

Supplementary Materials: RNA Interference of the Ecdysone Receptor Genes *EcR* and *USP* in Grain Aphid (*Sitobion avenae* F.) Affects Its Survival and Fecundity upon Feeding on Wheat Plants

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Table S1. Primer sets used in this study.

Use of Primers		Sequences of Primers (5' → 3')	Annealing Tempreture (°C)	Amplicon Length (bp)
cDNA cloning	SaEcR-F	CCACCGACTTGTCTATTTCAGG	55 °C	429 bp
	SaEcR-R	GCATATTCTGCGTTATCCACCTTC		
	SaUSP-F	AGAGCTGGTTGGAATGAGTTG	52 °C	371 bp
	SaUSP-R	AATGAAGGAAGCCGAAGAAGT		
	SaC002-F	CCGACAAATTCAAAGAGACGAAGAC	55 °C	267 bp
	SaC002-R	AGAAACTTCCAAACTTATTCACGGC		
	GFP-F	CGGGAACTACAAGACACG	50 °C	320 bp
	GFP-R	CTTTGGAAAGGGCAGATT		
	USPP-F	TGGTTGGAATGAGTTGATG	56 °C	493 bp
	USPP-R	CATGTAGCTACTTGAACGTCATG		
dsRNA synthesis	T7SaEcR-F	<u>TAATACGACTCACTATAGGGCCACCGACTTGTCTATTTCAGG</u>	55 °C	469 bp
	T7SaEcR-R	<u>TAATACGACTCACTATAGGGGCATATTCTGCGTTATCCACCTTC</u>		
	T7SaUSP-F	<u>TAATACGACTCACTATAGGGAGAGCTGGTTGGAATGAGTTG</u>	55 °C	411 bp
	T7SaUSP-R	<u>TAATACGACTCACTATAGGGAATGAAGGAAGCCGAAGAAGT</u>		
	T7SaC002-F	<u>TAATACGACTCACTATAGGCGACAAATTCAAAGAGACGAAGAC</u>	55 °C	307 bp
	T7SaC002-R	<u>TAATACGACTCACTATAGGAGAACTTCCAACTTATTCACGGC</u>		
	T7GFP-F	<u>TAATACGACTCACTATAGGGCGGGAACTACAAGACACG</u>	50 °C	360 bp
	T7GFP-R	<u>TAATACGACTCACTATAGGGCTTTGGAAAGGGCAGATT</u>		
qRT-PCR	qactin-F	CGGTTCAAAAACCCAAACCAG	57 °C	231 bp
	qactin-R	TGGTGATGATTCCCGTGTTT		
	L27-F	CCGAAAAGCTGTCATAATGAAGAC	57 °C	246 bp
	L27-R	GGTGAAACCTTGCTACTGTTACATCTTG		
	qSaEcR-F	TGATGTTCAGGGTAGCAAGG	57 °C	137 bp
	qSaEcR-R	CCGACTGAATGACAGTTGGT		
	qSaUSP-F	ATGGGTATGGATAGAACAG	57 °C	198 bp
	qSaUSP-R	TAATGAAGGAAGCCGAAG		
	qSaC002-F	CCGACAAATTCAAAGAGACGAAGAC	57 °C	267 bp
	qSaC002-R	AGAAACTTCCAAACTTATTCACGGC		

T7 RNA polymerase promoter is underline.

