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Double-Stranded RNA-Mediated Silencing of Sodium Channel and Ultraspiracle Genes in *Aphis gossypii* (Hemiptera: Aphididae)

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Abstract

Overuse of chemical insecticides such as organophosphates, pyrethroids, and endosulfan in managing notorious sap-sucking insect pests such as the melon aphid, *Aphis gossypii* Glover, resulted in insecticide resistance. To combat the above and bring out the novel pest control strategy, we implemented ribonucleic acid interference (RNAi), a reverse genetics tool in silencing the target genes. RNAi is a sequence-specific gene-silencing mechanism triggered by double-stranded ribonucleic acid (dsRNA). The efficiency of RNAi is reliant upon concentration of dsRNA, mode of delivery, gene targets, insect species, etc. The objective of the present study was to assess the extent of down-regulation by using two different concentrations of dsRNA specific to sodium channel (*AgSCN*) and ultraspiracle genes (*AgUSP*) (0.0625 and 0.125 μg/μl) with two time intervals (48 and 96 h) through oral delivery. The extent of declined gene expression and percentage mortality chronicled for sodium channel and USP is proportional to the dsRNA concentration. Thus, our study affirms the outlook in the development of RNAi which can form a potent species-specific tool in the management of insect pests such as *A. gossypii*.

Key words: *Aphis gossypii*, RNAi, dsRNA, sodium channel, ultraspiracle gene

Melon aphid, *Aphis gossypii* Glover, an invasive phloem feeder belonging to the order Hemiptera, is considered as the number one pest of agricultural crops because of its cosmopolitan distribution with broad host range, polyphenism, parthenogenetic reproduction, seasonal host alteration, and vectoring ability (Leclant and Deguine 1994, Hogenhout et al. 2008, Rebijith et al. 2013). Control strategies rely on use of synthetic insecticides like organophosphates and pyrethroids, which target central nervous system of the insects by hindering the conduction of the nerve impulses at synaptic cleft. Their extensive use resulted in substantial environmental hazards and insecticide resistance in *A. gossypii* (Herron et al. 2001, Amad et al. 2002, Andrews et al. 2004, Toda et al. 2004, Chuan-Wang et al. 2007, Pan et al. 2010). Thus, alternative, safe, and advanced management strategies need to be developed for the control of many insect pests such as *A. gossypii*. Ribonucleic acid interference (RNAi) technology, which upholds its specificity in silencing the target genes, is adopted resulting in pest control.

With the discovery of RNAi by Fire et al. (1998), it has now been a decade and a half implementing the technique in several fields of research, including agriculture. RNAi, also familiar as reverse genetics tool (Hannon 2002), is a strategy that encompasses the injection of exogenous cognate double-stranded ribonucleic acid (dsRNA) into the cells or tissues (Zhao et al. 2001), which are then cleaved into fragments of around 21–23 nucleotides termed as small interfering RNA (siRNA) by Dicer (an RNase III enzyme). Later, siRNAs are loaded onto a multiprotein complex known as RNA-induced silencing complex, wherein the catalytic component Argonaute protein identifies and chops the target mRNA (Meister and Tuschl 2004). Thus, sequence specificity is achieved by degrading the target mRNA, making RNAi as the powerful tool in genetics (Dykxhoorn et al. 2003).

Gene-silencing technology was well demonstrated in insects, such as *Drosophila melanogaster* Meigen (Miller et al. 2008), *Aphis gossypii* Glover (Rebijith et al. 2015), *Tribolium castaneum* (Herbst) (Konopova and Jindra 2008, Minakuchi et al. 2008a, Parthasarathy and Palli 2009), *Bombyx mori* (L.) (Hossain et al. 2008), *Acyrthosiphon pisum* (Harris) (Shakesby et al. 2009), *Bemisia tabaci* (Gennadius) (Asokan et al. 2014), and *Sitobion avenae* (F.) (Xu et al. 2014). Though, some fast-evolving viruses are resistant to RNAi through mutation of the target region using viral suppressors (Zheng et al. 2005), RNAi resistance in arthropods has not been

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reported (Pittendrigh et al. 2013). So, the usage of RNAi technology is effective in the management of *A. gossypii*.

