

Localized efficacy of environmental RNAi in *Tetranychus urticae*

Authors: Nicolas Bensoussan^{a,1}, Maja Milojevic^a, Kristie Bruinsma^a, Sameer Dixit^{a,2}, Sean Pham^a, Vinayak Singh^a, Vladimir Zhurov^a, Miodrag Grbić^a, Vojislava Grbić^{a,*}

^aDepartment of Biology, The University of Western Ontario, 1151 Richmond Street, London, ON N6A 5B7, Canada.

*corresponding author

Current addresses: ¹Institut national de recherche pour l'agriculture, l'alimentation et l'environnement, 33882 Villenave d'Ornon, France; ²National Institute of Plant Genome Research, New Delhi-110067, India.

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Additional information

Supplemental Table 1. Genes with consecutive 21 bp-sequence identity to dsRNA used for the RNAi.

Supplemental Table 2. Primers used in this study.

Supplemental Table 3. Expression patterns of RNAi-targeted genes.

Supplemental Figure 1. Expression analysis and pairwise sequence alignments of genes with consecutive 21 bp-sequence identity to dsRNA-*TuRpt3*.

Supplemental Table 1. Genes with consecutive 21 bp-sequence identity to dsRNA used for the RNAi

gene name	Tetur ID	# of off-targets (21 nt consecutive)	Sequences producing significant alignments	Length (nt)	Score (Bits)	E Value
<i>TuRpt3</i>	<i>tetur32g01800</i>	2	<i>tetur32g01800</i>	length:1248	1079	0
			<i>tetur35g00760</i>	length:1356	221	3.00E-57
			<i>tetur02g06210</i>	length:2394	84.2	5.00E-16
<i>TuHsc70-3</i>	<i>tetur08g01320</i>	0	<i>tetur08g01320</i>	length:1986	1025	0
<i>TuRpn7</i>	<i>tetur33g01390</i>	0	<i>tetur33g01390</i>	length:1170	1153	0
<i>TuSnap</i>	<i>tetur06g05400</i>	0	<i>tetur06g05400</i>	length:882	1139	0
<i>TuSrp54</i>	<i>tetur17g03110</i>	0	<i>tetur17g03110</i>	length:1494	1236	0
<i>TuRop</i>	<i>tetur13g00570</i>	0	<i>tetur13g00570</i>	length:1761	1187	0
<i>TuCOPB2_100</i>	<i>tetur24g00150</i>	0	<i>tetur24g00150</i>	length:2421	176	1.00E-44
<i>TuCOPB2_400</i>		0	<i>tetur24g00150</i>	length:2421	704	0
<i>TuCOPB2_600</i>		0	<i>tetur24g00150</i>	length:2421	1104	0
<i>TuVATPase_600</i>	<i>tetur09g04140</i>	0	<i>tetur09g04140</i>	length:1866	1133	0

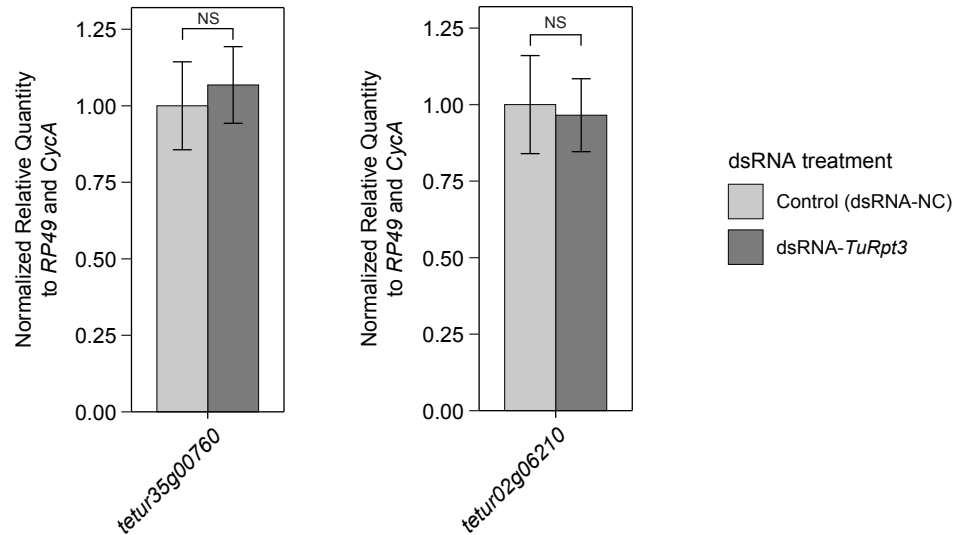
Supplemental Table 2. Primers used in this study

