Chapter 23

RNAi based system a new tool for insects' control

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Abstract

One of the molecular devices practised recently has been the fusion of RNA interference (RNAi) into some agricultural products. It is a definite genetic controlling system recognised in eukaryotes. Through this system, certain exogenous pathogens are neutralized by deactivating the expression of target genes. The decisive factor for the progress of this gene defence machinery is the double-stranded RNA (dsRNA). The effectiveness and specificity of the RNAi tool in gene silencing have been approved with great precision in small-scale guide tests. The development of this molecular tool as bioinsecticides has started to attract the biotechnology industries. Once the safety and certainty actions respecting the regulatory framework are established by researchers and biotechnology industries for crop protection, for example; sustainability and particularity of defence, healthiness of all health dangers, and removal of all unexpected influences on the environment, genetically modified crops incorporating dsRNA can be marketed. The current chapter discuss the RNAi tool and its role in protecting crops from insect pest attacks, dsRNA transfer methods in plant cells, and critical points affecting the achievement of the molecular tool. Finally, some environmental risks identified in the small-scale guide tests are discussed.

Keywords: RNAi; dsRNA; bioinsecticide; crop protection.

23.1. Introduction

The global population explosion and the increasing demand for high quality agricultural products from consumers and food processing industries in recent decades has obliged control services to establish and to apply new protection strategies to maintain food security (Godfray

and Garnett 2014). Plant pathogens and pests are the most relevant causes of loss and crop damage in the fields. In front of this critical situation, the use of pesticides is among the most applied handling policies, these agrochemicals used in agricultural land are very harmful to the health of consumers (Nicolopoulou-Stamati et al., 2016). Pesticides are also harmful to the environment; they can persist in water and soil ecosystems and interact with living things by inducing harmful effects (Damalas and Eleftherohorinos, 2011).

Biotechnological progress has revealed that the installation of RNA interference (RNAi) into biological cells may be a trick to interfere with exogenous genes. The first research reporting this consequence was published in 1998 by Fire 's team. The team used RNAi tool to modify gene expression. The interference mechanism depends on the hybridization between the nucleic acids of the injected RNA and the targeted messenger RNA (mRNA) in *Caenorhabditis elegans* (Fire et al., 1998). Another study indicated that RNAi regulates transcription through its interaction with transcriptional machinery, it is able to neutralize the transposable elements, its role appears in heterochromatin creation, developmental gene control and genome stability (Guang et al., 2010; Castel and Martienssen, 2013). Other RNAs are capable of acting at the genome level, for example; microRNA (miRNA) acts to regulate endogenous genes and to prevent invasive nucleic acids (Carthew and Sontheimer, 2009), and Hairpin RNA (hpRNAs) which form a commanding device inducing gene silencing (Schumann et al., 2013).

Molecular biology research aimed at controlling agricultural pathogens demonstrated that RNAi has significant potential for controlling insects. To restrict the employ of genetically modified organisms (GMOs), great attention has been paid to this molecular instrument in order to recommend it against insects ravaging plants and crops. Unexpected efficacy was demonstrated against harmful insects (Rosa et al., 2018; Zotti and Smagghe, 2015). The identification of new genes indispensable for the growth and development of insects supports the progress and success of RNAi in the biological fight against insects (Liu et al., 2020). Understanding inactivation steps by the double-stranded RNA (dsRNA) at the molecular level, and the crucial factors in initiating interference steps has increased in recent years. Now that the scientists have resolved the interference mechanism and the delivery methods of dsRNAs, it is time to apply the knowledge acquired for crop protection (Koch et al., 2016; Zotti et al., 2018). This chapter focuses on the applications of RNAi technology as a new molecular alternative for the control of field pests, dsRNA transport systems into insect cells, and the key factors increasing the success of interference. Potential risks to the environment are also discussed.

23.2. The effectiveness of RNAi in biological control and its working mechanism in the attenuation of genes which is essential for the life of insects

RNA interference is a conserved system in eukaryotes. It is responsible for the repression of genes by small non-coding nucleic acid fragments (siRNAs) of around 20 to 30 nucleotides (Nts) (Nicolás et al., 2013). RNAi is involved in cellular protection against attacks of extracellular nucleic acids (Jeang, 2012). Three main pathways are involved in the regulatory mechanism of RNAi, they are mediated by the siRNA, the miRNA and the RNAs interacting with Piwi (piRNA) (Blair and Olson, 2015). Dicer, Argonaute (Argo), and RNA-dependent RNA polymerase are the indispensable elements involved in the RNAi process (Dang et al.,

2011). siRNAs are produced endogenously, however, exogenous dsRNAs can also be inoculated into insect cells and then treated in small duplexes under the action of ribonucleases-III (Dicer) (Zhang et al., 2004). In the cytoplasm, the siRNAs formed a complex with RNA-binding proteins, members of the Argo family, leading to the establishment of the RNA-induced silencing complex (RISC) (Lee et al., 2006). The assembled structure contains essentially the siRNA and Ago-2 which has a catalytic cleavage activity. The assembled structure is activated by discharging the passenger RNA strand and then moves towards target recognition by complementing the bases with the single guide RNA strand (Kim and Rossi, 2008). Target recognition leads to transcriptional gene silencing or post-transcriptional gene silencing (Liu et al., 2020).

miRNAs are small, non-coding RNAs. They collectively control the expression of genomic genes in cells. More than 2,000 regulatory Nts sequences are recorded (Hammond, 2015). Three main factors are identified in the biosynthesis of miRNAs, they are: dsRNA-specific endoribonuclease (Drosha), DiGeorge syndrome critical region 8 (DGCR8) and Dicer. Primary miRNA (pri-miRNA) is treated under the action of Drosha and Pasha to subsequently shape the pre-miRNA precursor. The latter forms the miRNA duplex after its cleavage by the Dicer. The miRNA duplex is processed into a single strand miRNA by an RNA-helicase. The mature miRNA is loaded over Argo to form the RISC. The post-transcriptional silencing of genes is carried out by Argo which pilots the miRNA to the target mRNAs. Knockdown is induced by translational repression or degradation of mRNA (Bagga et al., 2005; Pong and Gullerova, 2018; Zhu and Palli, 2020).

Regarding the piwi pathway (piRNA), from piRNA clusters, RNA polymerase II transcribes a long single-stranded antisense RNA. The designed RNA is a pre-piRNA precursor which will after be transformed into primary piRNA (pri-piRNA) under the action of an endonuclease (Zuc). The piRNA is then loaded into specific proteins to produce the Piwi complex, which degrades the target and generates new sense piRNAs. The new sense piRNAs are loaded another time on Argo-3. The corresponding sequences are cleaved with the genesis of new antisense piRNAs and the cycle begins again (Zhu and Palli, 2020). Fig 23.1 summarizes the three gene regulatory pathways.

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Fig 23.1. Gene regulation in insect cells via siRNA, miRNA and piRNA.

23.3. Application of RNAi gene technology in the preservation of crops against harmful insects

With current approximation and statistical systems, scientists have estimated the existence of 5.5 million distinct insect species on Earth (Stork, 2018). A major problem is posed by insect pest populations due to the emergence of resistance to insecticides applied in agricultural fields (Sudo et al., 2017). This situation notably leads to significant crop losses, in particular, rice, wheat and maize known by their wide consumption, and an inability to meet growing food needs due to the global population explosion (Deutsch et al., 2018; Bodirsky et al., 2015). In addition, studies and research continue to point out the dangers and health risks linked to the application of pesticides, without neglecting their impacts on the receiving

environment and non-targeted beneficial insects (Chowdhury et al., 2008; Nagami et al., 2017; Fernandes et al., 2016). The uncontrollable situation prevented researchers to leave the habitual research platforms for new strategies to develop more effective alternatives capable of protecting food security and guaranteeing healthy and sustainable crops preservation in the next years. One of the practical molecular alternatives in this area is RNAi technology. The molecular tool is based on the transfer into insect cells of a dsRNA inducing knockdown of the transcribed target genes (Bally et al., 2018; Kunte et al., 2020). The rigorous selection of an dsRNA and the post-transcriptional disturbance of the transcribed sequence essential for growth and development induces the death of the insect. The eradication effect recorded against crop pests makes RNAi a gene tool capable of being introduced into the plant as a new control method (Wang et al., 2013; Kunte et al., 2020). Fig 23.2 demonstrates the mechanism of action of dsRNAs as insecticides introduced into transgenic plants.

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Fig 23.2. Transgenic plants express dsRNAs as an insecticide.

An unlimited number of experiments carried out in biological laboratories all over the world revealing the insecticidal effect of RNAi technology mediated by delivered dsRNAs. In the current section, dsRNA-mediated bioassays extinguish large numbers of insect species are discussed. The study by Baum and his group demonstrated the effectiveness of the nucleic acid introduced into coleopteran species responsible for damaging corn roots. Transgenic plants made capable of expressing dsRNAs have demonstrated resistance against insect damage (Baum et al., 2007). Expression of the CYP6AE14 gene coding for cytochrome P450 identified in *Helicoverpa armigera* (the agent responsible for cotton culture alterations) allows gossypol, a cotton metabolite, to be tolerated by the insect. Feeding the insect larvae with *Arabidopsis thaliana* and *Nicotiana tabacum* expressing ds-CYP6AE14 leads to retarded growth in insect (Mao et al., 2007). The expression of ds-CYP6AE14 by transgenic cotton, *Gossypium hirsutum*, also causes a delay in the growth of Bollworm larvae and enhances the resistance of cotton (Mao et al., 2011). Attenuation of RNAi-mediated genes can be improved by introducing other proteins in insect diet. The absorption GhCP1 (plant cysteine protease) of cotton by *H. armigera* larvae conducts to the attenuation of the peritrophic matrix, and accumulation of gossypol in the midgut which improved the suppression of CYP6AE14 mediated by ds-CYP6AE14 expressed by transformed cotton (Mao et al., 2013). A minimal survival rate was also recorded in *H. armiger* larvae during the knockdown of the coatomer β and V-ATPase-A genes by specific siRNAs (Mao et al., 2015).