AP-EcR ATGTTGCGACTAGCATCGCAGAACGACGGGGCCATGACTTCGTCTCGTCCGAAGTCACCTCGTCTCGTCTCGTCTCGTCTCGGCG
Sa-EcR ATGTTGCGACTCGCATCGCAGAACGATGGGGCCATGACTTCGTCTCGTCCGAAGTCACCTCGTCTCGTCTCGTCTCGTCTCGGCG

AP-EcR GCCGCTCCACCGGTTCTCAGCCACCAGCATGTTATCAACGCATTCTTCAGCACCAACATCAACAGCCCGATGACCCGG
Sa-EcR GCCGCTCCACCGGTTCTCGCCACCAGCATGTTATCAACGCATTCTTCAGCACCAACATCAACAGCCCGATGATCGG

AP-EcR GAGAGCTTTGAGTTCCTGCAGGACCTCGATGACAGCTTCGGCGAACAGCCCACTTACACCACCCACCAGCAGCGGTACCAC
Sa-EcR GAGAGCTTTGAGTTCCTGCAGGACCTCGATGACAGCTTCGGCGAACAGCCCACTTACACCACCCACCAGCAGCGGTACCAC

AP-EcR CAGGACACCATCATGAACCGGTTTCATGACACAGCACAAACAATTCTCCACCGTCCCAGTGATAACAACAGTCAAAGAA
Sa-EcR CAGGACACCATCATGAACCGGTTTCATGACACAGCACAAACAATTCTCCACCGTCCCCTTGATAACAACAGTCAAAGAA

AP-EcR GAGTTGTCTCCGCCAACAGCCTGTCGGGAGTCAGCAGCCATTCCGATGGGTTGAAGAAGAAGAACTCAACCACTCGCCC
Sa-EcR GAGTTGTCTCCGCCAACAGCCTGTCGGGAGTCAGCAGCCATTCCGATGGGTTGAAGAAGAAGAACTCAACCACTCGCCC

AP-EcR GTGACTGGCGTCGTCAACACCGCGCATCGGGCCCCGGAGCGCGCTTGGTGGCAACGTGCTGAACAACCGACCTCCCGAA
Sa-EcR GCGACTGGCGTCGTCAACACCGCGCATCGGGCCCCGGGGCGCGCTTGGTGGCAACGTGCTGAACAACCGACCTCCCGAA

AP-EcR GAGCTTTCTGGTGTGCGGCGACCGTCTCCGGTTACCATTACAACGCGCTGACATGCGAAGGGTGCAAGGGGTCTTTC
Sa-EcR GAGCTTTCTGGTGTGCGGCGACCGTCTCCGGTTACCATTACAACGCGCTGACATGCGAAGGGTGCAAGGGGTCTTTC

AP-EcR CGGAGGAGCATCACCAGAACGCCGTGTACCAGTGCAAGTACGGCAACAACGCGAAATCGACATGTACATGAGGCGGAAG
Sa-EcR CGGAGGAGCATCACCAGAACGCCGTGTACCAGTGCAAGTACGGCAACAACGCGAAATCGACATGTACATGAGGCGGAAG

AP-EcR TGCCAGGAGTGCCGCTGAAAAATGCCTCACCGTCGGCATGAGGCCTGAATGTGTTGTACCTGAAGTTCAATGTGCAGTA
Sa-EcR TGCCAGGAGTGCCGCTGAAAAATGCCTCACCGTCGGCATGAGGCCTGAATGTGTTGTACCTGAAGTTCAATGTGCAGTA

AP-EcR AAAAGAAAGGAGAAAAAGCTCAACGA GAAAAAGATAAACCAATTCTACTACAGATATTCTCCAGAAATAATAAATA
Sa-EcR AAAAGAAAGGAGAAAAAGCTCAACGA GAAAAAGATAAACCAATTCTACTACAGATATTACTCTAGAAATAATAAATA

AP-EcR GAACCTACAGAGATGAAGATTGAATGCGGTGAACCAATGATAATGGGCACGCCTATGCCAGCTGTACCTTACGTGAACCG
Sa-EcR GAACCTACAGAGATGAAGATTGAATGCGGTGAACCAATGATAATGGGCACGCCTATGCCAGCTGTACCTTACGTGAACCG

