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RESEARCH ARTICLE

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RNA interference-mediated control of cigarette beetle, *Lasioderma serricorne*

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Abstract

The cigarette beetle (CB; *Lasioderma serricorne*) is a pest on many stored products including tobacco. Fumigation is the common control method currently used. However, the options for controlling this pest are limited, due to resistance issues and phasing out of currently used chemical insecticides. Here, we evaluated RNA interference (RNAi) as a potential method for controlling the CB. RNA isolated from different stages was sequenced and assembled into a transcriptome. The CB RNA sequences showed the highest homology with those in the red flour beetle, *Tribolium castaneum*. Orthologs of proteins known to function in RNAi pathway were identified in the CB transcriptome, suggesting that RNAi may work well in this insect. Also, ^{32}P-labeled double-stranded RNA (dsRNA) injected into CB larvae and adults was processed to small interference RNAs. We selected 12 genes that were shown to be the effective RNAi targets in *T. castaneum* and other insects and identified orthologs of them in the CB by searching its transcriptome. Injection of dsRNA targeting genes coding for *GAWKY*, *Kinesin*, *Sec23*, *SNF7*, and *26S proteasome subunit 6B* into the CB larvae caused 100% mortality. Feeding dsRNA targeting *SNF7* and *26S proteasome subunit 6B* by sucrose droplet assay induced more than 90% mortality, which is 1.8 times higher than the mortality induced by dsGFP control (53%). These data demonstrate an efficient RNAi

response in CB, suggesting that RNAi could be developed as an efficient method to control this pest.

KEYWORDS
cigarette beetle, dsRNA, SNF7, transcriptome, Tribolium

1 | INTRODUCTION

The cigarette beetle (CB; *Lasioderma serricorne*) is a cosmopolitan stored product pest with considerable economic importance in tropical to temperate climate regions (USDA, 1962). The CB is a major pest on stored and processed tobacco (Runner, 1919), but it is ubiquitous due to a wide host range feeding on materials of both plant and animal origin (Hagstrum, Klejdysz, Subramanyam, & Nawrot, 2013). The CB can complete its life cycle feeding on tobacco alone, which is mostly attributed to CB becoming an important pest of tobacco. The larval feeding causes most of the damage (USDA, 1962). Adult feeding is limited, but it cuts holes in wrappers and packaging to enter or escape packaged commodities (Howe, 1965; Jones, 1913). Excreta, dead beetles, and other waste products cause loss of quality in stored tobacco (Powell, 1931; USDA, 1962). The damage and economic loss that occur by CB infestation in the tobacco industry are estimated to be about 0.7–1% of the total warehoused tobacco commodity (Farnham, Flora, Ingram, & Faustini, 2007; USDA, 1962). Fumigation is the most widely used method to control CB, and other stored product pests. Phosphine is the most popular fumigation agent, which can control all life stages of insects in most types of packaging, with minimal residue issues. However, lack of practical alternatives and repetitive use led storage pests including CB to evolve resistance to phosphine (Jagadeesan, Collins, Daglish, Ebert, & Schlipalius, 2012; Rajendran & Narasimhan, 1994; Saglam, Edde, & Phillips, 2015; Zettler, 1994). Therefore, there is an urgent need to develop novel methods to control this pest.

RNA interference (RNAi) is a posttranscriptional gene silencing method that provides an alternative strategy to control CB. Introduction of double-stranded RNA (dsRNA) into the cell initiates RNAi. In the cytoplasm, dicer protein works with other partners to process long dsRNA into 19–21bp small interference RNA (siRNA). siRNA is incorporated into a group of proteins called RNA-induced silencing complex (RISC). Argonautesare the key proteins in this complex, which executes targeted cleavage of messenger RNA (mRNA) with a complementary sequence. Largely, RNAi functions in endogenous gene regulation (Reinhart et al., 2000), a defense mechanism against virus and parasitic RNA (Li & Ding, 2005), and suppression of transposable elements (Buchon & Vaury, 2006). When dsRNA targeting the endogenous gene is administered by injection or feeding, the introduced dsRNA triggers RNAi resulting in knockdown of the target gene. If the target gene is essential for survival, the knockdown of the target gene results in its death. RNAi mechanisms are well conserved in eukaryotes (Shabalina & Koonin, 2008). Since its discovery in the nematode, *Caenorhabditis elegans* (Fire et al., 1998), RNAi response has been detected in plants and animals including insects. RNAi applications in both human and plant health are being pursued. RNAi works very efficiently in coleopteran insects (beetles). Many beetles are highly responsive to both injection and feeding dsRNA (Baum et al., 2007; Palli, 2014; Zhu & Palli, 2020; F. Zhu, Xu, Palli, Ferguson, & Palli, 2011). Commercial product for control of western corn rootworm is already registered (Head et al., 2017). As many stored product pests including CB are in the insect order Coleoptera, RNAi is a promising method for their control.

The objective of this study is to find target genes for RNAi-mediated control of CB. As there are no publicly available CB sequence data, we performed RNA sequencing and assembled transcriptome. To select target genes, we identified CB orthologs of 12 genes that are shown to be effective RNAi targets in *Tribolium castaneum* (Ulrich et al., 2015). We synthesized dsRNA for each target gene and evaluated them in CB larvae. Two genes were selected for sucrose droplet dsRNA feeding assay in neonates. Feeding dsRNA showed significant mortality in

neonates. Our data suggest that like other coleopteran insects, RNAi works efficiently in CB. When coupled with an efficient delivery method, RNAi can be a powerful tool to control this stored product pest.