In *Diabrotica virgifera* larvae, the injection of a dsRNA targeting DvSnf7 mRNA causes mortality. DvSnf7 gene codes for a protein involved in intracellular biological activities. The contact time and the length of the injected dsRNA sequence significantly affect the silencing activity (Bolognesi et al., 2012). A similar study was carried out by Vélez and his group, a dsRNA targeting V-ATPase-A in *D. v. virgifera* has shown that the injected sequence causes mortality in adult insects (Vélez et al., 2016a). Mevalonic acid pathway also called HMG-CoA reductase pathway is an important pathway in insect metabolism. 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is the main pathway enzyme. In *H. armigera* fed by leaves of transgenic plants expressing ds-HMGR, a downregulation is recorded for target gene

expression (Tian et al., 2015). Previous study found that the knockdown of HMGR results in reduced levels of vitellogenin mRNA and female fertility (Wang et al., 2013). The silencing of the molt-regulating transcription factor gene (HaHR3) by HaHR3-dsRNA expressed by transgenic cotton plants has led to deformations in adults and significant larval mortality (Han et al., 2017). Table 23.1 discloses several dsRNA sequences intended for the silencing of genes expressed by different insect pests.

Insert Table 23.1 here

23.4. Delivery methods of dsRNA into insect cells

It is clear and essential that before any application of RNAi technology against harmful insects, the efficacy and toxicity of administered nucleotide sequences must be ensured, on the one hand; to avoid any interference with beneficial insects, and on the other hand, to eradicate any danger to humans, animals and the receiving environment. Besides, the sensitivity of the administered dsRNAs must be studied in depth to facilitate their absorption by the target insect and to eliminate their possible enzymatic decomposition. In order to achieve this aim, packaging methods and nucleic acid delivery systems are proposed and published. In the current part of the chapter, an overview of some systems for efficiently conveying dsRNAs are discussed**.** Fig 23.3 summarizes the methods applied for the delivery of dsRNAs involved in the control of insect pests.

Insert Fig 23.3 here

Fig 23.3. Some effective methods applied for the introduction of dsRNAs involved in the control of crop insect pests.

23.4.1. Bacterial and fungal cells as carriers of dsRNA

Bacteria are the most widely used delivery microorganism, by its structure it can protect dsRNAs against decomposition. Among the first studies using bacteria as a vehicle for dsRNA is that of Timmons and Fire (1998) focused on *C. elegans*. The two researchers used *Escherichia coli* harbouring a vector that had a bidirectional transcription. In their study, *E. coli* serves as a nutrient for the nematode, which is absorbed in the intestines after being shredded (Timmons and Fire, 1998). The application of sonication on *E. coli* made capable of producing dsRNAs demonstrated an increase in efficiency against *Spodoptera exigua* with a significant decrease integrin β1 subunit (SeINT) expression. In addition, the treated larvae became more sensitive to the Crytoxin secreted by *Bacillus thuringiensis* (Kim et al., 2015).

In addition to sonication, other researchers have optimized other parameters to increase the effectiveness of dsRNA delivered by transformed bacteria. Vatanparast and Kim demonstrated that targeting *S. exigua* larvae with low RNase activity in the intestinal lumen and applying sonication before oral introduction significantly enhanced the efficacy of dsRNA targeting the SeCHY gene (Vatanparast and Kim, 2017). However, targeting multiple genes at the same time is very effective in eradicating damaging insects. The expression of dsRNAs targeting Actin, Sec23, V-ATPase-E, V-ATPase-B, and COPβ delivered by *E. coli* HT115 demonstrated a significant mortality in *Leptinotarsa decemlineata* (Zhu et al., 2011). In *Plagiodera versicolora*, the silencing of 6 genes, namely; ACT, SRP54, HSC70, SHI, CACT

and SNAP by the use of *E. coli* HT115 expressing dsRNAs has proven that the attenuation of actin and signal recognition particle protein 54k (SRP54) leads to a significant insecticidal effect (Zhang et al., 2019). In *L. decemlineata*, the use of Ldp5cdh1-dsRNA and Ldp5cdh2-dsRNA to target the Ldp5cdh gene which codes for a functional P5CDh enzyme used in the ATP biosynthesis pathway has demonstrated a decrease in ATP rates and an increase in insect mortality (Wan et al., 2015a).

The transfer of dsRNAs is possible through the use of symbiotic insect bacteria (Whitten et al., 2016). Nevertheless, the application of this delivery method requires detailed information and investigations, since the introduction of selected symbiotic bacteria as a vehicle for dsRNAs requires an understanding of the dynamics and potential interactions of the insect microbiome (Goodfellow et al., 2019).

Although the bacterial administration of dsRNAs is effective against certain insect species, for others it is limited or impotent. As demonstrated in a study carried out on *Sesamia nonagrioides* larvae, the knockdown of the juvenile hormone esterase (SnJHER) was effective while no negative effect on the development has been recorded (Kontogiannatos et al., 2013). However, Miller and his team have shown that the concentration and length of nucleotide sequence significantly affect the effectiveness of interference (Miller et al., 2012). Another crucial parameter that remains to be divulged to improve the effectiveness of dsRNA is the mechanism by which bacteria export transcribed RNA to the outside environment. High-throughput sequencing has revealed that bacterial cells can expel various types of RNA into the extracellular medium. Some of these nucleic acid sequences are linked to the outer membrane vesicle (OMV) (Ghosal et al., 2015), these types of interaction with the siRNAs formed require further study.

The delivery of dsRNAs can also be ensured by yeasts. The first study carried out in this context was realized by Murphye and his group. A modified *Saccharomyces cerevisiae* INVSc1 strains containing p406TEF1 DNA vectors carrying the target gene sequence (y-Tubulin) demonstrated a decrease in larval survival and a reduce in locomotor and reproductive activity in *Drosophila suzukii* (Murphy et al., 2016). The introduction of small hairpin RNA (shRNA) into *S. cerevisiae*, in an inactivated cell form, revealed sufficient silencing of target genes in *Anopheles gambiae* (Mysore et al., 2019a). In *Aedes aegypti*, the attenuation of axon guidance regulator semaphorin-1a (sema1a) by modified *S. cerevisiae* carrying shRNA induced larval mortality caused by severe neuronal abnormalities (Mysore et al., 2019b)**.** Table 23.2 shows the effectiveness of *E. coli* HT115 as a vehicle for dsRNAs targeting crucial genes included in the development of insect pests.

Insert Table 23.2 here

23.4.2. Viral vector as a delivery vehicle

Some viruses with their genomic material have a high ability to infect plant cells. The types of vectors carried by viruses are very interesting for the transport of nucleotide sequences which aims to increase plant immunity and knockdown the target genes in the vital organism of insect pests (Couto and High, 2010). The specificity of infection existing in viruses will create in the future a gene bank by precisely selecting and improving the recombinant vectors

allowing the knockdown of target genes (Liu et al., 2019). Despite their recommended effectiveness in the delivery of dsRNAs, it seems that some viruses are not useful as a delivery tool since some of them can prevent or disrupt interference steps (Mérai et al., 2006). Uhlirova's study is among the studies done in this area. A recombinant Sindbis virus members of the family Togaviridae is made capable to decrease mRNA levels of the transcription factor Broad-Complex BR-C in *Bombyx mori*. The resulting insects suffer from developmental defects (Uhlirova et al., 2003). Despite the recommendations recorded for the viral delivery method, its application in crop preservation has not been widely studied following the several obstacles of biosafety and genetic pollution which must be taken into consideration.

23.4.3. Nanoparticle as a delivery vehicle

dsRNA sequences are known for their sensitivity to enzymes, and to protect them against any enzymatic decomposition, they can be incorporated into nanostructure particles. Chitosan is the most used material for the design of nanostructured particles incorporating dsRNAs due to its lower toxicity to receptor cells. Chitosan is also cationic, which allows it to easily cross membranes. Its bio-decomposition and biocompatibility are also noted (Cao et al., 2019). This nanotechnology seems less expensive since chitosan and nucleic acids are prepared under electrostatic interactions (Ramesh Kumar et al., 2016). It is achievable with siRNAs or dsRNAs of a long strand (Zhang et al., 2015a). According to several publications, this delivery technique has shown high death rates in *A. gambiae* and *A. aegypti* (Ramesh Kumar et al., 2016; Zhang et al., 2015a; Mysore et al., 2013). Other nanoparticles such as for example; carbon quantum dot, and complex silica can incorporate dsRNA sequences and protect them from any alteration to increase the attenuation action of target genes (Das et al., 2015). In *Ostrinia furnacalis*, the oral administration of FNP / CHT10-dsRNA causes mortality in the insect (He et al., 2013). The combination of dsRNAs with guanidine-containing polymer nanoparticles (GPN) in order to preserve them against enzymatic nuclease deterioration in particular in a high pH environment such for example; *S. exigua* characterized by an alkaline intestinal environment showed better cellular uptake of dsRNA. By targeting the chitin synthase B gene, an increase in insect death of 53% against only 16% when applying naked dsRNA is recorded (Christiaens et al., 2018).