AP-EcR TTGAGTTCTGAACAAAAAGAACTGATCCATCGACTTGCTCTATTTCCAGGATCAATATGAAGCTCCTAGTGAAAAGGACATG
Sa-EcR TTGAGTTCTGAACAAAAAGAACTGATCCATCGACTTGCTCTATTTCCAGGATCAATATGAAGCTCCTAGTGAAAAGGACATG

AP-EcR AAACGTTTAAACAATAAATAATCAAAATATGGATGAATACGATGAAGAAAAACAAGTGACACCACATATCGAATCATCACT
Sa-EcR AAACGTTTAAACAATAAATAATCAAAATATGGATGAATACGATGAAGAAAAACAAGTGACACCACATATCGAATCATCACT

AP-EcR GAGATGACAATACTCACAGTTCAACTCATTGTAGAGTTTGCCAAACGATTACCAGGTTTTGATAAACTTGTAAGAGAAGAT
Sa-EcR GAGATGACAATACTCACAGTTCAACTCATTGTAGAGTTTGCCAAACGATTACCAGGTTTTGATAAACTTGTAAGAGAAGAT

AP-EcR CAAATCACTTTACTCAAGGCTTGCTCAAGTGAAGCTATGATGTTTCAGGGTAGCAAGGAAATATGACATCACCAGTACTCA
Sa-EcR CAAATCACTTTACTCAAGGCTTGCTCAAGTGAAGCTATGATGTTTCAGGGTAGTAGGAAATATGACATCACCAGTACTCA

AP-EcR ATAGTGTGCTAACAACAGCCATTTTCAGCCGATTCTTATAACAAAGCTGGGTTGGGAGATGCCATTGAAAACCACTG
Sa-EcR ATAGTGTGCTAACAACAGCCATTTTCAGCTGATTCTTATAACAAAGCTGGGTTGGGAGATGCCATTGAAAACCACTG

AP-EcR TCATTCACTCGGTTTATGTACAATATGAAAGTGGATAATGCAGAATATGCATTGTTGACCGCCATTGTCATATTTCAAGT
Sa-EcR TCATTCACTCGGTTTATGTACAATATGAAAGTGGATAATGCAGAATATGCATTGTTGACCGCCATTGTCATATTTCAAGT

AP-EcR AGGCCAAATTTACTAGATGGTTGGAAAGTGGAGAAAAATCAAGAGATCTACCTAGAGTCCTTAAAGCTTATGTAGATAAT
Sa-EcR AGGCCAAATTTACTAGATGGTTGGAAAGTGGAGAAAAATCAAGAGATCTACCTAGAGTCCTTAAAGCTTATGTAGATAAT

AP-EcR CGAGACCGTGACACAGCAACTGTGAGATATGCGCGACTTCTCTCGGTACTTACAGAGTTGCGTACATTGGGCAATGAAAC
Sa-EcR CGAGACCGTGACACAGCAACTGTGAGATATGCGCGACTTCTCTAGTACTTACAGAGTTGCGTACATTGGGCAATGAAAC

AP-EcR TCTGAGCTATGTATGACACTGAAACTGAAAAACCGAGTAGTACCCCCATTCTTGGCCGAAATATGGGATGTCATGCCATAG
Sa-EcR TCTGAGCTATGTATGACACTGAAACTGAAAAACCGAGTAGTACCCCCATTCTTGGCCGAAATATGGGATGTCATGCCATAG

(a)

Figure S1. Cont.

AP-USP ATGTTCAAGAAAGAAAACCCATGATGTCCGTGTC****GGCCATCATACAGAGCA**GGGCGGCGCATCACCCTGGGGC
 Sa-USP ATGCCACTCAGCGAGCTGTCACTGTCGCCCTGTGCATTCGGCTACCGCAATCTCCATTGAACGACCATGGACGGCACCGAA

AP-USP AGAGGATTAAGATTGGACAATAATATGTCACCTGGGTTCAATGGGTCCTCAGTCACCTCTAGACCTCAAACCCGACACGGCA
 Sa-USP CGAGGTTTAAGATTGGACAATAATATGTCACCTGGGTTCAATGGGTCCTCAGTCACCTCTAGACCTCAAACCCGACACAGCA

