNA; (**d**) Relative *hunchback* transcript expression for females and males exposed six times to *hb* dsRNA. Three biological replications per treatment and sex. Comparisons within each sex were performed with Dunnett's test (control group = water), * significance at $p < 0.05$. ** significance at $p < 0.001$. Comparisons between sexes within each treatment were performed with Student's *t*-test, * significant differences at $p < 0.05$.

Table S1. Primer pairs used to amplify DNA templates for *D. v. virgifera* dsRNA synthesis and qRT-PCR. Product size for dsRNA excludes T7 sequence [1].

Gene Name	Primer Sequences for dsRNA Synthesis				Product Length (bp)
brahma	Forward: TAATACGACTCACTATAGGGAACCTTCTTCATCTTCTG Reverse: TAATACGACTCACTATAGGGCTCTCCTAATACAGTTCAA				352
hunchback	Forward: TAATACGACTCACTATAGGGAAGTGTAAGCAATGTGATT Reverse: TAATACGACTCACTATAGGGTTATGGTACAAGGAGAGGA				405
GFP	Forward: TAATACGACTCACTATAGGGGGTGATGCTACATACGGAAAG Reverse: TAATACGACTCACTATAGGGTTGTTTGTCTGCCGTGAT				370
Gene Name	**Primer Sequence for qRT-PCR**	**Product Length (bp)**	**Slope**	**R^2**	**Primer Efficiency (%)**
brahma	Forward: TCGCTTGATTCTGCTTGTGGA Reverse: AGAACGAAGCGACAGGGTCT	166	−3.266	0.996	100.41
hunchback	Forward: TGCCCCAAGTGTCCTTTTGT Reverse: CAGTCAGAACAGCGGTATTGGT	179	−3.348	0.997	98.94
β-actin	Forward: TCCAGGCTGTACTCTCCTTG Reverse: CAAGTCCAAACGAAGGATTG	134	−3.419	0.999	96.1

Underlined sequence corresponds to T7 promoter.

Reference

1. Khajuria, C.; Vélez, A.M.; Rangasamy, M.; Wang, H.; Fishilevich, E.; Frey, M.L.F.; Carneiro, N.; Premchand, G.; Narva, K.E.; Siegfried, B.D. Parental RNA interference of genes involved in embryonic development of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. *Insect Biochem. Mol. Biol.* **2015**, *63*, 54–62.