2 | EXPERIMENTAL PROCEDURES

2.1 | Insect rearing

Cultures of CB were obtained from Dr. Peter Edde, Altria Corporation. The CB were maintained at 30 ± 2°C, 40 ± 10% relative humidity with a 16:8 hr (L:D) photoperiod, with a diet containing organic wheat flour mixed with 5% dried baker's yeast. Beetles were also maintained on dried tobacco powder for long-term culture.

2.2 | RNA sequencing and analysis

Total RNA was isolated from eggs, mixed larvae, pupae, male and female adults using TRI reagent (Molecular Research Center Inc., Cincinnati, OH). The complementary DNA (cDNA) libraries were prepared and sequenced using Illumina HiSeq 3000/4000 at the Duke University Sequencing Facility as described previously (Roy, George, & Palli, 2018). Following quality control (demultiplexing, trimming, adapter removal), the sequences were assembled and analyzed in CLC Genomic Workbench (version 9.5.9; Qiagen Bioinformatics, Valencia, CA). RNA-Seq data from CB were assembled using Trinity Transcriptome Assembler (Grabherr et al., 2011; Haas et al., 2013). The assembled transcriptome was checked for completeness using BUSCO analysis. Following BUSCO analysis, a local blast of the entire transcriptome was performed using the nonredundant protein database from NCBI. The local blast output was then imported to Blast2GO software for statistical analysis of blast hits (Gotz et al., 2008).

2.3 | ^{32}P-labeled dsRNA experiments

^{32}P-labeled dsRNA was synthesized as described previously (Shukla et al., 2016). For dsRNA processing experiments, 0.14 million CPM (count per million) of ^{32}P-labeled dsRNA was injected into each last instar larva or adult. Injected insects were incubated in 25°C for 2 days, and total RNA was extracted. RNA (2,000CPM) was resolved in 8M urea-16% polyacrylamide gels. The gels were dried and exposed overnight to a phosphorImager screen, and the screen was scanned in a phosphorImager (Typhoon 9500; GE Healthcare Life Sciences, Pittsburgh, PA). ImageJ program was used to determine relative levels of dsRNA and siRNA.

2.4 | dsRNA synthesis

Primers (Table S4) were designed to amplify fragments of target genes, based on the sequences of orthologs identified in searching assembled CB transcriptome. Total RNA was isolated from CB using the TRI reagent (Molecular Research Center Inc.). cDNAs were synthesized from the isolated total RNA, using M-MLV Reverse Transcriptase (Clontech Laboratories). Polymerase chain reaction (PCR) amplifications were conducted in 20 µl reactions containing 5 µM of each primer, 10 µl of 2× Taq premix (Promega) and 20 ng of cDNA. PCR conditions were 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 57°C for 30 s, and 68°C for 1 min, finishing with an extension step at 68°C for 5min. PCR products were purified using the QIAquick PCR purification kit (Qiagen). The purified PCR products were used as templates to synthesize dsRNAs using Megascript T7 RNA synthesis kit (Life Technologies, Carlsbad, CA).

2.5 | dsRNA injection bioassay

Two hundred nanograms of dsRNA in 200nl volume was injected into each final instar larva. Injected larvae were placed individually in each well of 96-well plates, provided with wheat flour mixed with 5% dried baker's yeast. Injected larvae were incubated in at 30 ± 2°C, 40 ± 1% relative humidity with a 16:8 hr (L:D) photoperiod, and mortality was recorded until adult emergence.

2.6 | Droplet feeding bioassay

Neonates (newly hatched, first instar larvae) were collected and placed in 60 × 15 mm Petri dish, provided with 0.5% sugar drop solution containing 2 μg/μl dsRNA. Sugar drop solution containing dsRNA was replenished every 2 days. Sterilized water was added in between to avoid drying of the droplet. Mortality was recorded for up to 20 days.

2.7 | Quantitative reverse-transcription polymerase chain reaction (RT-qPCR)

One μg of total RNA was used to synthesize cDNA using M-MLV Reverse Transcriptase. The cDNA was used for determining mRNA levels of the target gene using RT-qPCR. Briefly, 10 μl reaction containing 2 μl of diluted (5×) cDNA, 0.2 μl of each primer (10 μM; Table S5), 5 μl of the SYBR Green PCR Master Mix, and 2.6 μl of ddH_2O was assembled. StepOnePlus Real-Time PCR system (Life Technologies) was used to run the reactions, using the following cycling conditions: one cycle at 95°C (20s), followed by 40 cycles of denaturation at 95°C (3s), annealing and extension at 57°C for 30s. At the end of each RT-qPCR reaction, a melt curve was generated to confirm a single peak and rule out the possibility of primer dimer and nonspecific product formation. The translation elongation factor (*EF1a*) was used as a reference gene, and $2^{-\Delta\Delta C_t}$ method was used to calculate the relative mRNA levels of the target gene in dsRNA-treated samples compared with controls.

2.8 | Statistical analysis

A one-tailed *t* test was used for statistical analysis to compare the means of a single variable. *F* test with α value of .05 was used for determining equal variance among the treatment.