AP-USP ACTCTAATGGTCAATTTAGCCCTCCGGGAGCTCCTCTAAGTCTGCAGGATTATACAGTGTGACCGGAACAATATGATG
 Sa-USP ACTCTAATGGTCAATTTAGCCCTCCGGGAGCTCCTCTAAGTCTGCAGGATTATACAGCGTCGACCGGAACAATATGATG

AP-USP AATAATCTTGCAACGTACAAGACTCTCCGAATTACCCGCCCAACCATCCGCTCAGCGGTTGCAACATCTGTGCTCCATA
 Sa-USP AATAATCTTGCAACGTACAAGACTCTCCGAATTACCCGCCCAACCATCCGCTCAGCGGTTGCAACATCTGTGCTCCATA

AP-USP TGGCGCGATCGCGCCAGCGGAAACATTACGGAGTGTACAGCTGCGAGGGGTGCAAAGGGTTCTTCAACGTACCGTGAGG
 Sa-USP TGGCGCGATCGCGCCAGTGGGAAACATTACGGAGTGTACAGCTGCGAGGGGTGCAAAGGGTTCTTAAACGCAACCGTGAGG

AP-USP AAGAATTTGCTTTATGCGTGTGCGGAAGAAAACAAATGCATCATCGACAAGCGCCAACGAAATCGGTGCAGTACTGCAGG
 Sa-USP AAGAATTTGCTTTATGCGTGTGCGGAAGAAAACAAATGCATCATCGACAAGCGCCAACGAAATCGGTGTGAGTACTGCAGG

AP-USP TATCAAAATGTTTGACCATGGGCATGAAAAGAGAAGCCGTGCAGGAGGAAAGGCAACGTACAAAAGAACGAGATCATAAT
 Sa-USP TATCAAAATGTTTGACCATGGGCATGAAAAGAGAAGCCGTGCAGGAGGAAAGGCAACGTACAAAAGAACGAGATCATAAT

AP-USP AGCATAGAAGTTGAACCCACGAGCAGTTCTAATACTGATATGCCAGTGGAACCTTATATTAAGGGCTGAGAATAAAGCTGAT
 Sa-USP AACATAGAAGTTGAACCCACGAGCAGTTCTAATACTGATATGCCAGTGGAACCTTATATTAAGGGCTGAGAATAAAGCTGAT

AP-USP GCTATAAGACTGAACAACAGTATATAGAGCAACAACATCCTCAACATACTGTTGGTGCTATTGTGCAAGCAACTGACAAG
 Sa-USP GCTATAAGACTGAACAACAGTATATAGAGCAACAACATCCTCAACATACTGTTGGTGCTATTGTGCAAGCAACTGACAAG

AP-USP CAGTTAATACAACCTGTTGAGTGGGCCAAGCATATACCGCATTTTAAAAATTTACCTCTAGGCGATCAAGTTTGTATTG
 Sa-USP CAGCTAATACAACCTGTTGAGTGGGCCAAGCATATACCGCATTTTAAAAATTTACCTCTAGGCGATCAAGTTTGTATTG

AP-USP AGAGCTGGTTGGAATGAGTTGATGATTGCAGCATTTTCCCATAGATCAATCAGTGTAAGGATGGTATAGTCTTAGCTACT
 Sa-USP AGAGCTGGTTGGAATGAGTTGATGATTGCAGCATTTTCCCATAGATCAATCAGTGTAAGGATGGTATAGTCTTAGCTACT

AP-USP GGACTTACTGTTGACAGAGATTGAGCTACCAAGCTGGTGTGAAGCTATATTTGATCGTGACTCACTGAACTCGTTGCT
 Sa-USP GGACTTACTGTTGACAGAGATTGAGCTACCAAGCTGGTGTGAAGCTATATTTGATCGTGACTCACTGAACTCGTTGCT