23.4.4. Liposomes and protein as a delivery system

Another approach is being established by scientists to deliver intact and safe nucleic acids. This approach is based on liposomes which are spherical vesicles formed from one or multiple lipid bilayers. The sizes of the vesicles are between 30 nm to several micrometers. Their particularity is located in the polar heads oriented towards the aqueous phases. Liposomes are manipulated as envelopes to encapsulate biomolecules to deliver them intact to designated targets (Akbarzadeh et al., 2013). The liposomes are very operative for the encapsulation of nucleotide sequences (Barba et al., 2019), and their cationic character are helpful in cell transfection with less toxicity for eukaryotic tissue (Chien et al., 2005).

The application of liposomes in the transport of nucleotide sequences intended to eradicate insect pests demonstrated good performance with a high death rate. In soybean pests: neotropical stink bug *Euschistus heros*, the application of dsRNA encapsulated in liposomes targeting V-ATPase-A and muscle actin demonstrated after 14 days a 45% and 42% mortality

rate, respectively (Castellanos et al., 2019). In the Zhang study, the contact time with dsRNA was found to be the most critical parameter in comparison to the types of liposomes and the number of nucleic acids (Zhang et al., 2018b). Another study done on *Blattella germanica* showed that dsRNA wrapped in a liposome are better protected against enzymatic degradation. dsRNAs lead to the death of cockroaches after silencing tubulin genes in midgut (Lin et al., 2017; Huang et al., 2018). The liposome technique has also proved its competence in the eradication of four species of *Drosophila* namely: *D. melanogaster*, *D. sechellia*, *D. yakuba*, and *D. pseudoobscura*; the technique can be useful in the protection of fruit trees (Whyard et al., 2009).

The preservation of delivered nucleotide sequences against intestinal nucleases of insects by their consolidation with proteins is another approach to increase the potential of RNAi. In *Tribolium castaneum* and *Acyrthosiphon pisum*, the administration of branched amphiphilic peptide capsules (BAPCs) combined with dsRNAs (BiP-dsRNA and Armet-dsRNA) in nanometric form demonstrated significant mortality in both species compared to dsRNAs administered alone (Avila et al., 2018).

23.4.5. Genetically modified plants as a delivery system

Genetic modification of plants crops can be an effective solution to control plant insect pests. The introduction of nucleic acid sequences targeting desired genes into insects directly from modified plants**.** Many scientists have proved the ability of genetically modified, transplastomic and transgenic plants capable of expressing dsRNAs to be resistant to insect pests (Thakur et al., 2014; Poreddy et al., 2017; Zhang et al., 2017).

The knockdown of the juvenile hormone acid methyltransferase (JHAMT) by JHAMT-dsRNA in transformed potato plants via *Agrobacterium* demonstrated an accumulation of transcriptional RNA causing a significant decrease in JHAMT formation in *L. decemlineata* which negatively affects its development (Guo et al., 2018). The transfer of EcR-dsRNA by *Agrobacterium* to Agria and Lady Olympia potato cultivars has proved that genetically modified plants are capable of attenuating the expression of EcR gene (Ecdysone receptor) in *L. decemlineata*. The transformed plants exhibit high biotoxicity and mortality against the target insect (Hussain et al., 2019).

Plastid transformation is also an approach to make the plant capable of expressing dsRNAs. However, the length and the concentration of dsRNAs expressed by the genetically modified plant considerably influence the knockdown (Burke et al., 2019; Zhang et al., 2015). In potato transplastomic plants, the knockdown of CPB β-Actin gene from Colorado potato beetle demonstrated that dsRNAs of 200 bp induced high mortality compared to those of 60 bp which induced a weaker response (He et al., 2020). This dissimilarity in efficiency between the two strands results from their sensitivity to nucleases, which depends strongly on the length of the sequence (Wang et al., 2019).

23.4.6. Spraying as a delivery system

External application of dsRNAs can be another method of delivery. Spraying is the most practiced technique because it allows their absorption by plant cells. Spraying technique provides high protection against pests without the use of pesticides. The effectiveness of

spraying is demonstrated in the study of Yan and his group. dsRNAs/nanocarrier/detergent was performed as an insecticide against *Aphis glycines*. dsRNAs attenuating the four genes (TREH, ATPD, ATPE and CHS1) could penetrate the body wall of the aphid within 4 h. The delivered dsRNAs (ds-ATPD + ds-CHS1) silence the targets with 78.50% of deaths (Yan et al., 2020).

23.5. Parameter taken into account when applying dsRNA

Although the RNAi tool appears to be effective as an insecticide against several insect pests, several parameters and factors affect the interference and action of delivered dsRNAs with target genes which leads to a divergence in the success of the knockdown between species. Fig 23.4 summarizes the factors affecting the knockdown of dsRNA administered to insects or introduced into transgenic plants.

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Figure 23.4. Factors affecting the efficacy of dsRNA administered to insects or introduced into transgenic plants.

23.5.1. Influence of sensitivity and resistance of the target species

The difference in efficiency of dsRNA-induced knockdown between insect species and even within the same insect organism is a limiting factor for the application of the RNA tool (Vogel et al., 2019). Administration of dsRNAs targeting V-ATPase-E (TEV) and apoptosis inhibitor genes in *T. castaneum* and *A. pisum* has shown that the injection and ingestion of the nucleotide sequences resulted in up to 100% mortality of *T. castaneum* larvae. However, in *A. pisum*, the injection of VTE-dsRNA resulted in a death of 65% which confirms that the knockdown of the same genes in distinct species is accomplished by different consequences (Cao et al., 2018). Wang's team also demonstrated that the sensitivity of insect species to dsRNAs targeting the same gene (homologous chitinase) is variable. In descending order, *Periplaneta americana* was the most sensitive followed by *Zophobas atratus*, *Locusta migratoria*, and *Spodoptera litura* (Wang et al., 2016).

The dissimilarity in generating a systemic response is mainly due to several factors. Biomolecules and inhibitors are the primary agents responsible for the variation in sensitivity to dsRNA. In *L. decemlineata*, systemic RNA interference deficient-1 (Sid-1) transmembrane channel-mediated uptake and clathrin-mediated endocytosis are the two pathways involved in the absorption of dsRNAs (Cappelle et al., 2016). However, in *T. castaneum*, clathrin-dependent endocytosis is the major pathway by which the insect guarantees the transportation of dsRNAs and ensures the effectiveness of interference targeting TcLgl (Xiao et al., 2015). The resistance mechanism or its evolution in insects is another point that must be well developed to manage it effectively and generate lasting protection and healthy one (Khajuria et al., 2018).

Geographic origin is another essential parameter affecting the sensitivity of insect species to dsRNA as demonstrated in migratory locusts *L. migratoria* where the sensitivity to dsRNAs targeting corazonin (CRZ) and ecdysone receptor genes was distinct. The result explains that the genome of species resulting from different geographical regions is the key factor in this variation, admitting that several genes are supposed to control interference

(Sugahara et al., 2017). Treatment of three phenotypically dissimilar groups of *D. v. virgifera* with dsRNAs targeting DvRS5 (cysteine protease gene) also demonstrated variations in responses (Chu et al., 2014). Viruses infecting insects are another crucial factor that can affect the effectiveness of dsRNAs by affecting the RNAi machinery (Swevers et al., 2013).

23.5.2. The influence of enzymatic activity on the efficiency of knockdown

The nucleotide sequence delivery method targeting genes in insects plays an imperative role in the success of the interference. As demonstrated in many scientific papers, some insects are sensitive to orally administered dsRNAs, while others are insensitive (Spit et al., 2017). Variations in insect sensitivity make methods of administration and doses of dsRNA key factors for the success of the RNAi tool as a bio-insecticide because under certain conditions, the delivered sequence can be broken down enzymatically (Liu et al., 2012; Luo et al., 2013). Oral administration of dsRNA in *L. migratoria* is less effective, due to the enzymatic activities of nucleases expressed in the midgut, which results in rapid degradation of dsRNA (Song et al., 2017). In *Schistocerca gregaria*, four RNase sequences specifically expressed in the intestine are responsible for the decomposition of dsRNAs (Wynant et al., 2014). In the adult insect *L. decemlineata*, the suppression of nucleases increases crop preservation by increasing plant sensitivity to dsRNAs (Spit et al., 2017). In addition to RNase, the attenuation of gene expression in insects is also influenced by the physiological pH of gastric fluid (Song et al., 2019).

saliva is another factor that affects the success of RNAi. As it has been proven in *Lygus lineolaris*, administered dsRNA are quickly digested by salivary secretion (Allen and Walker, 2012). Salivary secretions have also been shown to be effective in degrading dsRNA targeting different genes in *A. pisum* (Christiaens et al., 2014). A study carried out on two insect species, namely: *Manduca sexta* and *B. germanica* showed that the administered dsRNA are rapidly broken down in the hemolymph plasma of *M. sexta*, whereas in *B. germanica*, the dsRNAs persisted much longer (Garbutt et al., 2013). In *A. pisum*, the introduction of dsRNA into the body showed no response following the decomposition of the nucleotide sequence by the hemolymph (Christiaens et al., 2014).