Name	Application	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	
RNAi				Fragment size
<i>TuSrp54</i>	RNAi	[T7]-AGCAGTTACCAACTCTCCCA	[T7]-AGCAGCCCCCTGTAATTGTC	685bp
<i>TuRop</i>	RNAi	[T7]-CGAGGCTCCAGAAAAACAC	[T7]-GATGCACTTATCTGCAGGCG	658bp
<i>TuSnap-α</i>	RNAi	[T7]-GATTGTTGCGGGGGCTCTTCT	[T7]-TCCCGGAATCACTGAAAGC	631bp
<i>TuHsc70-3</i>	RNAi	[T7]-AACCTACAGCTGCTGCCATT	[T7]-GGGTTAATGCCCGAGTAGG	568bp
<i>TuRpn7</i>	RNAi	[T7]-AACTGCTGGAGCGTATGAGG	[T7]-AATCTTCGACCCACGCAAGT	639bp
<i>TuRpt3</i>	RNAi	[T7]-CCTTCAGCTAGTGTCGCCTT	[T7]-CTGGACGGAGTAAAGCAGGG	598bp
<i>Control (NC)</i>	RNAi	[T7]-GCCCTCTCTGGTTGTAAACTT	[T7]-CGACCCCATCAGGCTATTGA	382bp
<i>TuCOPB2</i>	RNAi	[T7]-AGTTTGTGGTGACGGAGAAT	[T7]-TTTTCTTCGGCATGTATCC	612bp
<i>TuVATPase</i>	RNAi	[T7]-TCCAACAGTGATGTTATTGTTACG	[T7]-GAAGAGGTACGAAATCTGGG	628bp
<i>TuCOPB2_100</i>	Localisation	[T7]-AGTTTGTGGTGACGGAGAAT	[T7]-TGAGTCTAGGGCCCAACAA	97bp
<i>TuCOPB2_400</i>	Localisation	[T7]-AGTTTGTGGTGACGGAGAAT	[T7]-GCTTTGGCAACGTTATCAGG	390bp
<i>T7 promoter</i>		TAATACGACTCACTATAGGG		
RT-qPCR				Amplification efficiency
<i>TuSrp54</i>	RT-qPCR	TGGTCATGCTTGTGAAGCTC	ACCACCACCTTTAGCGTGTC	97%
<i>TuRop</i>	RT-qPCR	GCCTGCAGATAAGTGCATCA	TTTGCCTGAATTTGGTACA	91%
<i>TuSnap α</i>	RT-qPCR	TCAGGCGTAGTGAAGCAAA	CTCTAGAAGAGCCCCGAAC	93%
<i>TuHsc70-3</i>	RT-qPCR	CCTTCGCTCCAGAAGAGATTAG	GGTGACGACAGCATGAGTAA	101 %
<i>TuRpn7</i>	RT-qPCR	TGTTGCAGGACGCAATTAAG	ACGCTCCAGCAGTTCCTTGT	98 %
<i>TuRpt3</i>	RT-qPCR	TGAAAAAGCTACAACGGCAAT	TGTCCAATAACCAAGGGAACA	98%