3 | RESULTS

3.1 | RNA sequencing, transcriptome assembly, and analysis

Both total RNA and poly(A) RNA isolated from mixed life stages of CB were sequenced. For each sample 30–40 billion base pairs data were collected, with Q30 of more than 91.46 (Table S1), indicating that the quality of both samples are sufficient for further analysis. Sequences from total RNA and poly(A) RNA were assembled into transcriptome. N50 value shows more than half of the contigs are longer than 2,439bp (Table S2). The completeness of the transcriptome analyzed by BUSCO assessment showed 86% complete contigs (Figure S1). When blasted with Arthropoda database, CB transcripts showed the highest homology with *T. castaneum* sequence (26%), followed by six other coleopteran insects (Figure S2). In the fungal database, Clostridium showed the highest match hits (59%) followed by Eubacterium (22%) and Caedibacter (5%; Figure S3). In the bacterial database, Candida showed the highest match hits (94%), followed by Kloechera (5%), and Arthroderma (1%; Figure S4). The function of

CB sequences was annotated by using Gene Ontology (GO) assignments. In each of the three main categories including biological process, molecular function, and cellular component and of the GO classification, "cellular process," "binding," and "cell" terms are most dominant, respectively (Figure S5). Orthologs of genes coding for proteins known to be involved in RNAi pathway were identified in the CB transcriptome. Core siRNA pathway genes including Argonautes, Dicers, and Loquacious and others coding for auxiliary RISC-associated or regulatory factors and dsRNA transport were identified (Table S3). These data suggest CB contains genes coding for components for RNAi pathway and RNAi may work well in this insect.

3.2 | Processing of ^{32}P-labeled dsRNA

Initial steps of RNAi include entry of dsRNA into cell cytoplasm and processing of dsRNA into siRNA. Therefore, processing of dsRNA to siRNA could provide a quick assessment on whether or not RNAi functions well in a test organism. ^{32}P-labeled dsRNA was injected into larvae and adults, 2 days after injection, the total RNA was isolated and analyzed by gel electrophoresis. As shown in Figure 1, both larvae and adults efficiently processed dsRNA to siRNA. The siRNA band intensity produced by CB larvae and adults is comparable to the intensity of siRNA band produced by Colorado potato beetle cells (Lepd-SL1), which is known for having highly effective RNAi response (Yoon, Shukla, Gong, Mogilicherla, & Palli, 2016). These data suggest that RNAi could work efficiently in this insect.

3.3 | Screening of candidate genes by dsRNA injection

dsRNA targeting 12 candidate genes was injected into final instar larvae and the mortality was recorded. Injection of dsRNA targeting *GAWKY*, *Kinesin*, *Sec23*, *SNF7*, and *26S proteasome subunit 6B* induced 100% mortality (Figure 2). Except for *PPA2*, knockdown of rest of the 11 genes induced more than 80% mortality. About 36% mortality was observed in control insects injected with dsGFP (Figure 2). The control mortality is similar when dsRNA targeting the luciferase gene or distilled water alone was injected (data not shown). Most of the treatment showed LT50 ranging from 12 to 15 days. dsKinesin and dsSNF7 showed the lowest LT50 (8.56 and 8.44 days, respectively), which indicates their mortality effect started earlier in the insects injected with these dsRNAs than the other dsRNAs tested (Table S6). After dsIAP injection, 60% and 40% of total mortality was observed during pupal and larval stage, respectively (Figure 3a). In dsHsp70-injected larvae, 25% and 75% of total mortality were observed during pupal and larval stage, respectively (Figure 3b,c). For the rest of the dsRNAs tested, more than 90% total

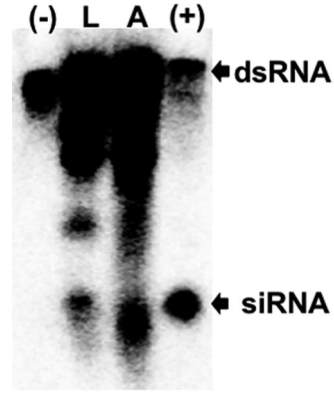

FIGURE 1 Both larvae and adults of cigarette beetle are able to process dsRNA into siRNA. ^{32}P-labeled dsRNA (0.14 million CPM) was injected in last instar larvae or adults. Two days after injection, total RNA was extracted using the whole body. Extracted RNA (2,000 CPM) was resolved on 8 M urea-16% polyacrylamide gel. siRNA produced by the Colorado potato beetle cell line (Lepd-SL1) was used as a positive control. dsRNA, double-stranded RNA; siRNA, small interference RNA

