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Advancing RNAi-based Strategies for Eco-friendly and Targeted Insect Pest Management in Sustainable Agriculture

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The RNA interference (RNAi) has emerged as a promising approach for targeted and eco-friendly insect pest management in sustainable agriculture. The RNAi involves the silencing of specific genes through the introduction of double-stranded RNA (dsRNA), leading to the degradation of complementary mRNA and subsequent reduction in the expression of targeted proteins. This chapter provides a comprehensive overview of the current state and future prospects of RNAi-based strategies for insect pest control. We discuss the molecular mechanisms underlying RNAi, delivery methods for dsRNA, and the design and selection of effective target genes. The application of RNAi in controlling various insect pests, including lepidopterans, coleopterans, and hemipterans is extensively reviewed. also highlight the potential challenges and limitations associated with RNAi-based pest management, such as off-target effects, variable efficacy across insect species, and the development of resistance. Strategies to overcome these challenges, including the use of nanoparticle-based delivery systems and the combination of RNAi with other pest control methods, are explored. Furthermore, discuss the environmental and ecological considerations surrounding the use of RNAi in agriculture, emphasizing the importance of assessing non-target effects and the need for appropriate risk assessment frameworks. The chapter concludes by outlining future research directions and the potential for RNAi to revolutionize insect pest management, contributing to the development of sustainable and resilient agricultural systems.

Keywords: *RNA interference; insect pest management; sustainable agriculture; dsRNA delivery; off-target effects.*

1. INTRODUCTION

Insect pests pose significant challenges to agricultural production, causing substantial yield losses and economic damage worldwide [1]. Traditional pest control methods, such as the use of chemical insecticides, have been widely employed to mitigate these losses. However, the excessive and indiscriminate use of insecticides has led to numerous adverse consequences, including the development of insecticide resistance, negative impacts on non-target organisms, and environmental contamination [2,3]. In response to these challenges, there is a growing need for alternative, eco-friendly, and targeted pest management strategies that can ensure sustainable agriculture while minimizing the reliance on chemical insecticides [4].

RNA interference (RNAi) has emerged as a promising approach for targeted insect pest control, offering a highly specific and environmentally benign alternative to conventional pest management methods [5,6]. The RNAi is a naturally occurring post-transcriptional gene silencing mechanism that involves the degradation of messenger RNA (mRNA) through the introduction of complementary double-stranded RNA (dsRNA) [7]. By exploiting this mechanism, RNAi-based strategies aim to silence specific genes essential for insect survival, development, or reproduction, leading to the suppression of target pest populations [8].

The application of RNAi in insect pest management has gained significant attention in recent years, with numerous studies demonstrating its potential in controlling a wide range of insect pests across various agricultural crops [9-11]. RNAi-based approaches offer several advantages over traditional pest control methods, including high specificity, reduced off-target effects, and the potential for long-term and sustainable pest suppression [12]. However, the successful implementation of RNAi in field conditions faces several challenges, such as the efficient delivery of dsRNA to target insects, the variable efficacy across different insect species, and the potential development of resistance [13,14]. This chapter provides a comprehensive overview of the current state and future prospects of RNAi-based strategies for eco-friendly and targeted insect pest management in sustainable agriculture. We discuss the molecular mechanisms underlying RNAi, the design and selection of effective target genes, and the various delivery methods for dsRNA. The application of RNAi in controlling major insect pests, including lepidopterans, coleopterans, and hemipterans, is extensively reviewed, highlighting the successes and challenges encountered in different agricultural systems. We also explore the potential environmental and