In the present study, we have selected voltage-gated sodium channel (*AgSCN*) and ultraspiracle gene (*AgUSP*) from *A. gossypii* as targets for silencing. As these genes play a vital role in conduction of nerve impulse and in normal development of insects, respectively, they could be excellent targets in pest management. The facilitation of conduction processes (nerve impulses) in neuronal as well as in excited cells is carried out by voltage-gated sodium channels. Unlike mammalian counterpart, which is composed of nine sodium channel genes with α- and β-subunits (Goldin 2002), insects have only one sodium channel gene (Dong 2010). The same acts as a substitute for splicing and editing of its ribonucleic acid entity, fetching in a more discrete variant of voltage-gated sodium channel gene (Dong 2007, 2010). Exploitation of insecticides such as pyrethroid has created insecticide resistance in *A. gossypii* resulting in decreased sensitivity toward sodium channel (Marshall et al. 2012). On silencing sodium channel gene, there is a disruption in induction and propagation of action potential in the nervous system.

Ultraspiracle (USP) is another gene that plays a vital role in a set of processes like insect development and reproduction. Gene transcription is brought about by USP, which belongs to a class of nuclear receptor family. USP can heterodimerize with another ligand-dependent transcription factor termed as ecdysteriod hormone receptor (ECR), resulting in ECR complex (Ec; Oro et al. 1990; Yao et al. 1992, 1993). This Ec in conjunction with Juvenile Hormone (JH) plays a crucial role in arthropod reproduction and development (Baldwin et al. 2001, Mu and LeBlanc 2002). Targeting such genes would disrupt the normal hormonal mechanism that controls the pest outburst (Dhadialla et al. 1998).

In the present study, we cloned and sequenced the *AgSCN* and *AgUSP* genes, and evaluated the effects of various concentrations of dsRNA in silencing these genes. The dsRNA region of *AgSCN* and *AgUSP* genes were aligned with other beneficial insects (*Apis mellifera* L. and Coccinellidae) using sequence alignment tool BOXSHADE 3.1.1 (http://mobyle.pasteur.fr/cgi-bin/portal.py?#forms::boxshade, accessed 8 October 2015). The degree of nucleotide conservation was not significant (Supp Figs. 3 and 4 [online only]). This suggests that our target dsRNA have no off-target effects. Thus, we hypothesize that this study aids in *A. gossypii* pest management using RNAi.

Materials and Methods

Culture Maintenance of *A. gossypii*

A. gossypii were collected on cotton (*Gossypium hirsutum* L.) from the experimental farm at Indian Institute of Horticultural Research, Bangalore, India. Morphological identification was carried out at National Bureau of Agriculturally Important Insects, Bangalore, India. Two to four weeks of *G. hirsutum*, which were maintained on presterilized soil at 25 ± 1°C with a photoperiod of 14:10 (L:D) h, were used for insect rearing. The apterous forms were transferred using a fine nylon Camlin brush onto the plants, which were kept inside a cage built using acrylic fibre, under controlled conditions—27 ± 1°C, 70 ± 5% relative humidity (RH), and a photoperiod of 12:12 (L:D) h (Shannag et al. 2007, Liu et al. 2008). Insects were left for accustoming to the ambient conditions for several generations before being used for further work.

Cloning of *AgSCN* and *AgUSP* Genes

Total RNA Isolation and cDNA Synthesis.

Total RNA was isolated from *A. gossypii* using MACHEREY-NAGEL GmbH kit (Germany) as per the manufacturer's protocol and quantified with NanoDropLite (Thermo scientific, Germany). DNA contamination was removed from the total RNA by treating it with RNase-free DNase-I (Fermentas Life Sciences, Glen Burnie, MD, USA). Complementary DNA (cDNA) synthesis was carried using M-MLV reverse transcriptase kit (Fermentas GmbH, Germany). Coding sequences of *AgSCN* and *AgUSP* were amplified from the first-strand cDNA using gene-specific primers *AgSCN*-F, *AgSCN*-R and *AgUSP*-F, *AgUSP*-R (Table 1). The amplicons were eluted, ligated onto a general purpose cloning vector (PTZ57R/T) (Fermentas, St. Leon-Rot, Germany), and transformed into DH5α strain (*Escherichia coli*) according to manufacturer's protocol (Fermentas, St. Leon-Rot, Germany). Plasmids were isolated using GenJET Plasmid MiniPrep kit (Fermentas GmbH), and the positive clones were sequenced in M13 forward and reverse direction (Xcelris Labs Ltd., Ahmedabad, India).