23.6.3. Influence of target genes on the efficiency of knockdown

The choice of targets is another key factor in the eradication of harmful insects since the silencing of many genes can attenuate the metabolism in the insect but does not cause death. On this, it is important to select with care the vital genes whose elimination induces the death of the insect. In *S. gregaria* larvae, the deactivation of halloween transcribed genes that code for cytochrome P450 enzymes by specific dsRNAs demonstrated a reduction in the rate of ecdysteroids (Marchal et al., 2011). In the same species, the knockdown of another halloween gene which codes for a 20-hydroxylase involved in the conversion of ecdysone to 20-hydroxyecdysone induced a downregulation of ecdysone-20- hydroxylation (Marchal et al., 2012). Also, in other insect species such as *T. castaneum*, the knockdown of TcLgl gene by injections of 100, 200 and 400 ng of TcLgl-dsRNA / larva caused 100% mortality after 20 days of injection (Xiao et al., 2014). Eradication of insect species can also be achieved by silencing chitinase genes, such as: TcCHT5, TcCHT10, TcCHT7, and TcIDGF4; they play an important

role in the development of insect and contribute in the decomposition of chitin during moulting (Zhu et al., 2008; Zhang et al., 2012). Otherwise, the knockdown of chymotrypsin-like peptidases (TcCTLP-5C and TcCTLP-6C) caused serious moult defects (Broehan et al., 2010).

The eradication of *S. exigua* is possible by the extinction of multiple genes important to the vital activities of the insect such as: SeChi and SeChi-h responsible for the synthesis of chitinases (Zhang et al., 2012), and the cuticular protein genes (PG316, CPG860 and CPG4855) essential in metamorphosis and development stages (Jan et al., 2017), as well as the transcriptional factor SeBRC1, which plays a vital role in pupal metamorphosis (Kim and Kim, 2012).

Reproductive control in *D. v. virgifera* by dsRNAs targeting two genes (dvvgr and dvbol) involved in reproduction demonstrated that dvvgr-dsRNA and dvbol-dsRNA cause reduced fertility in insects (Niu et al., 2017). However, targeting of the interference pathway genes (Dicer2 and Argo 2) showed a reduction in insect mortality even at lethal dsRNA concentrations, which leads to significant resistance (Vélez et al., 2016a). Another group of researchers found that the knockdown of drosha, dicer-1, dicer-2, pasha, loquacious, r2d2, Argo-1, and Argo-2 don't support previous studies and don't lead to the evolution of resistance (Davis-Vogel et al., 2018).

23.6. Risks des dsRNA to human health and environment

Some human gene sequences present perfect complementarities with the long endogenous dsRNAs with at least 21 Nts. Administered nucleotide sequence can knockdown non-target gene in the human genome only if dsRNAs are functional, however, the consumption of foods containing dsRNA does not pose a dietary risk (Ivashuta et al., 2009; Jensen et al., 2013). The gastric barrier by its acid pH and enzymes such as pepsin found in human gastric juice strongly contribute to the digestion of the nucleic acid sequence (Liu et al., 2015). In general, the harmful effects of dsRNA applied in agriculture have so far been negligible for humans, since dsRNAs can only trigger knockdown if they are functional and present inside the cell rather than on exterior receptors (Chen et al., 2018; Fletcher et al., 2020), even higher doses of dsRNAs pose negligible risks to mammals (Petrick et al., 2016). Oral V-ATPase-dsRNA sequence tests in mice for 28 days demonstrated that the high dose expresses no risks, and even the suppression of V-ATPase gene in digestive tissue is not recorded (Petrick et al., 2015). Furthermore, Bachman and his team also demonstrated that genetically modified maize (MON 87411) expressing ds-DvSnf7 intended against *D. v. virgifera* does not have toxic effects on soil biota, aquatic and terrestrial species (Bachman et al., 2016). Biosafety and security of RNAi tool is even extended to honey bees (*Apis mellifera* L) (Tan et al., 2016). The request that remains to be requested is: can the combination of dsRNA with other cellular components cause toxic effects in host cells? To answer this question, the knockdown of insect genes must be deepened at the molecular level and components supposed to be included in the interference must be well understood.

In agricultural ecosystems, the first consequences of administered nucleotide sequences are the targeting of non-target insects if the target sequences are similar to the delivered sequences or if their diet is closely similar to the diet of the target insect (Fletcher et al., 2020).

More than a hundred dsRNAs having an insecticidal potential are identified with a great similarity in the genomic sequence in bees (Mogren and Lundgren, 2017). However, in ladybug species, some of which are used in biological control in agriculture, the ds-V-ATPase-A introduced in corn and intended against western corn rootworm, negatively affect two species of *Coccinellidae* namely: *Adalia bipunctata* and *Coccinella septempunctata* (Haller et al., 2019). Otherwise, a strong Snf7 gene resemblance is noted between *Coleomegilla maculata* and *D. v. virgifera* species used in resistance development in transgenic corn. Another similarity was recorded between *C. maculata* and *L. decemlineata* for lethal actin used to produce transgenic potato plants resistant to insects. This implies deepening the selectivity and specificity of dsRNA used to suppress insect genes in future research (Allen, 2017). Some dsRNAs involved in the knockdown don't affect other unintended targets such as: dsRNAs targeting V-ATPase in *D. v. virgifera*; the RNA sequence does not affect the survival of *Danaus plexippus* (Pan et al., 2017), or induces limited effects as demonstrated in *A. mellifer* bees exposed to transgenic corn pollen targeting V-ATPase-A gene in *D. v. virgifera* (Vélez et al., 2016b).

Before any application of RNA technology in the field in order to protect plant, the evolution of insect resistance against dsRNA and the resistance mechanisms involved must be elucidated to create sustainable and efficient molecular technology for pest management. Impaired absorption in intestinal cells of insects or reduced luminal absorption of dsRNA are responsible for increasing insensitivity of insects (Khajuria et al., 2018). Reducing the expression levels of certain proteins which bind to dsRNA such as StaufenC in *L. decemlineata* decrease the resistance of the insect (Yoon et al., 2018).

23.7. Conclusion

Researchers have identified more than 700 species of insects resistant to one or more insecticides while the resistance continues to increase each year. As a result, the development of new biological pest control technologies is an urgent concern. Besides, these new control technologies are required to be respectful of the environment and safe for humans, animals, beneficial insects, water and crops. RNA interference is a natural approach tool that can solve agricultural losses either by improving the resistance of plant species or by knockdown the vital genes in insects. Further studies are needed to develop and improve the efficiency and stability of the interfering nucleotide sequences as well as their conservation to ensure the functional properties in the target organisms.

References

1. Akbarzadeh, A., Rezaei-Sadabady, R., Davaran, S., Joo, S.W., Zarghami, N., Hanifehpour, Y., Samiei, M., Kouhi, M., Nejati-Koshki, K. 2013. Liposome: classification, preparation, and applications. Nanoscale. Res. Lett. 8, 102.
2. Al-Ayedh, H., Rizwan-Ul-Haq, M., Hussain, A., Aljabr, A.M. 2016. Insecticidal potency of RNAi-based catalase knockdown in *Rhynchophorus ferrugineus* (Oliver) (Coleoptera: *Curculionidae*). Pest. Manag. Sci. 72, 2118-2127.
3. Allen, M.L. 2017. Comparison of RNAi sequences in insect-resistant plants to expressed sequences of a beneficial lady beetle: A closer look at off-target considerations. Insects. 8, 27.

4. Allen, M.L., Walker, W.B. 2012. Saliva of *Lygus lineolaris* digests double stranded ribonucleic acids. J. Insect. Physiol. 58, 391-6.

5. Andongma, A.A., Greig, C., Dyson, P.J., Flynn, N., Whitten, M.M.A. 2020. Optimization of dietary RNA interference delivery to western flower thrips *Frankliniella occidentalis* and onion thrips *Thrips tabaci*. Arch. Insect. Biochem. Physiol. 103, 21645.

6. Avila, L.A., Chandrasekar, R., Wilkinson, K.E., Balthazor, J., Heerman, M., Bechard, J., Brown, S., Park, Y., Dhar, S., Reeck, G.R., Tomich, J.M. 2018. Delivery of lethal dsRNAs in insect diets by branched amphiphilic peptide capsules. J. Control. Release. 273, 139-146.

7. Bachman, P.M., Huizinga, K.M., Jensen, P.D., Mueller, G., Tan, J., Uffman, J.P., Levine, S.L. 2016. Ecological risk assessment for DvSnf7 RNA: A plant-incorporated protectant with targeted activity against western corn rootworm. Regul. Toxicol. Pharmacol. 81, 77-88.

8. Bagga, S., Bracht, J., Hunter, S., Massirer, K., Holtz, J., Eachus, R., Pasquinelli, A.E. 2005. Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell. 122, 553-63.