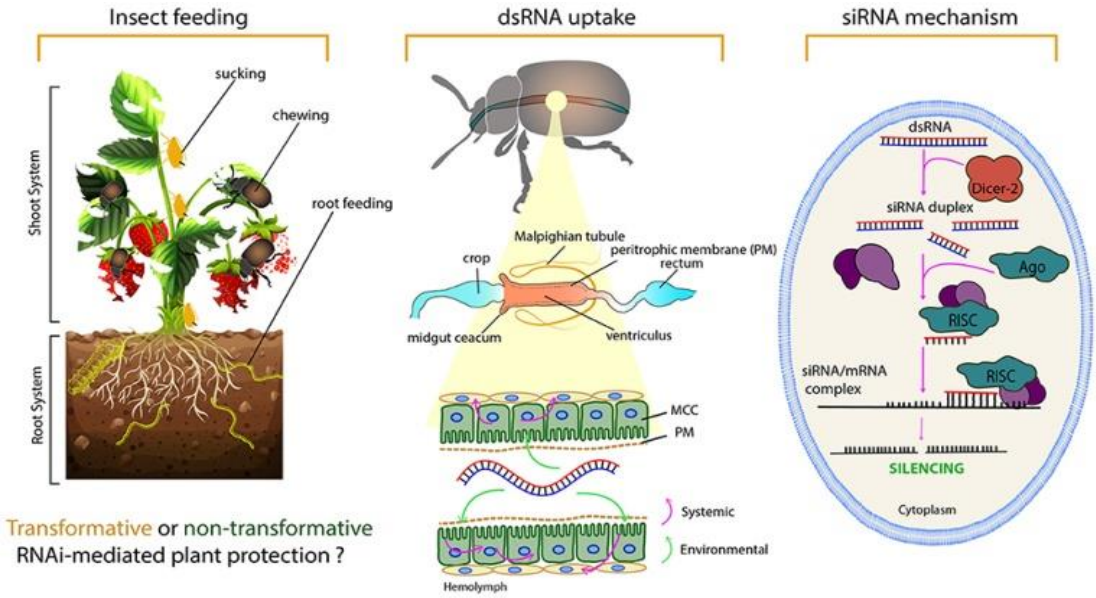

Fig. 1. Application of RNAi in insect pest management

ecological considerations associated with the use of RNAi in pest management, emphasizing the importance of assessing non-target effects and developing appropriate risk assessment frameworks. The chapter concludes by outlining future research directions and the potential for RNAi to revolutionize insect pest management, contributing to the development of sustainable and resilient agricultural systems.

2. MOLECULAR MECHANISMS OF RNA INTERFERENCE

RNA interference (RNAi) is a conserved eukaryotic mechanism that regulates gene expression through the sequence-specific degradation of messenger RNA (mRNA) [15]. The discovery of RNAi in the nematode *Caenorhabditis elegans* by Fire and Mello in 1998 [16] has revolutionized our understanding of gene regulation and opened up new avenues for targeted gene silencing in various organisms, including insects [17].

The RNAi pathway is triggered by the presence of double-stranded RNA (dsRNA) molecules, which can be introduced exogenously or generated endogenously within the cell [18]. The dsRNA is processed by the enzyme Dicer, an RNase III-type endonuclease, into short interfering RNAs (siRNAs) of approximately 21-23 nucleotides in length [19]. These siRNAs are then incorporated into the RNA-induced silencing complex (RISC), where they guide the sequence-specific degradation of complementary mRNA targets [20].

The core component of RISC is the Argonaute (AGO) protein, which contains both RNA-binding and endonuclease domains [21]. Within RISC, one strand of the siRNA (the guide strand) is retained, while the other strand (the passenger strand) is degraded [22]. The guide strand directs the RISC complex to the complementary mRNA target, leading to its cleavage and subsequent degradation [23]. This process results in the post-transcriptional silencing of the target gene, reducing its expression and ultimately affecting the corresponding protein levels [24].

In addition to the siRNA pathway, RNAi can also be mediated through microRNAs (miRNAs), which are endogenously encoded small RNAs that regulate gene expression through translational repression or mRNA degradation [25]. The miRNAs are processed from longer primary transcripts (pri-miRNAs) by the enzymes Drosha and Dicer, generating mature miRNAs of approximately 22 nucleotides in length [26]. Like siRNAs, miRNAs are incorporated into RISC and guide the sequence-specific regulation of target mRNAs [27].

The RNAi machinery is highly conserved across eukaryotic organisms, including insects [28]. In insects, the RNAi pathway plays crucial roles in various biological processes, such as development, reproduction, and defense against viruses [29,30]. The presence of a functional