Synthesis of dsRNA Specific to Cognate Genes

Selection of dsRNA region was performed with an online web tool, dsCheck (http://dscheck.RNAi.jpl) for minimizing the off-target effects. dsCheck allows comparison between the target gene sequence with that of its database, which comprises gene sequences of *Caenorhabditis elegans* Maupas, *D. melanogaster*, *Oryza sativa* L., *Rattus norvegicus* Berkenhout, and *Arabidopsis thaliana* L. (Naito et al. 2005), resulting in an output of a unique region of ≥500 bp. Sequence-specific primers for these regions ds-*AgSCN* and ds-*AgUSP* (Table 1) were synthesized by attaching T7 promoter sequences at 5′ end, which acts as a template for T7 RNA polymerase. dsRNA for Lac Z was used as nontarget control (Liu et al. 2010).

In total, 50 μl polymerase chain reaction (PCR) was performed to produce the DNA template for dsRNA synthesis using sequence-specific primer with the following combination: molecular biology grade water, 10× reaction buffer, 10 mM dNTP mix, 10 picomoles of each forward and reverse primer, respectively, for individual gene, 1:500 diluted plasmid template (~100 ng), and 1U of Taq DNA Polymerase (TaKaRa, Canada). PCR was carried out in a thermal cycler (AB-Applied Biosystems, Veriti 96 wells) with cycling parameters as: 94°C as initial denaturation for 4 min, followed by 35 cycles of 94°C for 40 s, annealing (ds-*AgSCN—58*°C and ds-*AgUSP—55*°C) for 40 s, extension at 72°C for 45 s, and 72°C for 10 min as final extension. The amplicons were resolved in 1.2% agarose gel and visualized under gel documentation system (UVP) for determining the product size (~500 bp); the gel was excised and purified using Nucleospin extract II kit (MN, Germany). One microgram of the amplicon was used for dsRNA synthesis using Mega Script T7 kit (Life Technologies, Darmstadt, Germany) as per the manufacturer's guidelines. The integrity of the dsRNA was verified by resolving it in 2.0% agarose gel and the quantitative entity through absorbance read at 260 nm on a NanoDropLite (Thermo scientific, Germany).

DsRNA Oral Delivery

Bioassay was carried out implementing parafilm feeding technique (Walters et al. 1990), using pyrogen, DNase, and RNase-free six-well tissue culture plate (Axygen, Life Sciences, CA). Individual wells were cut using a cutting blade so as to ease handling insects; mouth of each well measured 3.5 cm in diameter. Twenty-five apterous *A. gossypii* were transferred from the cotton plant using a fine camel hair brush into each well carefully without disrupting its stylet. Later, a parafilm (PARAFILM 'M'-Laboratory film), tailor cut to 3 cm^2, was stretched to form a thin film so that it was sealed onto the mouth of the bioassay plate. DsRNA specific to target genes was mixed with filter sterilized sucrose solution (2.5% prepared using molecular biology grade water, free from DNase and RNases) to

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Table 1. Primers adopted for cDNA synthesis, double-stranded RNA synthesis, and qRT-PCR analysis of sodium channel and ultraspiracle gene