9. Bally, J., Fishilevich, E., Bowling, A.J., Pence, H.E., Narva, K.E., Waterhouse, P.M. 2018. Improved insect-proofing: expressing double-stranded RNA in chloroplasts. Pest. Manag. Sci. 74, 1751-1758.

10. Barba, A.A., Bochicchio, S., Dalmoro, A., Lamberti, G. 2019. Lipid delivery systems for nucleic-acid-based-drugs: From production to clinical applications. Pharmaceutics. 11, 360.

11. Baum, J.A., Bogaert, T., Clinton, W., Heck, G.R., Feldmann, P., Ilagan, O., Johnson, S., Plaetinck, G., Munyikwa, T., Pleau, M., Vaughn, T., Roberts, J. 2007. Control of coleopteran insect pests through RNA interference. Nat. Biotechnol. 25, 1322-6.

12. Bento, F.M., Marques, R.N., Campana, F.B., Demétrio, C.G., Leandro, R.A., Parra, J.R.P., Figueira, A. 2020. Gene silencing by RNAi via oral delivery of dsRNA by bacteria in the South American tomato pinworm, *Tuta absoluta*. Pest. Manag. Sci. 76, 287-295.

13. Blair, C.D., Olson, K.E. 2015. The role of RNA interference (RNAi) in arbovirus-vector interactions. Viruses. 7, 820-43.

14. Bodirsky, B.L., Rolinski, S., Biewald, A., Weindl, I., Popp, A., Lotze-Campen, H. 2015. Global food demand scenarios for the 21st century. PLoS. One. 10, 0139201.

15. Bolognesi, R., Ramaseshadri, P., Anderson, J., Bachman, P., Clinton, W., Flannagan, R., Ilagan, O., Lawrence, C., Levine, S., Moar, W., Mueller, G., Tan, J., Uffman, J., Wiggins, E., Heck, G., Segers, G. 2012. Characterizing the mechanism of action of double-stranded RNA activity against western corn rootworm (*Diabrotica virgifera virgifera* LeConte). PLoS. One. 7, 47534.

16. Broehan, G., Arakane, Y., Beeman, R.W., Kramer, K.J., Muthukrishnan, S., Merzendorfer, H. 2010. Chymotrypsin-like peptidases from *Tribolium castaneum*: a role in molting revealed by RNA interference. Insect. Biochem. Mol. Biol. 40, 274-83.

17. Burke, W.G., Kaplanoglu, E., Kolotilin, I., Menassa, R., Donly, C. 2019. RNA Interference in the tobacco hornworm, *Manduca sexta*, using plastid-encoded long double-stranded RNA. Front. Plant. Sci. 10, 313.

18. Camargo, R.A., Barbosa, G.O., Possignolo, I.P., Peres, L.E., Lam, E., Lima, J.E., Figueira, A., Marques-Souza, H. 2016. RNA interference as a gene silencing tool to control *Tuta absoluta* in tomato (*Solanum lycopersicum*). Peer. J. 4, 2673.

19. Cao, M., Gatehouse, J.A., Fitches, E.C. 2018. A systematic study of RNAi effects and dsRNA stability in *Tribolium castaneum* and *Acyrthosiphon pisum*, following injection and ingestion of analogous dsRNAs. Int. J. Mol. Sci. 19, E1079.

20. Cao, Y., Tan, Y.F., Wong, Y.S., Liew, M., Venkatraman, S. 2019. Recent advances in chitosan-based carriers for gene delivery. Mar. Drugs. 17, 381.

21. Cappelle, K., de Oliveira, C.F., Van Eynde, B., Christiaens, O., Smagghe, G. 2016. The involvement of clathrin-mediated endocytosis and two Sid-1-like transmembrane proteins in double-stranded RNA uptake in the Colorado potato beetle midgut. Insect. Mol. Biol. 25, 315-23.

22. Carthew, R.W., Sontheimer, E.J. 2009. Origins and mechanisms of miRNAs and siRNAs. Cell. 136, 642-55.

23. Castel, S.E., Martienssen, R.A. 2013. RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. Nat. Rev. Genet. 14, 100-12.

24. 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. Manag. Sci. 75, 537-548.

25. Chen, X., Mangala, L.S., Rodriguez-Aguayo, C., Kong, X., Lopez-Berestein, G., Sood, A.K. 2018. RNA interference-based therapy and its delivery systems. Cancer. Metastasis. Rev. 37, 107-124.

26. Chien, P.Y., Wang, J., Carbonaro, D., Lei, S., Miller, B., Sheikh, S., Ali, S.M., Ahmad, M.U., Ahmad, I. 2005. Novel cationic cardiolipin analogue-based liposome for efficient DNA and small interfering RNA delivery in vitro and in vivo. Cancer. Gene. Ther. 12, 321-8.

27. Chowdhury, A., Pradhan, S., Saha, M., Sanyal, N. 2008. Impact of pesticides on soil microbiological parameters and possible bioremediation strategies. Indian. J. Microbiol. 48, 114-27.

28. Christiaens, O., Swevers, L., Smagghe, G. 2014. dsRNA degradation in the pea aphid (*Acyrthosiphon pisum*) associated with lack of response in RNAi feeding and injection assay. Peptides. 53, 307-14.

29. Christiaens, O., Tardajos, M.G., Martinez Reyna, Z.L., Dash, M., Dubruel, P., Smagghe, G. 2018. Increased RNAi efficacy in *Spodoptera exigua* via the formulation of dsRNA with guanylated polymers. Front. Physiol. 9, 316.

30. Chu, C.C., Sun, W., Spencer, J.L., Pittendrigh, B.R., Seufferheld, M.J. 2014. Differential effects of RNAi treatments on field populations of the western corn rootworm. Pestic. Biochem. Physiol. 110, 1-6.

31. Couto, L.B., High, K.A. 2010. Viral vector-mediated RNA interference. Curr. Opin. Pharmacol. 10, 534-42.

32. Damalas, C.A., Eleftherohorinos, I.G. 2011. Pesticide exposure, safety issues, and risk assessment indicators. Int. J. Environ. Res. Public. Health. 8, 1402-19.

33. Dang, Y., Yang, Q., Xue, Z., Liu, Y. 2011. RNA interference in fungi: pathways, functions, and applications. Eukaryot. Cell. 10, 1148-55.

34. Das, S., Debnath, N., Cui, Y., Unrine, J., Palli, S.R. 2015. Chitosan, carbon quantum dot, and silica nanoparticle mediated dsRNA delivery for gene silencing in *Aedes aegypti*: A comparative analysis. ACS. Appl. Mater. Interfaces. 7, 19530-5.

35. Davis-Vogel, C., Ortiz, A., Procyk, L., Robeson, J., Kassa, A., Wang, Y., Huang, E., Walker, C., Sethi, A., Nelson, M.E., Sashital, D.G. 2018. Knockdown of RNA interference pathway genes impacts the fitness of western corn rootworm. Sci. Rep. 8, 7858.

36. Deutsch, C.A., Tewksbury, J.J., Tigchelaar, M., Battisti, D.S., Merrill, S.C., Huey, R.B., Naylor, R.L. 2018. Increase in crop losses to insect pests in a warming climate. Science. 361, 916-919.

37. Fernandes, M.E.S., Alves, F.M., Pereira, R.C., Aquino, L.A., Fernandes, F.L., Zanuncio, J.C. 2016. Lethal and sublethal effects of seven insecticides on three beneficial insects in laboratory assays and field trials. Chemosphere. 156, 45-55.

38. 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, 806-11.

39. Fletcher, S.J., Reeves, P.T., Hoang, B.T., Mitter, N. 2020. A Perspective on RNAi-based biopesticides. Front. Plant. Sci. 11, 51.

40. Fu, K.Y., Li, Q., Zhou, L.T., Meng, Q.W., Lü, F.G., Guo, W.C., Li, G.Q. 2016. Knockdown of juvenile hormone acid methyl transferase severely affects the performance of *Leptinotarsa decemlineata* (Say) larvae and adults. Pest. Manag. Sci. 72, 1231-41.

41. Ganbaatar, O., Cao, B., Zhang, Y., Bao, D., Bao, W., Wuriyanghan, H. 2017. Knockdown of *Mythimna separata* chitinase genes via bacterial expression and oral delivery of RNAi effectors. BMC. biotechnology, 17, 9.

42. Garbutt, J.S., Bellés, X., Richards, E.H., Reynolds, S.E. 2013. Persistence of double-stranded RNA in insect hemolymph as a potential determiner of RNA interference success: evidence from *Manduca sexta* and *Blattella germanica*. J. Insect. Physiol. 59, 171-178.

43. Ghosal, A., Upadhyaya, B.B., Fritz, J.V., Heintz-Buschart, A., Desai, M.S., Yusuf, D., Huang, D., Baumuratov, A., Wang, K., Galas, D., Wilmes, P. 2015. The extracellular RNA complement of *Escherichia coli*. Microbiologyopen. 4, 252-266.

44. Godfray, H.C., Garnett, T. 2014. Food security and sustainable intensification. Philos. Trans. R Soc. Lond B. Biol. Sci. 369, 20120273.

45. Goodfellow, S., Zhang, D., Wang, M.B., Zhang, R. 2019. Bacterium-mediated RNA interference: Potential application in plant protection. Plants. 8, 572.