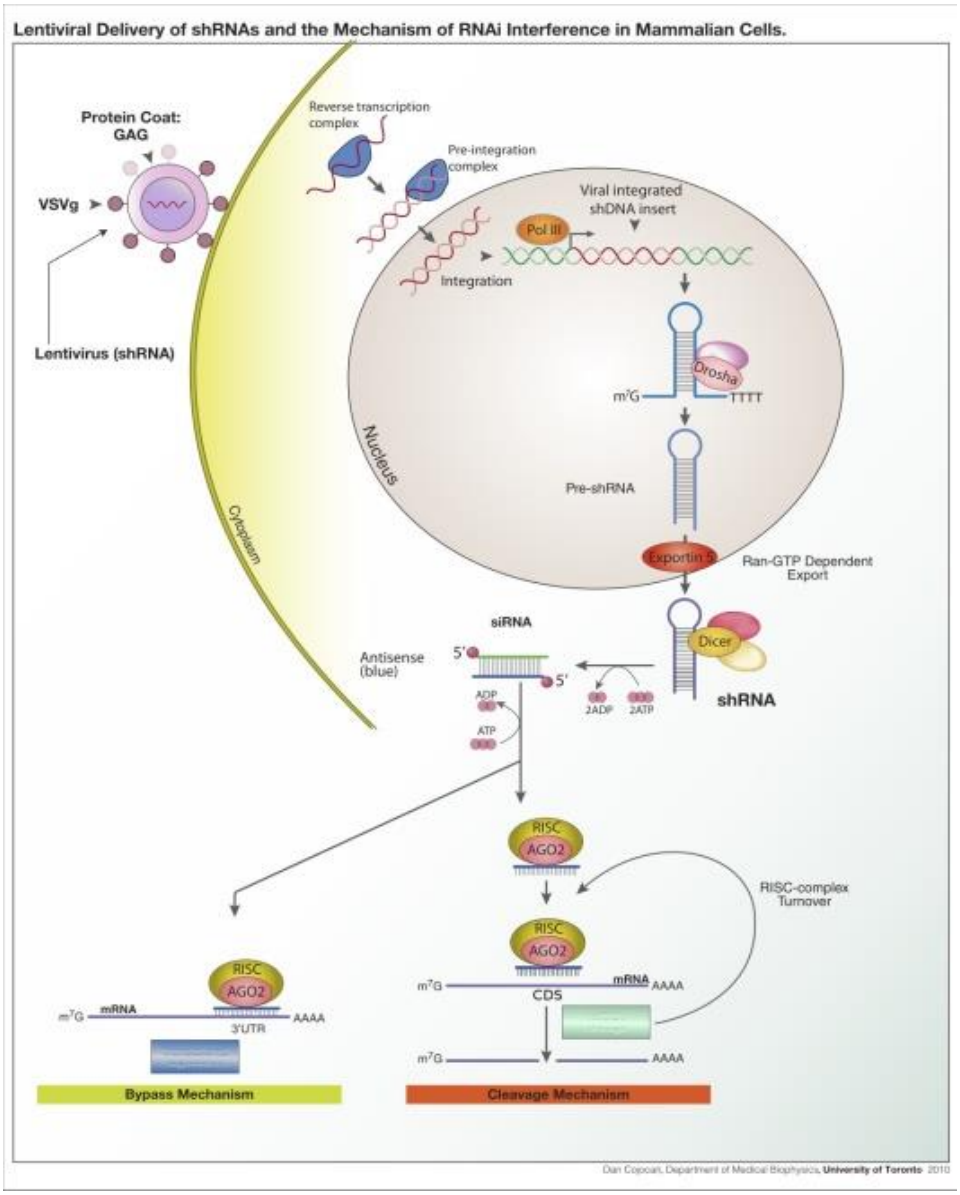

Fig. 2. Molecular mechanisms of RNA interference

RNAi pathway in insects has opened up the possibility of exploiting this mechanism for targeted pest management [31].

The efficiency of RNAi in insects varies among different species and depends on several factors, such as the delivery method of dsRNA, the stability of dsRNA in the insect gut, and the presence of RNAi-inhibiting proteins [32,33]. Some insect orders, such as Coleoptera and Lepidoptera, exhibit a robust RNAi response, while others, like Diptera and Hemiptera, show a more variable response [34,35]. Understanding the molecular basis of these differences is crucial for the successful application of RNAi in insect pest management.

Recent advances in understanding of the RNAi pathway in insects have provided valuable insights into the design and optimization of RNAi-based strategies for pest control [36]. The identification of key genes involved in the RNAi machinery, such as Dicer, Argonaute, and systemic RNA interference deficient (SID) proteins, has enabled the development of more efficient dsRNA delivery methods and the enhancement of RNAi efficacy in target insects [37,38].