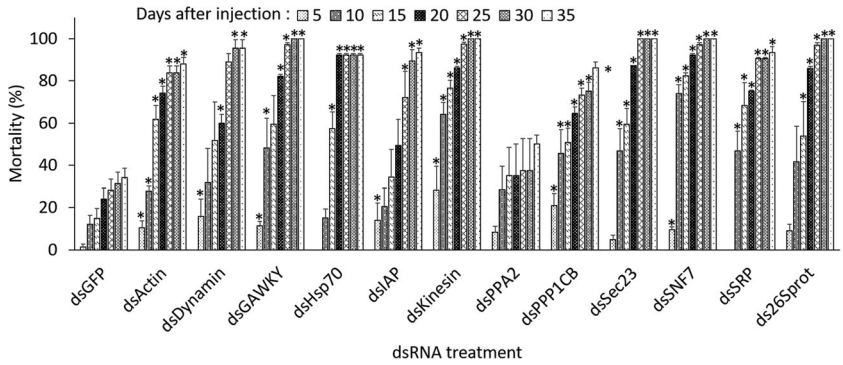

FIGURE 2 Double-stranded RNA (dsRNA) induced mortality after injection. Two hundred nanograms of dsRNA in 200 nl was injected into fourth instar (final instar) larvae. Injected larvae were reared at 30 ± 2°C, and mortality was recorded until adult emergence. Mean+standard error (*S.E.*; n = 10–15) of three replicates are shown. One-tailed Student's t test was used to determine the significance of mortality rate in each treatment compared with control (dsRNA targeting green fluorescence protein) in respective time points after injection. F test with α value of .05 was used for determining equal variance among the treatment. The significance of mean is shown above each bar (*< .05)

mortality was observed during the larval stage (data not shown). Knockdown efficiency of dsRNA tested varied between 40% and 90% (Figure 4).

3.4 | Droplet feeding bioassay

Based on their effectiveness in injection bioassay, we selected dsSNF7 and ds26S proteasome subunit 6B for droplet feeding assay in neonate larvae. We first confirmed that neonates ingest 0.5% sucrose solution by feeding these larvae with the sucrose solution traced with purple food color dye. After feeding on sucrose droplet for 24 hr, the alimentary canal from neonates showed purple color suggesting that these larvae ingested sucrose solution (Figure 5). The neonate larvae stayed alive by feeding on sucrose up to 20 days, but they did not grow and molt to the next stage. Feeding of dsRNA targeting *SNF7* and *26S proteasome subunit 6B* induced 95% and 93% mortality, respectively (Figure 6), which is a 1.83 and 1.78 times increase in mortality compared with that in dsGFP-fed control larvae (53%). LT50 for *SNF7* and *26S proteasome subunit 6B* was 13.34 and 13.52, respectively (Table S7).

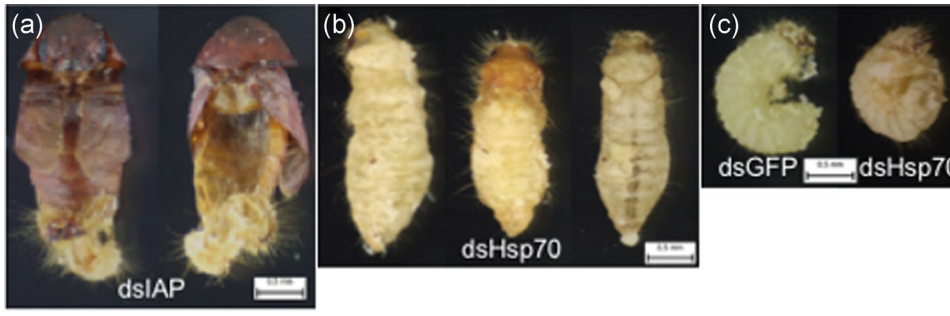

FIGURE 3 Phenotype after double-stranded RNA injection. (a) In dsIAP injection, 60% and 40% of the total mortality was observed in pupal and larval stages, respectively. (b, c) In dsHsp70 injection, 25% and 75% of total mortality were observed in pupal and larval stages, respectively

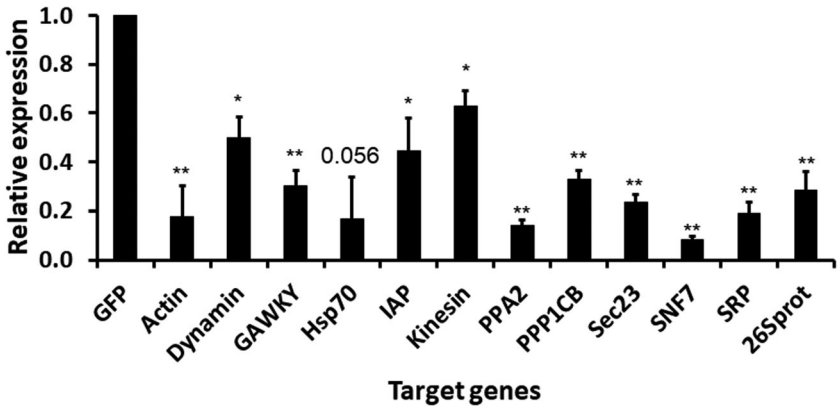

FIGURE 4 Knockdown efficiency of injected double-stranded RNA. Two hundred nanograms of dsRNA in 200 nl volume was injected into each fourth instar (final instar) larvae. Injected larvae were reared at 30 ± 2°C for 2 or 4 days. Total RNA was extracted from the whole body samples. Extracted total RNA was used in quantitative reverse-transcription polymerase chain reaction. Data were analyzed with $2^{-\Delta\Delta C_t}$ method, using *EF1a* as the reference gene. Mean+standard error (*S.E.*) of three replicates are shown. Statistical analysis was performed by one-tailed Student's *t* test. *F* test with α value of .05 was used for determining equal variance among the treatment. The significance of means and p value for *Hsp70* are shown above bars (*< .05, **< .01)

4 | DISCUSSION

Although, CB is an important and difficult to control storage pest, no information on its sequences is publicly available except for its mitochondrial genome sequence (Yang et al., 2017). To fill this knowledge gap, we sequenced CB RNA and assembled it into transcriptome. Transcriptome sequences are useful for high-throughput screening of RNAi target genes (H. Li, Jiang, Zhang, Xing, & Li, 2013; Wang, Zhang, Li, & Miao, 2011). Moreover, transcriptome can be a useful genetic resource for future research aimed at developing advanced methods to control CB.