<i>RP49 (tetur18g03590)</i>	RT-qPCR	CTTCAAGCGGCATCAGAGC	CGCATCTGACCTTGAACCTC	102 %
<i>CycA (tetur01g12670)</i>	RT-qPCR	GCTTCAAGGCGGTGACTTT	ACCTGGTCCAGTGTTTGAG	102%
<i>tetur35g00760</i>	RT-qPCR	GGAGGGGATTTCAGGAGAAGG	GCATCAGGACCTTGCCCTT	101%
<i>tetur02g06210</i>	RT-qPCR	ACAATGTGCTTGGTTTCGCTC	ATTCAGCAACTCTACCGCCT	93%
In situ hybridization				Probe size
<i>TuCOPB2</i>	ISH_Sense	[T7]-GTGAGGTTCTCTGTTGCGGTGT	TTCAATTCTTCCGGATCGAC	577bp
	ISH_Anti sense	GTGAGGTTCTCTGTTGCGGTGT	[T7]- TTCAATTCTTCCGGATCGAC	
<i>TuSrp54</i>	ISH_Sense	[T7]-AGCAGTTACCAACTCTCCCA	[AGCAGCCCCCTGTAATTGTC	685bp
	ISH_Anti sense	[AGCAGTTACCAACTCTCCCA	[T7]-AGCAGCCCCCTGTAATTGTC	
<i>TuRop</i>	ISH_Sense	[T7]-CGAGGCTCCAGAAAAACAC	GATGCACTTATCTGCAGGCG	658bp
	ISH_Anti sense	CGAGGCTCCAGAAAAACAC	[T7]-GATGCACTTATCTGCAGGCG	
<i>TuSnapα</i>	ISH_Sense	[T7]-GATTGTTGCGGGGGCTCTTCT	TCCCGGAATCACTGAAAGC	631bp
	ISH_Anti sense	GATTGTTGCGGGGGCTCTTCT	[T7]-TCCCGGAATCACTGAAAGC	
<i>TuHsc70-3</i>	ISH_Sense	[T7]-AACCTACAGCTGCTGCCATT	GGGTTAATGCCCGAGTAGG	568bp
	ISH_Anti sense	AACCTACAGCTGCTGCCATT	[T7]-GGGTTAATGCCCGAGTAGG	
<i>TuRpn7</i>	ISH_Sense	[T7]-AACTGCTGGAGCGTATGAGG	AATCTTCGACCCACGCAAGT	639bp
	ISH_Anti sense	AACTGCTGGAGCGTATGAGG	[T7]-AATCTTCGACCCACGCAAGT	
<i>TuRpt3</i>	ISH_Sense	[T7]-CCTTCAGCTAGTGTCGCCTT	CTGGACGGAGTAAAGCAGGG	598bp
	ISH_Anti sense	CCTTCAGCTAGTGTCGCCTT	[T7]-CTGGACGGAGTAAAGCAGGG	
<i>TuVATPase</i>	ISH_Sense	[T7]-CCGTGATATGGGTTACCATG	GAAGAGGTACGAAATCTGGG	416bp
	ISH_Anti sense	CCGTGATATGGGTTACCATG	[T7]-GAAGAGGTACGAAATCTGGG	