3. DESIGN AND SELECTION OF TARGET GENES FOR RNAI-BASED PEST CONTROL

The success of RNAi-based insect pest management relies heavily on the proper design and selection of target genes [39]. Ideal target genes should be essential for insect survival, development, or reproduction, and their silencing should result in significant pest suppression without causing off-target effects on non-target organisms [40]. The identification of suitable target genes requires a thorough understanding of insect biology, genomics, and the RNAi pathway [41].

3.1 Identification of Essential Genes

One approach to identify potential target genes is to focus on essential genes that are critical for insect survival and development [42]. These genes can be involved in various biological processes, such as embryonic development, molting, metamorphosis, and reproduction [43]. Silencing of essential genes through RNAi can lead to lethal or sublethal effects on the target insect, resulting in population suppression [44]

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Examples of essential genes that have been successfully targeted for RNAi-based pest control:

- Chitin synthase (CHS): CHS is a key enzyme involved in the synthesis of chitin, a major component of the insect exoskeleton [45]. Silencing of CHS genes has been shown to disrupt molting and cause mortality in various insect species, such as the African cotton leafworm (*Spodoptera littoralis*) [46] and the Colorado potato beetle (*Leptinotarsa decemlineata*) [47].
- Ecdysone receptor (EcR): EcR is a nuclear receptor that mediates the action of the molting hormone ecdysone, which regulates insect growth and development [48]. RNAi-mediated knockdown of EcR has been demonstrated to inhibit molting and cause developmental abnormalities in insects, such as the red flour beetle (*Tribolium castaneum*) [49] and the tobacco cutworm,*Spodoptera litura* [50].
- Vitellogenin (Vg): Vg is a precursor protein of egg yolk that plays a crucial role in insect reproduction [51]. Silencing of Vg genes through RNAi has been shown to reduce fecundity and egg viability in various insect species, including the brown planthopper,*Nilaparvata lugens* [52] and the diamondback moth,*Plutella xylostella*, [53].

3.2 Comparative Genomics and Transcriptomics

Comparative genomics and transcriptomics approaches can be employed to identify conserved and species-specific genes that are potential targets for RNAi-based pest control [54]. By comparing the genomes or transcriptomes of target and non-target species, researchers can identify genes that are unique to the target pest or have divergent sequences, reducing the risk of off-target effects [55].