Gene	Application	Primer Id	Primer sequence (5′–3′)	Product size (bp)	GenBank
Sodium channel	PCR	*AgSCN*-F	GCTAAATCGTGGCCCACACTT	586	KT365907
		AgSCN-R	TCATCTCTACTGGTTCCTTAG		
	dsRNA synthesis	ds-*AgSCN*-F	*TAATACGACTCACTATAGGG*T CGCCGTTATGGGTATGCAGTT	500	KT365907
		ds-*AgSCN*-R	*TAATACGACTCACTATAGGG*G GCGCAATAACATCTACAATTGTT		
	RT-qPCR	qRT-*AgSCN*-F	AGTATTATGTGGTGAATGGATTGAA	125	KT365907
		qRT-*AgSCN*-R	GCCAAGAAAAGATTAAGTACCACA		
Ultraspiracle	PCR	*AgUSP*-F	ATGGGTCCTCAGTCACCTCTA	1,146	KT365906
		AgUSP-R	TCATGTAGCTACTTGAACGTC		
	dsRNA synthesis	ds-*AgUSP*-F	*TAATACGACTCACTATAGGG* GATGATGATTCAAGAACTTCCATT	444	KT365906
		ds-*AgUSP*-R	*TAATACGACTCACTATAGGG* ATCAAGTTTTATTGTTGAGAGC		
	RT-qPCR	qRT-*AgUSP*-F	TAATGAAGGGAGCCGAAGAAGT	124	KT365906
		qRT-*AgUSP*-R	AGTCTGTGAATGAAGTGGAAG TACTA		
Actin	PCR	*AgACT*-F	ATGTGTGACGAWGAWGTAGCMGC	1,120	KJ018754
		AgACT-R	AGCAYTTWCKGTGSACAAT		
	RT-qPCR	qRT-*AgUSP*-F	ATGGAAGATTCCGTTGCCCA	143	KJ018754
		qRT-*AgUSP*-R	ACAATGCAGTGTTGGCGTACAAGT		
LacZ	PCR	*LacZ*-F	ACAATTTCCATTCGCCATTCA	534	–
		LacZ-R	ATGACCATGATTACGCCA		
	RT-qPCR	ds-LacZ-F	*TAATACGACTCACTATAGGG* ACAATTTCCATTCGCCATTCA	452	–
		ds-LacZ-R	*TAATACGACTCACTATAGGG* ATGACCATGATTACGCCA		

F, forward primer; R, reverse primer.

T7 RNA polymerase promoter sequences are italicized.

AgSCN, *A. gossypii* sodium channel gene; *AgUSP*, *A. gossypii* ultraspiracle gene; *AgACT*, *A. gossypii* actin; and *LacZ*, *E. coli* Lac Z.

produce different concentrations such as 0.0625 and 0.125 µg/µl (Similar concentrations were used in earlier studies on RNAi in *A. gossypii* by Gong et al. 2014). Using another stretched parafilm, dsRNA–sucrose mixture of different concentrations was sandwiched between two layers. All the above procedures were carried out aseptically in a laminar air flow. Bioassay was set for each gene and for each concentration along with nontarget control (LacZ) and untreated control (2.5% sucrose). The insects were exposed to 1.562 and 3.125 µl of dsRNA at 0.0625 and 0.125 µg/µl concentrations, respectively, in each parafilm sandwich. The bioassay plates or wells were placed in a culture room under controlled conditions of temperature 21 ± 1°C and 65–70% RH. Mortality in individual gene treatment (concentration wise) was observed and scored at 48- and 96-h time intervals.

RT-qPCR Analysis

Target gene silencing was assessed both in terms of concentration (0.0625 and 0.125 µg/µl) and posttreatment intervals (48 and 96 h) by performing reverse transcriptase quantitative real-time PCR (RT-qPCR). RNA isolation, cDNA synthesis, and quantification were performed as described previously. RT-qPCR was performed as per, the Minimum Information for Publication of Quantitative Real-Time PCR (MIQE) Compliances (Bustin et al. 2009). The reaction set up was for 20.0 µl final volume and comprised 10.0 µl of 2 × SYBR Green PCR master mix buffer (Takara, Japan), 0.5 µl (10 mM) of each gene primers qRT-*AgSCN* and qRT-*AgUSP* (Table 1) designed as per the guidelines of Herbert et al. 2011, and 2.0 µl of diluted cDNA as template, along with actin (GenBank KJ018754), a house-keeping gene expressing constituently, used as reference gene for normalization purpose. Three sets of technical replicates of each reaction were carried out along with no template control in the experiment with the following temperature cycles: 95°C for 5 min for initial denaturation; followed by 40 cycles of 95°C for 30 s, 58°C for 40 s, and 70°C for 30 s; annealing at 60°C for 1 min in a Light Cycler 480II (Roche Applied Science, Switzerland). Using $2^{-\Delta\Delta CT}$ method, relative expression of genes was analyzed (Livak and Schmittgen 2001).