Supplemental Table 3. Expression patterns of RNAi-targeted genes

gene name	<i>T. urticae</i>		<i>T. castaneum</i>		<i>D. melanogaster</i>	
	ID	Expression pattern (ISH)	ID	Transcriptomic profiling (RNAseq, enrichment >1 compared to whole beetle)	ID	Expression pattern (ISH)
TuRop	tetur13g00570	ovaries, nervous mass	TC011120	ubiquitous; enriched in brain; head; midgut; gonads; tubule; rectal complex; carcass	CG15811	central nervous system (embryonic stage; Salzberg et al., 1993) ubiquitous; larval midgut (embryonic stage; Fisher et al., 2012)
TuSnap a	tetur06g05400	ovaries, nurse cells, DC	TC013571	ubiquitous; enriched in brain; midgut; head; tubule; rectal complex; carcass; fat body; gonads	CG6625	larval nervous system (embryonic stage; Fisher et al., 2012; Ordway et al., 1994)
TuRpt3	tetur32g01800	ovaries; caeca midgut epithelial	TC007999	ubiquitous; enriched in gonads; fat body	CG16916	N/A
TuRpn7	tetur33g01390	ovaries, caeca midgut epithelial	TC006375	ubiquitous; enriched in midgut; carcass; gonads; tubule; fat body; rectal complex; brain; head	CG5378	ubiquitous (embryonic stage; Fisher et al., 2012)
TuHsc70-3	tetur08g01320	ovaries, caeca midgut epithelial, epithelium	TC004425	ubiquitous; enriched in midgut; gonads; carcass; fat body	CG4147	N/A
TuSrp54	tetur17g03110	ovaries, adjacent nurse cells	TC002574	ubiquitous; enriched in midgut; gonads; fat body	CG4659	salivary gland body primordium; presumptive embryonic salivary gland (embryonic stage; Abrams and Andrew, 2005)
TuCOPB2	tetur24g00150	ovaries, caeca midgut epithelial	TC013867	ubiquitous; enriched in midgut; tubule; rectal complex; gonads; brain	CG6699	ubiquitous; midgut; salivary gland; embryonic dorsal epidermis (embryonic stage; Fisher et al., 2012)
TuVATPase	tetur09g04140	ovaries; posterior midgut; caeca midgut epithelial; digestive cells	TC008354	ubiquitous; enriched in midgut; rectal complex; brain; tubule; carcass; gonads	CG3762	ubiquitous; midgut; Malpighian tubule; gastric caecum; muscles (embryonic stage; Fisher et al., 2012) Malpighian tubule; ovariole (adult stage; Allan et al., 2005)

A



B Pairwise sequence alignment between *tetur35g00760* CDS and dsRNA-*TuRpt3*

dsRNA_TuRpt3	1	-----CCTTC-----AGCTA-----GTGTC-----GCCTT-----	20
tetur35g00760	401	TATCAACTTCTGTGGGAAGTGAACATTATGTGTCTATATTATCCTTTGTG	450
dsRNA_TuRpt3	21	-----ACATAAGCACAGTAATGC----TTTGGTTGA-----	47
tetur35g00760	451	GACAAGGAACAACCTTGAGCCCA--ATTGCTCAGTTTGTGTTGAATCATAAA	498
dsRNA_TuRpt3	48	-----TGTTTTACCA--CCTGA-AGCTGACTC---TTCTATCG-CTA	82
tetur35g00760	499	GTCCATGCTGTTGTAGGAGTCCTCAGTGATGATACAGATCCAATGGTCAA	548
dsRNA_TuRpt3	83	TGT--TGAGAGCTGATGAAAAA---CCTGATGTTAGTTATGCAGATATTG	127
tetur35g00760	549	TGTAATGA-AGTT--AGAAAAAGCTCCTCAAGAACTTATGCTGATATTG	595
dsRNA_TuRpt3	128	GTGGTCTCGA-CATTCAAAGCAAGAAATTCGTGAAGCAGTAGAGCTTCC	176
tetur35g00760	596	GAGGTCTCGATCA-GCAAATTCAAGAAATTAAAGAATCAGTAGAGCTTCC	644
dsRNA_TuRpt3	177	ATTAACCTACTTTGAATTGTATAAAACAATCGGTATTGACCCACCTCGTG	226
tetur35g00760	645	TTTGACTCATCCAGAATACTATGAGGAAATGGGTATTAAACCACCTAAAG	694
dsRNA_TuRpt3	227	GTGTTCTCATGTATGGTCTCCAGGTTGTGGTAAACTATGTTAGCCAAA	276
tetur35g00760	695	GTGTCATTCTTTATGGCCACCTGGTACCGGTAAACACTTTTAGCTAAA	744
dsRNA_TuRpt3	277	GCAGTTGCTCATCATACCACTGCATCTTTCATTGCTGTTGTTGGTTCTGA	326
tetur35g00760	745	GCTGTGGCTAATCAAACATCAGCAACCTTCTTGAGAGTAGTTGGGTCCGA	794
dsRNA_TuRpt3	327	GTTTGTCCAAAAGTACCTTGGTGAAGGTCTAGGATGGTCCGAGATGTGT	376
tetur35g00760	795	GTTGATTCAAAAATATCTCGGAGATGGACCAAAATTGGTTAGAGAATTAT	844