In *T. castaneum*, 5,000 genes were screened to find lethal RNAi target genes (Ulrich et al., 2015). We selected 12 genes that are shown to be effective in *T. castaneum*. We identified orthologs of these 12 genes and tested dsRNA targeting these genes in CB. Out of 12 genes, 11 genes were highly effective in inducing mortality over 80% (Figure 2). These results suggest that the function of these selected genes and their essential roles for survival are conserved between *T. castaneum* and CB. *T. castaneum* information was also used for the identification of RNAi targets in *Diabrotica virgifera*. Out of 50 selected genes, 21 induced mortality and 36 showed growth inhibition in *D. v. virgifera* diet-based RNAi bioassays (Knorr et al., 2018). A similar approach was used in other studies, and they

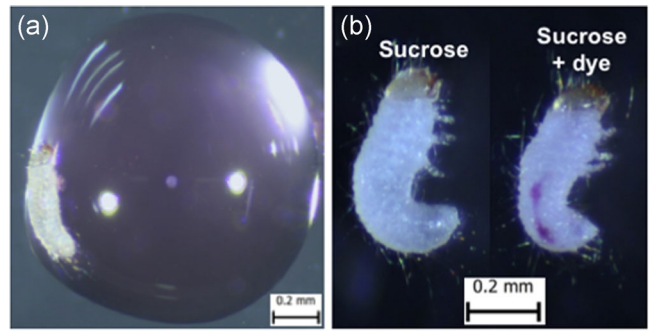

FIGURE 5 Confirmation of neonate larvae ingestion on 0.5% sucrose solution. (a) Neonate ingesting in sucrose solution. (b) Neonate fed on 0.5% sucrose solution alone (left), and neonate fed on 0.5% sucrose solution with 0.5% purple dye (right)

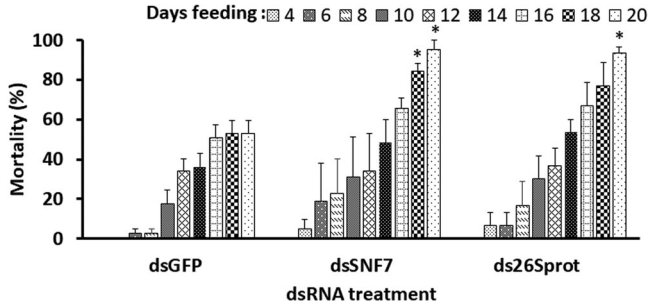

FIGURE 6 Mortality induced by fed double-stranded RNA (dsRNA). Neonates (newborn, first instar larvae) were placed in 60 × 15mm Petri dishes, with 0.5% sugar drop solution containing 2 μg/μl dsRNA. Sugar drop solution containing dsRNA was resupplemented every 2 days. Sterilized water was added in between to avoid drying of the solution. Mortality was recorded up to 20 days. Mean + standard error (*S.E.*; $n = 7–11$) of three replicates are shown. One-tailed Student's *t* test was used to determine the significance of mortality rate in each treatment compared with control (dsRNA targeting green fluorescence protein) in respective feeding periods. *F* test with α value of .05 was used for determining equal variance among the treatment. The significance of mean is shown above each bar (*< .05)

were able to identify efficient RNAi target genes (Castellanos, Smagghe, Sharma, Oliveira, & Christiaens, 2019; Dhandapani, Gurusamy, Duan, & Palli, 2020; Rodrigues, Duan, Palli, & Rieske, 2018).

Our study also expanded the target gene variety used as lethal RNAi target genes in Coleoptera and Hemiptera. These include *actin* (Castellanos et al., 2019; Liu, Yang, Zhang, Ding, & Wang, 2019; Mogilicherla, Howell, & Palli, 2018; Rodrigues et al., 2018; Rosa, Kamita, & Falk, 2012; F. Zhu et al., 2011), *IAP* (Galdeano, Breton, Lopes, Falk, & Machado, 2017; Liu et al., 2019; Mogilicherla et al., 2018; Rodrigues, Rieske, Jian, Mogilicherla, & Palli, 2017; Walker & Allen, 2011), *SNF7* (Mogilicherla et al., 2018; Ramaseshadri et al., 2013), and *vATPase* (Baum et al., 2007; Castellanos et al., 2019; Mogilicherla et al., 2018; F. Zhu et al., 2011). Similar to our study, high-throughput screening of lethal RNAi target genes was performed in other coleopteran insects, *Agrilus planipennis* (Rodrigues et al., 2018), *Anoplophora glabripennis* (Dhandapani et al., 2020) and in two hemipteran insects, *Halyomorpha halys* (Mogilicherla et al., 2018), and *Euschistus heros* (Castellanos et al., 2019). The widely used target genes, *actin*, *IAP*, and *SNF7* are effective in all four species including CB. However, *Dynamin* and *Hsp70* are only effective in two coleopteran species, whereas in hemipteran insect *H. halys*, they are not effective.