Statistical Analysis

Statistical analysis was carried out using Graphpad Prism V.6 (GraphPad Prism Software, Inc.). Two-way ANOVA was followed to analyze the data to chronicle the variance. Post hoc comparisons were carried out using Sidak test to identify significant differences between time intervals and concentrations.

Results

Cloning and Sequencing of Sodium Channel and Ultraspiracle Genes

RT-PCR was employed to amplify both *AgSCN* and *AgUSP* with gene-specific primers. Amplified products were cloned, sequenced, and submitted to NCBI GenBank (Table 1). Sequencing results revealed a total length of 586 bp and 1,146 bp for *AgSCN* and *AgUSP* genes, respectively. Further, the BLAST analysis revealed 100% sequence similarity with previously submitted sodium channel and USP genes of *A. gossypii* in NCBI GenBank.

Efficiency and Specificity of RT-qPCR

The melt curve analysis in the RT-qPCR for both *AgSCN* and *AgUSP* genes exhibited single melt peak indicating single product, which was resolved on 2% agarose gel. The products were cloned and sequenced. Further, BLAST analysis revealed 100% similarity with the corresponding gene. The RT-qPCR efficiency resulted from the present study was found to be: 1.918 (95.9%) and 1.968 (98.4%) for *AgSCN* and *AgUSP* (target genes), respectively, and 1.922 (96.1%) for Actin (reference gene).

In the present study, we have carried out gene silencing in *A. gossypii* employing dsRNA for *AgSCN* and *AgUSP* with two concentrations viz. 0.0625 and 0.125 µg/µl at 48 h and 96 h through

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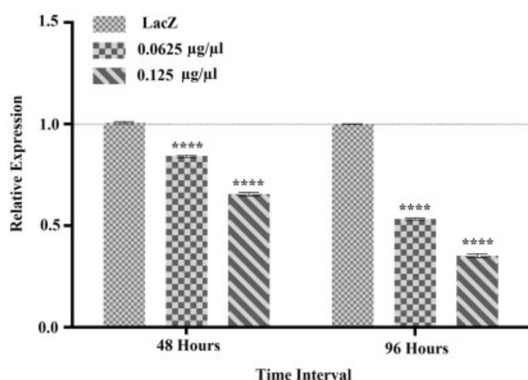

Fig. 1. Relative expression of sodium channel gene in response to dsRNA treatment. Expression levels of target gene were determined by RT-qPCR at 48 and 96 h post dsRNA treatment at two different concentrations. The expression levels of the target gene in the control (sucrose fed) were set to 1; relative expression levels were determined with respect to control. Results were analyzed by two-way ANOVA followed by post hoc test (****$P < 0.0001$; Sidak multiple comparison test; $n = 3$). Standard error of the mean (SEM) are indicated by error bars.

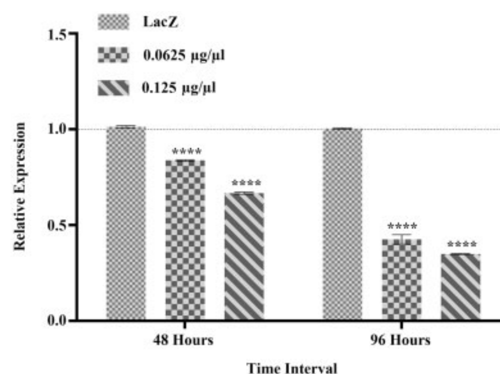

Fig. 2. Relative expression of USP gene on post dsRNA treatment. RT-qPCR was used to determine expression levels at 48 and 96 h post dsRNA treatment at two different concentrations. The expression levels of the target gene in the control (sucrose fed) were set to 1; relative expression levels were determined with respect to control. Analysis was carried out by two-way ANOVA followed by post hoc test (****$P < 0.0001$; Sidak multiple comparison test; $n = 3$). Standard error of the mean (SEM) are indicated by error bars.

oral feeding. Our results also revealed that the expression levels for both *AgSCN* and *AgUSP* did not vary in the nontarget (dsLacZ) and nontreated (sucrose) controls. Feeding of dsRNA induced reduction in the transcript levels for both *AgSCN* and *AgUSP* genes in *A. gossypii*. Silencing of sodium channel augmented with increase in time and dsRNA concentration. At 0.0625 and 0.125 µg/µl concentration, the percentage silencing observed for *AgSCN* were 15.75, 46.62 and 34.47, 64.77, respectively, after 48 and 96 h (Fig. 1). Similarly with *AgUSP*, at 0.0625 and 0.125 µg/µl concentration, the percentage silencing observed were 16.17, 57.4 and 33.28, 65.05 at 48 and 96 h, respectively (Fig. 2). The extent of silencing was directly proportional to the concentration of dsRNA.