dsRNA_TuRpt3	377	TTAGACTAGCGCGAGAAAATGCCCCGGCAATTATATTTATTGATGAAATC	426
tetur35g00760	845	TTAGAGTAGCTGAAGAACACGCACCGTCCATCGTTTTATTGATGAAATC	894
dsRNA_TuRpt3	427	GATGCTATAGCAACCAAGAGATTTGATGCTCAAA--CTGGTGCTGACAGA	474
tetur35g00760	895	GATGCTATTGGTACTAAACGATATGAT--TCAAATCTGGAGGAGAAAGG	942
dsRNA_TuRpt3	475	GAGGTTCAAAGAATCTTGTTAGAATTATTGAATCAAATGGATGGGTTTGA	524
tetur35g00760	943	GAAATCCAAAGAACCATGTTGGAGTTACTTAACCAATTGGATGGTTTTGA	992
dsRNA_TuRpt3	525	TCAAAGTACTAATGTAAAGTAATCATGGCCACT-----AATCGGGCAGA	569
tetur35g00760	993	TTCTCGTGGTGATGTCAAAGTTGTGATGGCAACTAATAGAATCG---AAA	1039
dsRNA_TuRpt3	570	TACTTTGGACCTG-CTTTACTCCGTCCAG-----	598
tetur35g00760	1040	CACTT--GATCCTGCCCTTA-TTCGGCCTGGTCGTATCGATCGTAAATTT	1086

C, Pairwise sequence alignment between *tetur02g06210* CDS and dsRNA-*TuRpt3*

dsRNA_TuRpt3	1	-----CC-----TTC-----AGCT	9
tetur02g06210	701	ATCTGGGAATAAGCTCCGAAAATGGTGTTTCCTGTTTCTATGAAAGAGGT	750
dsRNA_TuRpt3	10	AGTGTCGCCTTACATAAGC-ACAGTAATGCTTTGGTTG-----ATGT--	50
tetur02g06210	751	GGTG-----ACAATAGCTACA-TCACTCTGTTGTTAATTATGTTA	792
dsRNA_TuRpt3	51	-----TTTACCAC-----CTGAAGCTGAC---TCTT	73
tetur02g06210	793	GCCTTATACGCTCTCTCTTTAACACAAGTCCGATCAAG-TGTCTCATCTT	841
dsRNA_TuRpt3	74	CTATCGCTATGTT-----GAGAGCTGATGAAAAAC-----	103
tetur02g06210	842	CTA--GTAATGTTTTCTCCAAACTTGGAAGAGC-----ACGTTTCA	880
dsRNA_TuRpt3	104	CTGATGTT-----AGTTA----TGCAGAT	123
tetur02g06210	881	CTGTTGTTGATTCATTAACAGGGGCAGGTAAAGGAGTTAAATTTGCCGAT	930
dsRNA_TuRpt3	124	ATTGGTGGTCTCGACATTCAAAGCAAGAAATTCGTGAAGCAGTA--GAG	171
tetur02g06210	931	GTTGCAGGTCTCAA-----AGAAGCAA-AAAT---TGAA--ATTATGGAG	969
dsRNA_TuRpt3	172	CT--TCCATTAACCTCA---CT-TTGAATTGTATAAA--CAAATCGGTATT	213
tetur02g06210	970	TTTGTGCGATTATCTCAAAGCTCCTGAA-----AAATTCAA---GGCATT	1010
dsRNA_TuRpt3	214	--GACC-----CACCTCGTGGTGTCT-CATGTATGGTCCTCCAGGTTGT	255
tetur02g06210	1011	AGGAGCTAAAGTACCTCGAGGTGTTTGTCTTTTA-GGTCCTCCTGGTTGT	1059
dsRNA_TuRpt3	256	GGTAAAACTATGTTAGCCAAAGCAGTTG-CTCATCATACCACTGC--ATC	302
tetur02g06210	1060	GGTAAAACTATGTTAGCCAAAGCAGTTGCCCTCAGAA-----GCAAATG	1102
dsRNA_TuRpt3	303	TTTCATTTCGTG---TTGTTGGTTCTGAGTTTGTCCAAAAGTACCTTGGT	348
tetur02g06210	1103	TTCCGTTTTTGGCGATGGCTGGAAGTTCATCGAAATG---ATTGGT	1149