Although, dsKinesin showed the lowest knockdown efficiency (36%), mortality was still high (100%). dsPPA2 showed high knockdown efficiency (86%), but the mortality induced was low (50%). This phenomenon was also observed in another study (H. Li et al., 2013). The authors suggested that this may be due to redundancy in the function of gene products. If a gene product has no redundancy, which means there are no other proteins with similar functions, even lower levels of knockdown could induce mortality. There could be other reasons including RNA and protein stability that could influence mortality induced by dsRNA. Further work is needed to address this discrepancy.

Care should be taken when interpreting dsRNA feeding assay in this study. dsRNA targeting *SNF7* and *26S proteasome subunit 6B* were force-fed to CB neonates by sucrose droplet feeding assay. They caused significant mortality (95% and 93%, respectively) compared with control (53%) (Figure 6). Compared with dsRNA injection method, feeding dsRNA method tends to induce lower and variable RNAi efficiency as reported in other insects (Mogilicherla et al., 2018; Prentice et al., 2017). The stability of dsRNA in the midgut environment and uptake of dsRNA by midgut epithelial cell may also affect the efficiency of fed dsRNA. Although dsRNA is quite stable in midgut lumen contents from coleopteran insects compared with lepidopteran insects, considerable variation in dsRNA stability still exists among different species of coleopteran insects (Singh, Singh, Mogilicherla, Shukla, & Palli, 2017). Knockdown of nuclease activity in the gut of Colorado potato beetle, *Leptinotarsa decemlineata* enhanced RNAi efficiency (Spit et al., 2017).

RNAi has great potential in stored product pest management. Several stored product pests are coleopteran insects, which are known to have a systemic RNAi response (Palli, 2014). The major issue of RNAi application in stored product pest management will be the delivery of dsRNA. Possible dsRNA delivery methods include formulating dsRNA with grain protectants, products applied to surroundings (surfaces or cracks and crevices in empty bins, equipment and structures where food material accumulates), and attractants (Perkin, Adrianos, & Oppert, 2016). Transgenic approach can also open opportunities for RNAi applications. Transgenic bacterial symbiont expressing dsRNA targeting fecundity gene in *Rhodnius prolixus* successfully colonized in the gut and caused significant reduction in host reproduction (Whitten et al., 2016). Bacterial symbionts are identified in some stored product pests (Van Wyk, Hodson, & Christensen, 1959; Vazquez-Arista et al., 1997). Also, CB is known to have symbiotic yeast, *Symbiotaphrina kochii* (Noda & Kodama, 1996), which can be utilized as a carrier of dsRNA targeting the host. The expression of dsRNA in the transgenic plant could be another option. Transgenic corn expressing dsRNA targeting the *SNF7* gene in western corn rootworm (Head et al., 2017) is being used to control this pest.

In summary, we assembled transcriptome of CB to find effective target genes for RNAi-mediated control of CB. CB transcriptome data contains orthologs of genes coding for proteins known to function in RNAi pathway. We also confirmed the conversion of dsRNA into siRNA in CB larvae and adults, which is important initial step of RNAi. Out of 12 candidate target genes tested, injected dsRNA targeting 11 genes induced significant knockdown and mortality. Feeding of dsRNA targeting two genes (*SNF7* and *26S proteasome subunit 6B*) induced significant mortality in neonate larvae. Our results suggest that CB has an efficient RNAi response, and combined with an efficient delivery method, RNAi can be developed as a method to control this pest.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Jinmo Koo: Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Writing-original draft (lead). **Shankar C. R. R. Chereddy:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Writing-original draft (supporting). **Subba R. Palli:** Conceptualization (lead); Funding acquisition (lead); Resources (Lead); Supervision (lead); Writing-original draft (supporting); Writing-review & editing (lead).

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REFERENCES

Baum, J. A., Bogaert, T., Clinton, W., Heck, G. R., Feldmann, P., Ilagan, O., … Roberts, J. (2007). Control of coleopteran insect pests through RNA interference. *Nature Biotechnology*, *25*(11), 1322–1326.

Buchon, N., & Vaury, C. (2006). RNAi: A defensive RNA-silencing against viruses and transposable elements. *Heredity*, *96*(2), 195–202. https://doi.org/10.1038/sj.hdy.6800789

Castellanos, N. L., Smagghe, G., Sharma, R., Oliveira, E. E., & Christiaens, O. (2019). Liposome encapsulation and EDTA formulation of dsRNA targeting essential genes increase oral RNAi-caused mortality in the Neotropical stink bug *Euschistus heros*. *Pest Management Science*, *75*(2), 537–548.

Dhandapani, R. K., Gurusamy, D., Duan, J. J., & Palli, S. R. (2020). RNAi for management of Asian long-horned beetle, *Anoplophora glabripennis*: Identification of target genes. *Journal of Pest Science*, *93*, 823–832.

Farnham, A. S., Flora, J. W., Ingram, S. S., & Faustini, D. L. (2007). No evidence of substantial nicotine metabolism by *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae) reared on tobacco. *Journal of Stored Products Research*, *43*(2), 171–176.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., & Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*, *391*(6669), 806–811.

Galdeano, D. M., Breton, M. C., Lopes, J. R., Falk, B. W., & Machado, M. A. (2017). Oral delivery of double-stranded RNAs induces mortality in nymphs and adults of the Asian citrus psyllid, *Diaphorina citri*. *PLOS One*, *12*(3):e0171847.