Effect of dsRNA Oral Delivery on Mortality

Target gene silencing (*AgSCN* and *AgUSP*) resulted in substantial mortality. The mortality was noted against two concentrations of dsRNA (0.0625 and 0.125 µg/µl) at 48 and 96 h (Supp Tables 1 and 2 [online only]). Mortality increased with increase in sodium channel dsRNA concentration and reached maximum i.e., 69.33% mortality with 0.125 µg/µl concentration at 96 h (Fig. 3). As the concentration of USP dsRNA increased, 57% mortality was observed at 96 h with 0.125 µg/µl concentration (Fig. 4).

Hence, greater mortality was observed at 96 h as compared with 48 h post dsRNA treatment. At the same time, the control (sucrose) and nontarget control (dsLacZ) had less mortality as compared with the treatments. The overall results showed that the down regulation and mortality were positively correlated (Supp Table 3; Supp Figs. 1 and 2 [online only]).

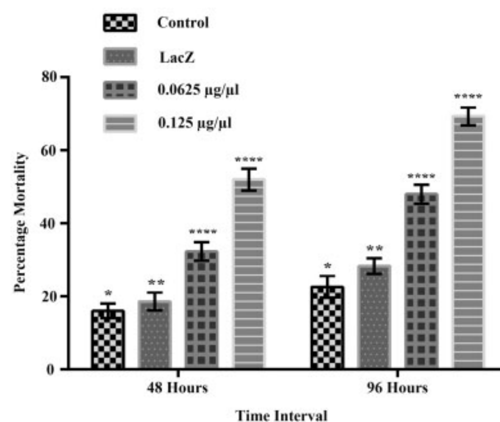

Fig. 3. Mortality induced upon oral delivery of sodium channel dsRNA. Percentage mortality was reported for 0.125 and 0.0625 μg/μl concentrations at 48 and 96 h. Mortality was also chronicled for control (sucrose fed) and nontarget (LacZ dsRNA). Analysis was performed using two-way ANOVA (*, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$; Sidak multiple comparison test; $n = 3$). Standard error of the mean (SEM) are indicated by error bars.

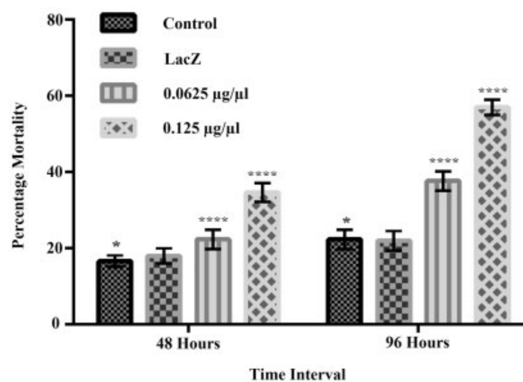

Fig. 4. Mortality induced upon oral delivery of USP dsRNA. Percentage mortality was reported for 0.125 and 0.0625 μg/μl concentrations at 48 and 96. Mortality was also chronicled for control (sucrose fed) and nontarget (LacZ dsRNA). Analysis was performed using two-way ANOVA (*$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$; Sidak multiple comparison test; $n = 3$). Standard error of the mean (SEM) are indicated by error bars.