AP-USP AAAATGAGAGATATGGGTATGGATAGAACAGAGCTTGGCTGTTTACGTACTATTATCTTTTTAATCCAGGTTCAAAAGGT
 Sa-USP AAAATGAGAGATATGGGTATGGATAGAACAGAGCTTGGCTGTTTACGTACTATTATCTTTTTAATCCAGGTTCAAAAGGT

AP-USP TTGCAGTCTGTGAATGAAGTGAAGTACTACGTGATAAGGTTTATGTTGCGTTAGAAGAATATTGTCGTACAACACATCCA
 Sa-USP TTGCAGTCTGTGAATGAAGTGAAGTACTACGTGATAAGGTTTATGTTGCGTTAGAAGAATATTGTCGTACAACACATCCA

AP-USP GAAGAACCTGGGCGATTGCTAAACTACTTCTTCGGCTTCCTTCATTACGTTCAATTGGTTTAAATGTCTGGAACATTTA
 Sa-USP GAAGAACCTGGGCGATTGCTAAACTACTTCTTCGGCTTCCTTCATTACGTTCAATTGGTTTAAATGTCTGGAACATTTA

AP-USP TTCTTTTATAAACTTATTGGAGATTCACCAATCGATACATTTTAAATGGAAGTCTTGAATCATCGTCACATGACGTTCAA
 Sa-USP TTCTTTTATAAACTTATTGGAGATTCACCAATCGATACATTTTAAATGGAAGTCTTGAATCATCGTCACATGACGTTCAA

AP-USP GTAGCTACATGA
 Sa-USP GTAGCTACATGA

(b)

Figure S1. Cont.

AP-EcR MLRLASQNDGAMTSSSEVTSSSSSSSAAASTGFSATSMFINAFFSTNINSPMTRESFEFLQDLDDSFGEQPTYTTHQQRHY
 Sa-EcR MLRLASQNDGAMTSSSEVTSSSSSSSAAASTGFSATSMFINAFFSTNINSPMTRESFEFLQDLDDSFGEQPTYTTHQQRHY

AP-EcR QDTIMNRFMTQHNNNSSTVPVITTVKEELSPPNLSGVSSSHDGLKKKKLNHSPVITGVVNTAASGPGGGVGGNVLNNRPPE
 Sa-EcR QDTIMNRFMTQHNNNSSTVPLITTVKEELSPPNLSGVSSSHDGLKKKKLNHSPATGVVNTAASGPGGGVGGNVLNNRPPE

AP-EcR ELCLVCGDRSSGYHYNALTCEGCKGFFRRSITKNAVYQCKYGNNCEIDMYMRKQCERLKKCLTVGMRPECVVPVQCAV
 Sa-EcR ELCLVCGDRSSGYHYNALTCEGCKGFFRRSITKNAVYQCKYGNNCEIDMYMRKQCERLKKCLTVGMRPECVVPVQCAV

AP-EcR KRKEKKAQREKDKPNSTTDISTPEIIKIEPTMKIECGEPMIMGTPMPTVPYVKPLSSEQKELIHRLVYFQDQYEAPSEKDM
 Sa-EcR KRKEKKAQREKDKPNSTTDITPEIIKIEPTMKIECGEPMIMGTPMPTVPYVKPLSSEQKELIHRLVYFQDQYEAPSEKDM

AP-EcR KRLTINNQNMDYDEEKQSDTTYRIITEMTILTVQLIVEFAKRLPGFDKLVREDQITLLKACSSEAMFRVARKYDITDS
 Sa-EcR KRLTINNQNMDYDEEKQSDTTYRIITEMTILTVQLIVEFAKRLPGFDKLVREDQITLLKACSSEAMFRVARKYDITDS