Gotz, S., Garcia-Gomez, J. M., Terol, J., Williams, T. D., Nagaraj, S. H., Nueda, M. J., … Conesa, A. (2008). High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids Research*, *36*(10), 3420–3435.

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., … Regev, A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology*, *29*(7), 644–652.

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., … Regev, A. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols*, *8*, 1494–1512.

Hagstrum, D. W., Klejdysz, T., Subramanyam, B., & Nawrot, J. (2013). Stored-product insects. In D. W.Hagstrum, T.Klejdysz, B. Subramanyam, & J.Nawrot (Eds.), *Atlas of Stored-Product Insects and Mites* (pp. 3–198). Eagan, MN: AACC International Press.

Head, G. P., Carroll, M. W., Evans, S. P., Rule, D. M., Willse, A. R., Clark, T. L., … Meinke, L. J. (2017). Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: Efficacy and resistance management. *Pest Management Science*, *73*(9), 1883–1899.

Howe, R. W. (1965). A summary of estimates of optimal and minimal conditions for population increase of some stored products insects. *Journal of Stored Products Research*, *1*(2), 177–184.

Jagadeesan, R., Collins, P. J., Daglish, G. J., Ebert, P. R., & Schlipalius, D. I. (2012). Phosphine resistance in the rust red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae): Inheritance, gene interactions and fitness costs. *PLOS One*, *7*(2):e31582.

Jones, C. R. (1913). The cigarette beetle (*Lasioderma serricorne* Fabr.) in the Philippine Islands. *Phillippine Journal of Science, General Biology, Ethnology, and Anthropology*, *8*, 1–51.

Knorr, E., Fishilevich, E., Tenbusch, L., Frey, M. L. F., Rangasamy, M., Billion, A., … Narva, K. E. (2018). Gene silencing in *Tribolium castaneum* as a tool for the targeted identification of candidate RNAi targets in crop pests. *Scientific Reports*, *8*(1), 2061.

Li, H., Jiang, W., Zhang, Z., Xing, Y., & Li, F. (2013). Transcriptome analysis and screening for potential target genes for RNAi-mediated pest control of the beet armyworm, *Spodoptera exigua*. *PLOS One*, *8*(6):e65931.

Li, H. W., & Ding, S. W. (2005). Antiviral silencing in animals. *FEBS Letters*, *579*(26), 5965–5973.

Liu, F., Yang, B., Zhang, A., Ding, D., & Wang, G. (2019). Plant-mediated RNAi for controlling *Apolygus lucorum*. *Frontiers of Plant Science*, *10*, 64.

Mogilicherla, K., Howell, J. L., & Palli, S. R. (2018). Improving RNAi in the brown marmorated stink bug: Identification of target genes and reference genes for RT-qPCR. *Scientific Reports*, *8*(1), 3720.

Noda, H., & Kodama, K. (1996). Phylogenetic position of yeastlike endosymbionts of anobiid beetles. *Applied and Environmental Microbiology*, *62*(1), 162–167.

Palli, S. R. (2014). RNA interference in Colorado potato beetle: Steps toward development of dsRNA as a commercial insecticide. *Current Opinion in Insect Science*, *6*, 1–8.

Perkin, C. L., Adrianos, L. S., & Oppert, B. (2016). Gene disruption technologies have the potential to transform stored product insect pest control. *Insects*, *7*(3), 46.

Powell, T. E., Jr. (1931). An ecological study of the tobacco beetle, *Lasioderma serricorne* Fabr., with special reference to its life history and control. *Ecological Monographs*, *1*(3), 333–393.

Prentice, K., Christiaens, O., Pertry, I., Bailey, A., Niblett, C., Ghislain, M., … Smagghe, G. (2017). RNAi-based gene silencing through dsRNA injection or ingestion against the African sweet potato weevil *Cylas puncticollis* (Coleoptera: Brentidae). *Pest Management Science*, *73*(1), 44–52.

Rajendran, S., & Narasimhan, K. S. (1994). Phosphine resistance in the cigarette beetle *Lasioderma serricorne* (Coleoptera: Anobiidae) and overcoming control failures during fumigation of stored tobacco. *International Journal of Pest Management*, *40*(2), 207–210.

Ramaseshadri, P., Segers, G., Flannagan, R., Wiggins, E., Clinton, W., Ilagan, O., … Bolognesi, R. (2013). Physiological and cellular responses caused by RNAi-mediated suppression of Snf7 orthologue in western corn rootworm (*Diabrotica virgifera virgifera*) larvae. *PLOS One*, *8*(1):e54270.

Reinhart, B. J., Slack, F. J., Basson, M., Pasquinelli, A. E., Bettinger, J. C., Rougvie, A. E., … Ruvkun, G. (2000). The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature*, *403*(6772), 901–906.

Rodrigues, T. B., Duan, J. J., Palli, S. R., & Rieske, L. K. (2018). Identification of highly effective target genes for RNAi-mediated control of emerald ash borer, *Agrilus planipennis*. *Scientific Reports*, *8*(1), 5020.

Rodrigues, T. B., Rieske, L. K., Jian, J. D., Mogilicherla, K., & Palli, S. R. (2017). Development of RNAi method for screening candidate genes to control emerald ash borer, *Agrilus planipennis*. *Scientific Reports*, *7*(1), 7379.