dsRNA_TuRpt3	349	GAAGGTCCTAGGATG-----GTCCGAGATGTGTTTAGACTAGCGCGA	390
			
tetur02g06210	1150	GGA---CTTGGA-GCATCTAGAGTGAGAGATCTTTCAAAGAAGCTCGA	1194
dsRNA_TuRpt3	391	GAAGGTCCTAGGATG-----GTCCGAGATGTGTTTAGACTAGCGCGA	434
			
tetur02g06210	1195	AAAAGAGCACC-----TTGTATAGTTTATATTGACGAAATCGATGCAATC	1239
dsRNA_TuRpt3	435	---AGCAACCAAG-----AGATTTGATGCT-----	456
		. . .	
tetur02g06210	1240	GGAAGGAA--AAGGAGTGGAAC TAGAGTCGAACTTCAGGAGAAGAAGAA	1287
dsRNA_TuRpt3	457	CAAAC-----TGGT---GCTG-----	469
tetur02g06210	1288	CAAACCTCTAAATCAATTGCTAGTTGAAATGGATGGTATGGCTGGCCGAGA	1337
dsRNA_TuRpt3	470	-----ACAGA---GAGGTTC-----	481
		.	
tetur02g06210	1338	AGGTGTCATTCTTTTAGGGTCGACAAACAGAGCTGAAGTTCTGGACAAAG	1387
dsRNA_TuRpt3	482	-----	481
tetur02g06210	1388	CACTACTTCGACCTGGTAGATTGATCGACATATTCTCATAGATTACCT	1437
dsRNA_TuRpt3	482	-----AAAGAATCTTGTAGAAATTA-TTGAATCAAATGGA	515
			
tetur02g06210	1438	ACACTAGAAGAAAGAAAAGAACTTTTGAGAAATACTTGAA--AAATATA	1485
dsRNA_TuRpt3	516	TGGGTTT-GATCAA---AGTACTAATGTTAAAGTAATCA---TGGCCAC	557
			
tetur02g06210	1486	AACTTTTCGATAAAACCAGAA--AATGT----CTCATCACGTCTGGCCTC	1529
dsRNA_TuRpt3	558	T---AATCGGGCAGATACTTTGGACCC-----TG-----	583
			
tetur02g06210	1530	TTTAACTCCTG--GATTCAGTGGAGCCGATATCGCCAATGTGTGCAATGA	1577
dsRNA_TuRpt3	584	-----CTTTACTCCGTCCAG-----	598
		. . .	
tetur02g06210	1578	ATCAGCCCTTAATGCGGCTAGATATAAACGAAAAGCAGTTACTCCTAATG	1627

Supplemental Figure 1. Expression analysis and pairwise sequence alignments of genes with consecutive 21 bp-sequence identity to dsRNA-*TuRpt3*. **A**, Expression analysis of *tetur35g00760* and *tetur02g06210* displaying consecutive 21 bp-sequence identity to dsRNA-*TuRpt3*. Average gene expression level relative to the expression of the reference control genes *RP49* and *CycA*. Data represent the mean $\pm$ SE. The RT-qPCR analysis was conducted in three independent experimental runs. Statistical analysis was performed using unpaired two-tailed t-test (exact P-values corresponding to each pairwise comparison between the control and the treatment are displayed; NS: not significant). **B**, Pairwise sequence alignment between *tetur35g00760* CDS and *TuRpt3* cDNA sequence used to generate dsRNA-*TuRpt3*. EMBOSS Needle was used to perform the alignment. Consecutive 21 bp-sequence identity to dsRNA-*TuRpt3* is displayed in yellow. **C**, Pairwise sequence alignment between *tetur35g00760* CDS and *TuRpt3* cDNA sequence used to generate dsRNA-*TuRpt3*. EMBOSS Needle was used to perform the alignment. Consecutive 21 bp-sequence identity to dsRNA-*TuRpt3* is displayed in yellow.