Discussion

With the better understanding of genome information from pea aphids in 2010, research is being carried out in various related species of aphids, such as *A. gossypii*, *Macrosiphum euphorbiae* (Thomas), *Diuraphis noxia* (Kurdjumov), *Myzus persicae* (Sulzer) etc. (International Aphid Genomics Consortium, 13th September 2013). *A. gossypii*, an invasive sap sucker, causes significant crop loss by direct feeding and also by transmitting numerous plant pathogenic viruses. The global crop loss by *A. gossypii* was estimated as

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one million US dollar (Oerke et al. 1994, Morrison and Peairs 1998). Continuous use of synthetic insecticides such as organophosphates and pyrethroids has resulted in increased insecticide resistance. Thus, there is a need to look for an alternative, effective, and safe strategy for insect pest management like RNAi, which is a sequence-specific mechanism that degrades the target mRNA with the aid of exogenous or endogenous dsRNA.

In order to induce RNAi-mediated gene silencing, exogenous dsRNA can be delivered using the following methods: parafilm feeding (Gong et al. 2014), artificial diet (Turner et al. 2006), soaking (Huvenne and Smagghe 2010), microinjection (Blandin et al. 2002), and through transgenic plant (Pitino et al. 2011). In the present study, we demonstrated the effectiveness of RNAi employing oral delivery of dsRNA for two genes viz. sodium channel (neuronal conduction) and USP (involved in development and reproduction). Our results showed the down regulation of the respective gene expression in *A. gossypii* on dsRNA treatment. However, there are several factors influencing successful RNAi, such as the dsRNA concentration, mode of delivery, dose frequency, insect species, and gene targets (Terenius et al. 2011). The study by Terenius et al. (2011) validated nearly 130 genes from Lepidoptera for gene silencing of which only 14% of the target genes were futile and 48% were proven silenced at high levels. The root cause behind the failure may be the functional nature of the candidate genes. The extent of silencing was varying with the dsRNA concentration. RNAi experiments with *Spodoptera frugiperda* (J.E. Smith), employing 2.0 μg dsRNA for allotostatin gene (neuropeptide hormone), yielded 60% mortality, whereas 3.0 μg yielded only 21.3% mortality through microinjection (Griebler et al. 2008). In some species like *Manduca sexta* (L.) (BettenCourt et al. 2002), *Antheraea pernyi* Guérin-Méneville (Hirai et al. 2004), and *Hyalophora cecropia* (L.) (Terenius et al. 2007), lower concentration of dsRNA elucidated higher extent of silencing, i.e., <10 ng/ml was used for injecting purpose into tissues. Our experiment showed a clear-cut difference in silencing pattern between *AgSCN* and *AgUSP* genes, which indicates that the degree of silencing is directly proportional to the concentration of the dsRNA treatment.

With respect to the application frequency of the dsRNA, single application in our experiment with two concentrations for both the candidate genes resulted in silencing after 48 h of treatment. The maximum silencing was observed after 96 h of feeding with a concentration of 0.125 μg/μl in both the candidate genes. Similar results were observed in apple moth for *Epiphyas postvittana* (Walker) pheromone-binding protein (*EposPBP1*; Turner et al. 2006). Maximum silencing duration in insects differs from species to species. It can be varied from 25 d as in case of *A. mellifera* (Amdam et al. 2003) to even more than 4 mo as in the case of *T. castaneum* (Tomoyasu and Denell 2004). Numerous parameters can be altered in achieving efficient RNAi. The most common one is to increase the frequency of the dose. But in case of *Rhodnius prolixus* Stål, increasing dose has brought down the extent of silencing (Araujo et al. 2006). However, our study revealed that single application is effective in bringing about efficient gene silencing in *A. gossypii*.

The present study affirms the potential utility of exogenously administered cognate dsRNA specific for sodium channel and ultraspiracle gene of *A. gossypii*, which is an invasive pest of global importance. The simplicity associated in designing the administration of cognate dsRNA was less expensive yielding in mortality which was a cause of silencing. In our study, percentage mortality was assessed by setting up three technical replicates for bioassay at both the concentrations.

In a nutshell, RNAi provides a unique approach by acting as a potential species-specific tool in managing insect pest such as *A. gossypii*. It can even outreach the present scenario associated with *Bacillus thuringiensis* insecticidal protein and synthetic pesticides resistance, as many of the insects are becoming resistant toward them. In midst of all, our study will impart a foot mark ahead in the journey of development and management of pest control strategy for insect pests like *A. gossypii* through RNAi.

Supplementary Data

Supplementary data are available at *Annals of the Entomological Society of America* online.

Acknowledgments

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