Rosa, C., Kamita, S. G., & Falk, B. W. (2012). RNA interference is induced in the glassy winged sharpshooter *Homalodisca vitripennis* by actin dsRNA. *Pest Management Science*, *68*(7), 995–1002.

Roy, A., George, S., & Palli, S. R. (2018). Correction to: Multiple functions of CREB-binding protein during postembryonic development: Identification of target genes. *BMC Genomics*, *19*(1), 584.

Runner, G. A. (1919). *The tobacco beetle: An important pest in tobacco products* (Vol. 737, 1–88). Washington, DC: U.S. Dept. of Agriculture.

Saglam, O., Edde, P. A., & Phillips, T. W. (2015). Resistance of *Lasioderma serricorne* (Coleoptera: Anobiidae) to fumigation with phosphine. *Journal of Economic Entomology*, *108*(5), 2489–2495.

Shabalina, S. A., & Koonin, E. V. (2008). Origins and evolution of eukaryotic RNA interference. *Trends in Ecology and Evolution*, *23*(10), 578–587.

Shukla, J. N., Kalsi, M., Sethi, A., Narva, K. E., Fishilevich, E., Singh, S., … Palli, S. R. (2016). Reduced stability and intracellular transport of dsRNA contribute to poor RNAi response in lepidopteran insects. *RNA Biology*, *13*(7), 656–669.

Singh, I. K., Singh, S., Mogilicherla, K., Shukla, J. N., & Palli, S. R. (2017). Comparative analysis of double-stranded RNA degradation and processing in insects. *Scientific Reports*, *7*(1), 17059.

Spit, J., Philips, A., Wynant, N., Santos, D., Plaetinck, G., & Vanden Broeck, J. (2017). Knockdown of nuclease activity in the gut enhances RNAi efficiency in the Colorado potato beetle, *Leptinotarsa decemlineata*, but not in the desert locust, *Schistocerca gregaria*. *Insect Biochemistry and Molecular Biology*, *81*, 103–116.

Ulrich, J., Dao, V. A., Majumdar, U., Schmitt-Engel, C., Schwirz, J., Schultheis, D., … Bucher, G. (2015). Large scale RNAi screen in Tribolium reveals novel target genes for pest control and the proteasome as prime target. *BMC Genomics*, *16*(1), 674.

United States. Stored-Product Insect Research Branch. (1962). *Stored-tobacco insects: biology and control*, Agriculture handbook (United States. Department of Agriculture), no. 233(1–39). Washington, DC: U.S. Dept. of Agriculture, Agricultural Marketing Service.

Van Wyk, J. H., Hodson, A. C., & Christensen, C. M. (1959). Microflora associated with the confused flour beetle, *Tribolium Confusum*. *Annals of the Entomological Society of America*, *52*(4), 452–463.

Vazquez-Arista, M., Smith, R. H., Olalde-Portugal, V., Hinojosa, R. E., Hernandez-Delgadillo, R., & Blanco-Labra, A. (1997). Cellulolytic bacteria in the digestive system of *Prostephanus truncatus* (Coleoptera: Bostrichidae). *Journal of Economic Entomology*, *90*(5), 1371–1376.

Walker, W., & Allen, M. (2011). RNA interference-mediated knockdown of IAP in *Lygus lineolaris* induces mortality in adult and pre-adult life stages. *Entomologia Experimentalis et Applicata*, *138*, 83–92.

Wang, Y., Zhang, H., Li, H., & Miao, X. (2011). Second-generation sequencing supply an effective way to screen RNAi targets in large scale for potential application in pest insect control. *PLOS One*, *6*(4):e18644.

Whitten, M. M., Facey, P. D., Del Sol, R., Fernandez-Martinez, L. T., Evans, M. C., Mitchell, J. J., … Dyson, P. J. (2016). Symbiont-mediated RNA interference in insects. *Proceedings. Biological Sciences*, *283*(1825), 20160042.

Yang, W.-J., Xu, K.-K., Zhu, X.-Y., Chen, C.-X., Cai, X.-Y., Cao, Y., … Li, C. (2017). The complete mitochondrial genome of the cigarette beetle, *Lasioderma serricorne* (Coleoptera: Anobiidae). *Mitochondrial DNA Part B*, *2*(2), 430–431.

Yoon, J. S., Shukla, J. N., Gong, Z. J., Mogilicherla, K., & Palli, S. R. (2016). RNA interference in the Colorado potato beetle, *Leptinotarsa decemlineata*: Identification of key contributors. *Insect Biochemistry and Molecular Biology*, *78*, 78–88.

Zettler, J. L. (1994). Phosphine resistance in cigarette beetle (Coleoptera: Anobiidae) associated with tobacco storage in the southeastern United States. *Journal of Economic Entomology*, *87*(3), 546–550.

Zhu, F., Xu, J., Palli, R., Ferguson, J., & Palli, S. R. (2011). Ingested RNA interference for managing the populations of the Colorado potato beetle, *Leptinotarsa decemlineata*. *Pest Management Science*, *67*(2), 175–182.

Zhu, K. Y., & Palli, S. R. (2020). Mechanisms, applications, and challenges of insect RNA interference. *Annual Review of Entomology*, *65*, 293–311.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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