46. Gregoriou, M.E., Mathiopoulos, K.D. 2020. Knocking down the sex peptide receptor by dsRNA feeding results in reduced oviposition rate in olive fruit flies. Arch. Insect. Biochem. Physiol. 2020, 21665.

47. Guang, S., Bochner, AF., Burkhart, K.B., Burton, N., Pavelec, D.M., Kennedy, S. 2010. Small regulatory RNAs inhibit RNA polymerase II during the elongation phase of transcription. Nature. 465, 1097-101.

48. Guo, W., Bai, C., Wang, Z., Wang, P., Fan, Q., Mi, X., Wang, L., He, J., Pang, J., Luo, X., Fu, W., Tian, Y., Si, H., Zhang, G., Wu, J. 2018. Double-stranded RNAs high-efficiently protect transgenic potato from *Leptinotarsa decemlineata* by disrupting juvenile hormone biosynthesis. J. Agric. Food. Chem. 66, 11990-11999.

49. Haller, S., Widmer, F., Siegfried, B.D., Zhuo, X., Romeis, J. 2019. Responses of two ladybird beetle species (Coleoptera: *Coccinellidae*) to dietary RNAi. Pest. Manag. Sci. 75, 2652-2662.

50. Hammond, SM. 2015. An overview of microRNAs. Adv. Drug. Deliv. Rev. 87, 3-14.

51. Han, Q., Wang, Z., He, Y., Xiong, Y., Lv, S., Li, S., Zhang, Z., Qiu, D., Zeng, H. 2017. Transgenic cotton plants expressing the HaHR3 gene conferred enhanced resistance to *Helicoverpa armigera* and improved cotton yield. Int. J. Mol. Sci. 18, 1874.

52. He, B., Chu, Y., Yin, M., Müllen, K., An, C., Shen, J. 2013. Fluorescent nanoparticle delivered dsRNA toward genetic control of insect pests. Adv. Mater. 25, 4580-4.

53. He, W., Xu, W., Xu, L., Fu, K., Guo, W., Bock, R., Zhang, J. 2020. Length-dependent accumulation of double-stranded RNAs in plastids affects RNA interference efficiency in the Colorado potato beetle. J. Exp. Bot. eraa001.

54. huang, j.h., liu, y., lin, y.h., belles, x., lee, h.j. 2018. practical use of rna interference: oral delivery of double-stranded rna in liposome carriers for cockroaches. j. vis. exp. 135.

55. Hussain, T., Aksoy, E., Çalışkan, M.E., Bakhsh, A. 2019. Transgenic potato lines expressing hairpin RNAi construct of molting-associated EcR gene exhibit enhanced resistance against Colorado potato beetle (*Leptinotarsa decemlineata*, Say). Transgenic. Res. 28, 151-164.

56. Ivashuta, S.I., Petrick, J.S., Heisel, S.E., Zhang, Y., Guo, L., Reynolds, T.L., Rice, J.F., Allen, E., Roberts, J.K. 2009. Endogenous small RNAs in grain: semi-quantification and sequence homology to human and animal genes. Food. Chem. Toxicol. 47, 353-60.

57. Jan, S., Liu, S., Hafeez, M., Zhang, X., Dawar, F.U., Guo, J., Gao, C., Wang, M. 2017. Isolation and functional identification of three cuticle protein genes during metamorphosis of the beet armyworm, *Spodoptera exigua*. Sci. Rep. 7, 16061.

58. Jeang, K.T. 2012. RNAi in the regulation of mammalian viral infections. BMC. Biol. 10, 58.

59. Jensen, P.D., Zhang, Y., Wiggins, B.E., Petrick, J.S., Zhu, J., Kerstetter, R.A., Heck, G.R., Ivashuta, S.I. 2013. Computational sequence analysis of predicted long dsRNA transcriptomes of major crops reveals sequence complementarity with human genes. GM. Crops. Food. 4, 90-7.

60. Khajuria, C., Ivashuta, S., Wiggins, E., Flagel, L., Moar, W., Pleau, M., Miller, K., Zhang, Y., Ramaseshadri, P., Jiang, C., Hodge, T., Jensen, P., Chen, M., Gowda, A., McNulty, B., Vazquez, C., Bolognesi, R., Haas, J., Head, G., Clark, T. 2018. Development and characterization of the first dsRNA-resistant insect population from western corn rootworm, *Diabrotica virgifera virgifera* LeConte. PloS. one, 13, 0197059.

61. Kim, D., Rossi, J. 2008. RNAi mechanisms and applications. Biotechniques. 44, 613-6.

62. Kim, E., Park, Y., Kim, Y. 2015. A transformed bacterium expressing double-stranded RNA specific to integrin β1 enhances Bt toxin efficacy against a polyphagous insect pest, *Spodoptera exigua*. PLoS. One. 10, 0132631.

63. Kim, J., Kim, Y. 2012. RNA interference of broad gene expression mimics antimetamorphic effect of pyriproxyfen on the beet armyworm, *Spodoptera exigua*. Arch. Insect. Biochem. Physiol. 81, 214-27.

64. Koch, A., Biedenkopf, D., Furch, A., Weber, L., Rossbach, O., Abdellatef, E., Linicus, L., Johannsmeier, J., Jelonek, L., Goesmann, A., Cardoza, V., McMillan, J., Mentzel, T., Kogel, K.H. 2016. An RNAi-based control of *Fusarium graminearum* infections through spraying of

long dsRNAs involves a plant passage and is controlled by the fungal silencing machinery. PLoS. Pathog. 12, 1005901.

65. Kontogiannatos, D., Swevers, L., Maenaka, K., Park, E.Y., Iatrou, K., Kourti, A. 2013. Functional characterization of a juvenile hormone esterase related gene in the moth *Sesamia nonagrioides* through RNA interference. PloS. One, 8, 73834.

66. Kunte, N., McGraw, E., Bell, S., Held, D., Avila, L.A. 2020. Prospects, challenges and current status of RNAi through insect feeding. Pest. Manag. Sci. 76, 26-41.

67. Lee, Y., Hur, I., Park, S.Y., Kim, Y.K., Suh, M.R., Kim, V.N. 2006. The role of PACT in the RNA silencing pathway. EMBO. J. 25, 522-32.

68. Li, Y., Wang, K., Lu, Q., Du, J., Wang, Z., Wang, D., Sun, B., Li, H. 2017. Transgenic *Nicotiana benthamiana* plants expressing a hairpin RNAi construct of a nematode Rs-cps gene exhibit enhanced resistance to *Radopholus similis*. Sci. Rep. 7, 13126.

69. Lin, Y,H., Huang, J.H., Liu, Y., Belles, X., Lee, H.J. 2017. Oral delivery of dsRNA lipoplexes to German cockroach protects dsRNA from degradation and induces RNAi response. Pest. Manag. Sci. 73, 960-966.

70. Liu, J., Swevers, L., Iatrou, K., Huvenne, H., Smagghe, G. 2012. *Bombyx mori* DNA/RNA non-specific nuclease: expression of isoforms in insect culture cells, subcellular localization and functional assays. J. Insect. Physiol. 58, 1166-76.

71. Liu, J., Swevers, L., Kolliopoulou, A., Smagghe, G. 2019. Arboviruses and the challenge to establish systemic and persistent infections in competent mosquito vectors: The interaction with the RNAi mechanism. Front. Physiol. 10, 890.

72. Liu, S., Jaouannet, M., Dempsey, D.A., Imani, J., Coustau, C., Kogel, K.H. 2020. RNA-based technologies for insect control in plant production. Biotechnol. Adv. 39, 107463.

73. Liu, Y., Zhang, Y., Dong, P., An, R., Xue, C., Ge, Y., Wei, L., Liang, X. 2015. Digestion of nucleic acids starts in the stomach. Sci. Rep. 5, 11936.

74. Luo, Y., Wang, X., Wang, X., Yu, D., Chen, B., Kang, L. 2013. Differential responses of migratory locusts to systemic RNA interference via double-stranded RNA injection and feeding. Insect. Mol. Biol. 22, 574-83.

75. Mao, J., Zhang, P., Liu, C., Zeng, F. 2015. Co-silence of the coatomer β and v-ATPase A genes by siRNA feeding reduces larval survival rate and weight gain of cotton bollworm, *Helicoverpa armigera*. Pestic. Biochem. Physiol. 118, 71-6.

76. Mao, Y.B., Cai, W.J., Wang, J.W., Hong, G.J., Tao, X.Y., Wang, L.J., Huang, Y.P., Chen, X.Y. 2007. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nat. Biotechnol. 25, 1307-13.

77. Mao, Y.B., Tao, X.Y., Xue, X.Y., Wang, L.J., Chen, X.Y. 2011. Cotton plants expressing CYP6AE14 double-stranded RNA show enhanced resistance to bollworms. Transgenic. Res. 20, 665-73.

78. Mao, Y.B., Xue, X.Y., Tao, X.Y., Yang, C.Q., Wang, L.J., Chen, X.Y. 2013. Cysteine protease enhances plant-mediated bollworm RNA interference. Plant. Mol. Biol. 83, 119-29.

79. Marchal, E., Badisco, L., Verlinden, H., Vandersmissen, T., Van Soest, S., Van Wielendaele, P., Vanden Broeck, J. 2011. Role of the Halloween genes, spook and phantom in ecdysteroidogenesis in the desert locust, *Schistocerca gregaria*. J. Insect. Physiol. 57, 1240-8.