AP-EcR IVFANNQPFADSYNKAGLDAIENQLSFSRFMYNMKVDNAEYALLTAIVIFSSRPNLLDGWKVEIKIYEIYLESLKAYVDN
 Sa-EcR IVFANNQPFADSYNKAGLDAIENQLSFSRFMYNMKVDNAEYALLTAIVIFSSRPNLLDGWKVEIKIYEIYLESLKAYVDN

AP-EcR RDRDTATVRYARLLSVLTELRTLGNENSELCMTLKLKNRVVPPFLAEIWDVMP*
 Sa-EcR RDRDTATVRYARLLSVLTELRTLGNENSELCMTLKLKNRVVPPFLAEIWDVMP*

(c)

AP-USP *MFKKEKPMMSVSAIIQSRAHHHWGRGLRLDNNMSLGSMPQSPDLKPDATLMVNFSPPGAPLSPAGLYSVDRNMMN
 Sa-USP MPLSELSSLSPSFGYRNLTMDGTERGLRLDNNMSLGSMPQSPDLKPDATLMVNFSPPGAPLSPAGLYSVDRNSMMN

AP-USP NSCNVQDSPNYPPNHPLSGSKHLCSICGDRASGKHGYGVYSCGCKGFFKRTVRKNLSYACREENKCIIDKQRNRCQYCRY
 Sa-USP NSCNVQDSPNYPPNHPLSGSKHLCSICGDRASGKHGYGVYSCGCKGFFKRTVRKNLSYACREENKCIIDKQRNRCQYCRY

AP-USP QKCLTMGMKREAVQEEQRQTKERDHNSEIEPTSSSNTDMPVELILRAENKADAITEQQYIEQQHPQHTVGAICQATDKQ
 Sa-USP QKCLTMGMKREAVQEEQRQTKERDHNSEIEPTSSSNTDMPVELILRAENKADAITEQQYIEQQHPQHTVGAICQATDKQ

AP-USP LIQLVEWAKHIPHFKNLPLGDQVLLLRAGWNELMIAAFSHRSISVKDGIVLATGLTVDRDSAQAGVEAIFDRVLTTELVAK
 Sa-USP LIQLVEWAKHIPHFKNLPLGDQVLLLRAGWNELMIAAFSHRSISVKDGIVLATGLTVDRDSAQAGVEAIFDRVLTTELVAK

AP-USP MRDMGMDRTELGLRTIILFNPGSKGLQSVNEVEVLDRDKVYVALEEYCRTHPEEPGRFAKLLLRPLSLRSIGLKCIEHLE
 Sa-USP MRDMGMDRTELGLRTIILFNPGSKGLQSVNEVEVLDRDKVYVALEEYCRTHPEEPGRFAKLLLRPLSLRSIGLKCIEHLE

AP-USP FYKLIGDSPIDTFIMEVLESSSHVDQVAT*
 Sa-USP FYKLIGDSPIDTFIMEVL*SSSHVDQVAT*

(d)

Figure S1. The alignments of the coding sequences and the deduced amino acid sequences of *EcR* and *USP* orthologs in grain aphid and pea aphid. (a) Alignment of the coding sequences of *EcR* between grain aphid and pea aphid; (b) Alignment of the coding sequences of *USP* between grain aphid and pea aphid; (c) Alignment of the amino acid sequences of *EcR* between grain aphid and pea aphid; (d) Alignment of the amino acid sequences of *USP* between grain aphid and pea aphid. The sequences shadowed with green color indicate C domain of *EcR* and *USP*, whereas these shadowed with pink color indicate E domain of *EcR* and *USP*. Differences between grain aphid and pea aphid are highlighted in yellow. The short sequences underlined are primer pairs. Primer pairs for amplification of the fragment for dsRNA synthesis in pea aphid are marked with blue lines, while these in grain aphid are marked with black lines. AP-EcR, Sa-EcR and AP-USP, AP-USP, represent the *EcR* and *USP* orthologs from pea aphid and grain aphid, respectively.