80. Marchal, E., Verlinden, H., Badisco, L., Van Wielendaele, P., Vanden Broeck, J. 2012. RNAi-mediated knockdown of Shade negatively affects ecdysone-20-hydroxylation in the desert locust, *Schistocerca gregaria*. J. Insect. Physiol. 58, 890-6.

81. Mérai, Z., Kerényi, Z., Kertész, S., Magna, M., Lakatos, L., Silhavy, D. 2006. Double-stranded RNA binding may be a general plant RNA viral strategy to suppress RNA silencing. J. Virol. 80, 5747-56.

82. Miller, S.C., Miyata, K., Brown, S.J., Tomoyasu, Y. 2012. Dissecting systemic RNA interference in the red flour beetle *Tribolium castaneum*: parameters affecting the efficiency of RNAi. PLoS. One. 7, 47431.

83. Mogren, C.L., Lundgren, J.G. 2017. In silico identification of off-target pesticidal dsRNA binding in honey bees (*Apis mellifera*). Peer. J. 5, 4131.

84. Murphy, K.A., Tabuloc, C.A., Cervantes, K.R., Chiu, J.C. 2016. Ingestion of genetically modified yeast symbiont reduces fitness of an insect pest via RNA interference. Sci. Rep. 6, 22587.

85. Mysore, K., Flannery, E.M., Tomchaney, M., Severson, D.W., Duman-Scheel, M. 2013. Disruption of *Aedes aegypti* olfactory system development through chitosan/siRNA nanoparticle targeting of semaphorin-1a. PLoS. Negl. Trop. Dis. 7, 2215.

86. Mysore, K., Hapairai, L.K., Wei, N., Realey, J.S., Scheel, N.D., Severson, D.W., Duman-Scheel, M. 2019a. Preparation and use of a yeast shRNA delivery system for gene silencing in mosquito larvae. Methods. Mol. Biol. 1858, 213-231.

87. Mysore, K., Li, P., Wang, C.W., Hapairai, L.K., Scheel, N.D., Realey, J.S., Sun, L., Severson, D.W., Wei, N., Duman-Scheel, M. 2019b. Characterization of a broad-based mosquito yeast interfering RNA larvicide with a conserved target site in mosquito semaphorin-1a genes. Parasit. Vectors. 12, 256.

88. Nagami, H., Suenaga, T., Nakazaki, M. 2017. Pesticide exposure and subjective symptoms of cut-flower farmers. J. Rural. Med. 12, 7-11.

89. Nicolás, F.E., Torres-Martínez, S., Ruiz-Vázquez, R.M. 2013. Loss and retention of RNA interference in fungi and parasites. PLoS. Pathog. 2013. 9, 1003089.

90. Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., Hens, L. 2016. Chemical pesticides and human health: the urgent need for a new concept in agriculture. Front. Public. Health. 4, 148.

91. Niu, X., Kassa, A., Hu, X., Robeson, J., McMahon, M., Richtman, N.M., Steimel, J.P., Kernodle, B.M., Crane, V.C., Sandahl, G., Ritland, J.L., Presnail, J.K., Lu, A.L., Wu, G. 2017. Control of western corn rootworm (*Diabrotica virgifera virgifera*) reproduction through plant-mediated RNA interference. Sci. Rep. 7, 12591.

92. Pan, H., Yang, X., Bidne, K., Hellmich, R.L., Siegfried, B.D., Zhou, X. 2017. Dietary risk assessment of v-ATPase A dsRNAs on monarch butterfly larvae. Front. Plant. Sci. 8, 242.

93. Petrick, J.S., Frierdich, G.E., Carleton, S.M., Kessenich, C.R., Silvanovich, A., Zhang, Y., Koch, M.S. 2016. Corn rootworm-active RNA DvSnf7: Repeat dose oral toxicology assessment in support of human and mammalian safety. Regul. Toxicol. Pharmacol. 81, 57-68.

94. Petrick, J.S., Moore, W.M., Heydens, W.F., Koch, M.S., Sherman, J.H., Lemke, S.L. 2015. A 28-day oral toxicity evaluation of small interfering RNAs and a long double-stranded RNA targeting vacuolar ATPase in mice. Regul. Toxicol. Pharmacol. 71, 8-23.

95. Pong, S.K., Gullerova, M. 2018. Noncanonical functions of microRNA pathway enzymes - Drosha, DGCR8, Dicer and Ago proteins. FEBS. Lett. 592, 2973-2986.

96. Poreddy, S., Li, J., Baldwin, I.T. 2017. Plant-mediated RNAi silences midgut-expressed genes in congeneric lepidopteran insects in nature. BMC. Plant. Biol. 17, 199.

97. Ramesh Kumar, D., Saravana Kumar, P., Gandhi, M.R., Al-Dhabi, N.A., Paulraj, M.G., Ignacimuthu, S. 2016. Delivery of chitosan/dsRNA nanoparticles for silencing of wing development vestigial (vg) gene in *Aedes aegypti* mosquitoes. Int. J. Biol. Macromol. 86, 89-95.

98. Roderick, H., Urwin, P.E., Atkinson, H.J. 2018. Rational design of biosafe crop resistance to a range of nematodes using RNA interference. Plant. Biotechnol. J. 16, 520-529.

99. Rosa, C., Kuo, Y.W., Wuriyanghan, H., Falk, B.W. 2018. RNA interference mechanisms and applications in plant pathology. Annu. Rev. Phytopathol. 56, 581-610.

100. Schumann, U., Smith, N.A., Kazan, K., Ayliffe, M., Wang, M.B. 2013. Analysis of hairpin RNA transgene-induced gene silencing in *Fusarium oxysporum*. Silence. 4, 3.

101. Song, H., Fan, Y., Zhang, J., Cooper, A.M., Silver, K., Li, D., Li, T., Ma, E., Zhu, K.Y., Zhang, J. 2019. Contributions of dsRNases to differential RNAi efficiencies between the injection and oral delivery of dsRNA in *Locusta migratoria*. Pest. Manag. Sci. 75, 1707-1717.

102. Song, H., Zhang, J., Li, D., Cooper, A.M.W., Silver, K., Li, T., Liu, X., Ma, E., Zhu, K.Y., Zhang, J. 2017. A double-stranded RNA degrading enzyme reduces the efficiency of oral RNA interference in migratory locust. Insect. Biochem. Mol. Biol. 86, 68-80.

103. 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. Biochem. Mol. Biol. 81, 103-116.

104. Stork, N.E. 2018. How many species of insects and other terrestrial arthropods are there on earth?. Annu. Rev. Entomol. 63, 31-45.

105. Sudo, M., Takahashi, D., Andow, D.A., Suzuki, Y., Yamanaka, T. 2017. Optimal management strategy of insecticide resistance under various insect life histories: Heterogeneous timing of selection and interpatch dispersal. Evol. Appl. 11, 271-283.

106. Sugahara, R., Tanaka, S., Jouraku, A., Shiotsuki, T. 2017. Geographic variation in RNAi sensitivity in the migratory locust. Gene. 605, 5-11.

107. Sun, Y., Sparks, C., Jones, H., Riley, M., Francis, F., Du, W., Xia, L. 2019. Silencing an essential gene involved in infestation and digestion in grain aphid through plant-mediated RNA interference generates aphid-resistant wheat plants. Plant. Biotechnol. J. 17, 852-854.

108. Swevers, L., Vanden Broeck, J., Smagghe, G. 2013. The possible impact of persistent virus infection on the function of the RNAi machinery in insects: A hypothesis. Front. Physiol. 4, 319.

109. Tan, J., Levine, S.L., Bachman, P.M., Jensen, P.D., Mueller, G.M., Uffman, J.P., Meng, C., Song, Z., Richards, K.B., Beevers, M.H. 2016. No impact of DvSnf7 RNA on honey bee

(*Apis mellifera* L.) adults and larvae in dietary feeding tests. Environ. Toxicol. Chem. 35, 287-94.

110. Thakur, N., Upadhyay, S.K., Verma, P.C., Chandrashekar, K., Tuli, R., Singh, P.K. 2014. Enhanced whitefly resistance in transgenic tobacco plants expressing double stranded RNA of v-ATPase A gene. PLoS. One. 9, 87235.

111. Tian, G., Cheng, L., Qi, X., Ge, Z., Niu, C., Zhang, X., Jin, S. 2015. Transgenic cotton plants expressing double-stranded RNAs target HMG-CoA reductase (HMGR) gene inhibits the growth, development and survival of cotton bollworms. Int. J. Biol. Sci. 11, 1296-305.

112. Tian, H., Peng, H., Yao, Q., Chen, H., Xie, Q., Tang, B., Zhang, W. 2009. Developmental control of a lepidopteran pest *Spodoptera exigua* by ingestion of bacteria expressing dsRNA of a non-midgut gene. PloS. One. 4, 6225.

113. Timmons, L., Fire, A. 1998. Specific interference by ingested dsRNA. Nature. 395, 854.

114. Turner, C.T., Davy, M.W., MacDiarmid, R.M., Plummer, K.M., Birch, N.P., Newcomb, R.D. 2006. RNA interference in the light brown apple moth, *Epiphyas postvittana* (Walker) induced by double-stranded RNA feeding. Insect. Mol. Biol. 15, 383-91.

115. Uhlirova, M., Foy, B.D., Beaty, B.J., Olson, K.E., Riddiford, L.M., Jindra, M. 2003. Use of Sindbis virus-mediated RNA interference to demonstrate a conserved role of Broad-Complex in insect metamorphosis. Proc. Natl. Acad. Sci. USA, 100, 15607-15612.

116. Vatanparast, M., Kim, Y. 2017. Optimization of recombinant bacteria expressing dsRNA to enhance insecticidal activity against a lepidopteran insect, *Spodoptera exigua*. PLoS. One. 12, 0183054.

117. Vélez, A.M., Jurzenski, J., Matz, N., Zhou, X., Wang, H., Ellis, M., Siegfried, B.D. 2016b. Developing an in vivo toxicity assay for RNAi risk assessment in honey bees, *Apis mellifera* L. Chemosphere. 144, 1083-90.

118. Vélez, A.M., Khajuria, C., Wang, H., Narva, K.E., Siegfried, B.D. 2016a. Knockdown of RNA Interference pathway genes in western corn rootworms (*Diabrotica virgifera virgifera* Le Conte) demonstrates a possible mechanism of resistance to lethal dsRNA. PLoS. One. 11, 0157520.

119. Vogel, E., Santos, D., Mingels, L., Verdonckt, T.W., Broeck, J.V. 2019. RNA interference in insects: Protecting beneficials and controlling pests. Front. Physiol. 9, 1912.

120. Wan, P.J., Fu, K.Y., Lü, F.G., Guo, W.C., Li, G.Q. 2015b. Knockdown of a putative alanine aminotransferase gene affects amino acid content and flight capacity in the Colorado potato beetle *Leptinotarsa decemlineata*. Amino. Acids. 47, 1445-54.

121. Wan, P.J., Fu, K.Y., Lü, F.G., Wang, X.X., Guo, W.C., Li, G.Q. 2015a. Knocking down a putative Δ(1) -pyrroline-5-carboxylate dehydrogenase gene by RNA interference inhibits flight and causes adult lethality in the Colorado potato beetle *Leptinotarsa decemlineata* (Say). Pest. Manag. Sci. 71, 1387-96.

122. Wang, J.D., Wang, Y.R., Wang, Y.Z., Wang, W.Z., Wang, R., Gao, S.J. 2018. RNA interference of tubulin genes has lethal effects in *Mythimna separate*. Gene. 670, 1-6.

123. Wang, K., Peng, Y., Fu, W., Shen, Z., Han, Z. 2019. Key factors determining variations in RNA interference efficacy mediated by different double-stranded RNA lengths in *Tribolium castaneum*. Insect. Mol. Biol. 28, 235-245.

124. Wang, K., Peng, Y., Pu, J., Fu, W., Wang, J., Han, Z. 2016. Variation in RNAi efficacy among insect species is attributable to dsRNA degradation in vivo. Insect. Biochem. Mol. Biol. 77, 1-9.

125. Wang, Z., Dong, Y., Desneux, N., Niu, C. 2013. RNAi silencing of the HaHMG-CoA reductase gene inhibits oviposition in the *Helicoverpa armigera* cotton bollworm. PLoS. One. 8, 67732.

126. Waris, M.I., Younas, A., Ul Qamar, M.T., Hao, L., Ameen, A., Ali, S., Abdelnabby, H.E., Zeng, F.F., Wang, M.Q. 2018. Silencing of chemosensory protein gene NlugCSP8 by RNAi induces declining behavioral responses of *Nilaparvata lugens*. Front. Physiol. 9, 379.

127. Whitten, M.M., Facey, P.D., Del, Sol, R., Fernández-Martínez, L.T., Evans, M.C., Mitchell, J.J., Bodger, O.G., Dyson, P.J. 2016. Symbiont-mediated RNA interference in insects. Proc. Biol. Sci. 283, 20160042.

128. Whyard, S., Singh, A.D., Wong, S. 2009. Ingested double-stranded RNAs can act as species-specific insecticides. Insect. Biochem. Mol. Biol. 39, 824-32.

129. Wynant, N., Santos, D., Verdonck, R., Spit, J., Van Wielendaele, P., Vanden Broeck, J. 2014. Identification, functional characterization and phylogenetic analysis of double stranded RNA degrading enzymes present in the gut of the desert locust, *Schistocerca gregaria*. Insect. Biochem. Mol. Biol. 46, 1-8.

130. Xiao, D., Gao, X., Xu, J., Liang, X., Li, Q., Yao, J., Zhu, K.Y. 2015. Clathrin-dependent endocytosis plays a predominant role in cellular uptake of double-stranded RNA in the red flour beetle. Insect. Biochem. Mol. Biol. 60, 68-77.

131. Xiao, D., Liang, X., Gao, X., Yao, J., Zhu, K.Y. 2014. The lethal giant larvae gene in *Tribolium castaneum*: molecular properties and roles in larval and pupal development as revealed by RNA interference. Int. J. Mol. Sci. 15, 6880-96.

132. Yan, S., Qian, J., Cai, C., Ma, Z., Li, J., Yin, M., Ren, B., Shen, J. 2020. Spray method application of transdermal dsRNA delivery system for efficient gene silencing and pest control on soybean aphid *Aphis glycines*. J. Pest. Sci. 93, 449-459.

133. Yan, T., Chen, H., Sun, Y., Yu, X., Xia, L. 2016. 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. Int. J. Mol. Sci. 17, 2098.

134. Yoon, J.S., Mogilicherla, K., Gurusamy, D., Chen, X., Chereddy, S., Palli, S.R. 2018. Double-stranded RNA binding protein, Staufen, is required for the initiation of RNAi in coleopteran insects. Proc. Natl. Acad. Sci. USA. 115, 8334-8339.

135. Yu, R., Xu, X., Liang, Y., Tian, H., Pan, Z., Jin, S., Wang, N., Zhang, W. 2014. The insect ecdysone receptor is a good potential target for RNAi-based pest control. Int. J. Biol. Sci. 10, 1171-1180.

136. Zha, W., Peng, X., Chen, R., Du, B., Zhu, L., He, G. 2011. Knockdown of midgut genes by dsRNA-transgenic plant-mediated RNA interference in the hemipteran insect *Nilaparvata lugens*. PLoS. One. 6, 20504.

137. Zhang, D., Chen, J., Yao, Q., Pan, Z., Chen, J., Zhang, W. 2012. Functional analysis of two chitinase genes during the pupation and eclosion stages of the beet armyworm *Spodoptera exigua* by RNA interference. Arch. Insect. Biochem. Physiol. 79, 220-34.

138. Zhang, H., Kolb, F.A., Jaskiewicz, L., Westhof, E., Filipowicz, W. 2004. Single processing center models for human Dicer and bacterial RNase III. Cell. 118, 57-68.

139. Zhang, J., Khan, S.A., Hasse, C., Ruf, S., Heckel, D.G., Bock, R. 2015a. Pest control. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. Science. 347, 991-4.

140. Zhang, J., Khan, S.A., Heckel, D.G., Bock, R. 2017. Next-generation insect-resistant plants: RNAi-mediated crop protection. Trends. Biotechnol. 35, 871-882.

141. Zhang, X., Mysore, K., Flannery, E., Michel, K., Severson, D.W., Zhu, K.Y., Duman-Scheel, M. 2015a. Chitosan/interfering RNA nanoparticle mediated gene silencing in disease vector mosquito larvae. J. Vis. Exp. 52523.

142. Zhang, Y., Cui, J., Zhou, Y., Cao, J., Gong, H., Zhang, H., Zhou, J. 2018b. Liposome mediated double-stranded RNA delivery to silence ribosomal protein P0 in the tick *Rhipicephalus haemaphysaloides*. Ticks. Tick. Borne. Dis. 9, 638-644.

143. Zhang, Y., Xu, L., Li, S., Zhang, J. 2019. Bacteria-mediated RNA interference for management of *Plagiodera versicolora* (Coleoptera: *Chrysomelidae*). Insects. 10, 415.

144. Zhang, Y., Zhang, Y., Fu, M., Yin, G., Sayre, R.T., Pennerman, K.K., Yang, F. 2018a. RNA interference to control asian corn borer using dsRNA from a novel glutathione-s-transferase gene of *Ostrinia furnacalis* (Lepidoptera: *Crambidae*). J. Insect. Sci. 18, 16.

145. 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. Manag. Sci. 67, 175-82.

146. Zhu, K.Y., Palli, S.R. 2020. Mechanisms, applications, and challenges of insect RNA interference. Annu. Rev. Entomol. 65, 293-311.

147. Zhu, Q., Arakane, Y., Beeman, R.W., Kramer, K.J., Muthukrishnan, S. 2008. Functional specialization among insect chitinase family genes revealed by RNA interference. Proc. Natl. Acad. Sci. USA. 105, 6650-5.

148. Zotti, M., Dos Santos, E.A., Cagliari, D., Christiaens, O., Taning, C.N.T., Smagghe, G. 2018. RNA interference technology in crop protection against arthropod pests, pathogens and nematodes. Pest. Manag. Sci. 74, 1239-1250.

149. Zotti, M.J., Smagghe, G. 2015. RNAi technology for insect management and protection of beneficial insects from diseases: Lessons, challenges and risk assessments. Neotrop. Entomol. 44, 197-213.