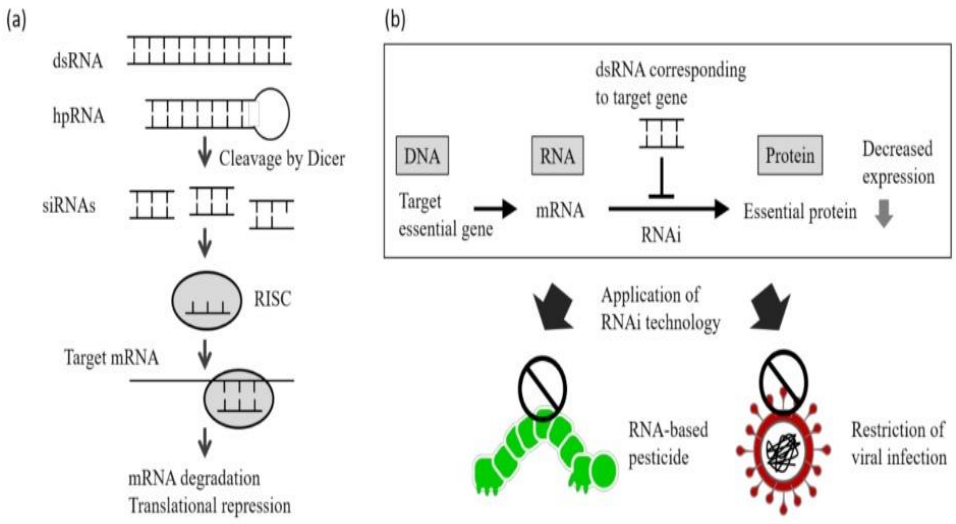

Fig. 3. Design and selection of target genes for RNAi-based pest control

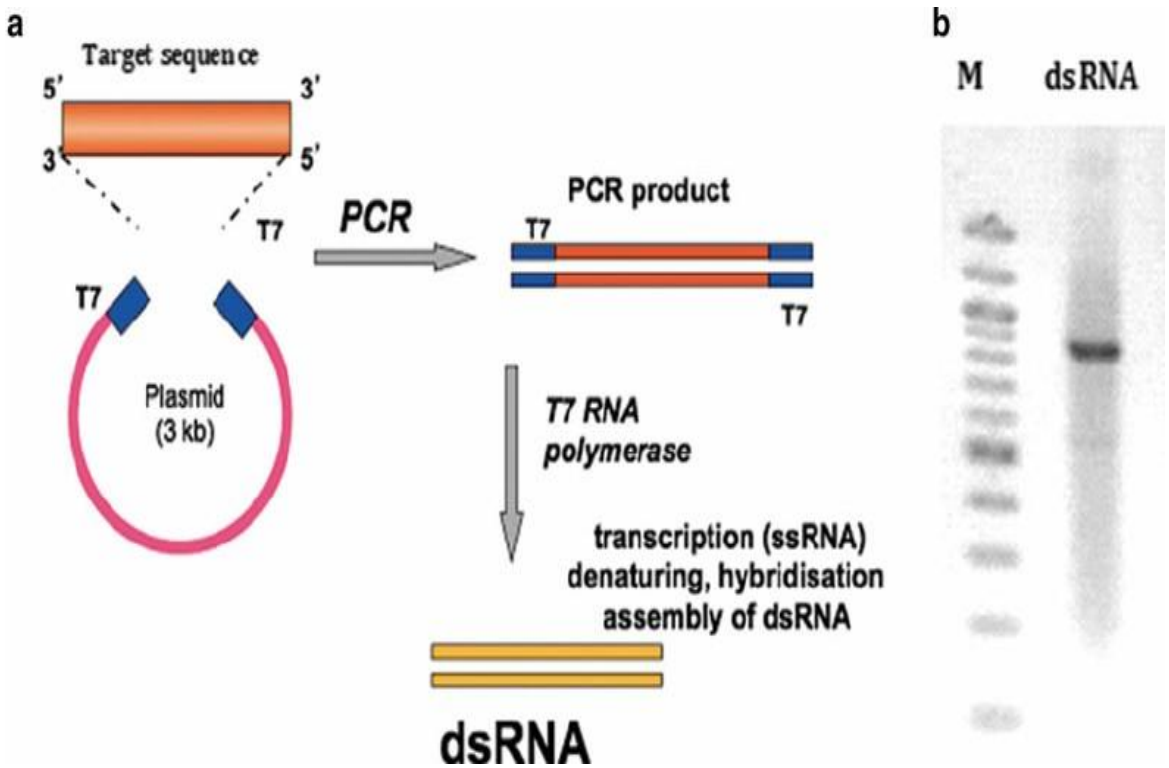

Fig. 4. Comparative genomics and transcriptomics

High-throughput sequencing technologies, such as RNA sequencing (RNA-seq), have greatly facilitated the identification of candidate genes for RNAi [56]. RNA-seq allows for the comprehensive analysis of gene expression profiles, revealing genes that are differentially expressed across different developmental stages, tissues, or in response to specific treatments [57]. This information can be used to prioritize target genes based on their expression patterns and potential roles in insect biology [58].

3.3 *In silico* Design of dsRNA

Once potential target genes have been identified, the next step is to design effective dsRNA sequences for RNAi [59]. The design of dsRNA involves several considerations, such as the length of the dsRNA, the target region within the gene, and the potential for off-target effects [60].

The length of the dsRNA can influence the efficiency of RNAi, with longer dsRNA molecules generally eliciting a stronger RNAi response [61]. However, longer dsRNAs also increase the risk of off-target effects, as they may contain sequences that are complementary to unintended genes [62]. A balance must be struck between dsRNA length and specificity to optimize RNAi efficiency while minimizing off-target effects [63].

The target region within the gene is another important consideration in dsRNA design [64]. Regions that are conserved across different isoforms or splice variants of the target gene are preferable, as they can ensure the silencing of all relevant transcripts [65]. Additionally, targeting regions with minimal secondary structure and high accessibility to the RNAi machinery can enhance the efficiency of gene silencing [66].

In silico tools and algorithms have been developed to aid in the design of effective and specific dsRNA sequences [67]. These tools can predict the secondary structure of the target mRNA, identify potential off-target genes, and optimize the dsRNA sequence for maximum RNAi efficiency [68,69]. Examples of such tools include siDirect [70], E-RNAi [71], and DSIR [72].

3.4 Experimental Validation

After the *in silico* design of dsRNA, experimental validation is necessary to confirm the efficacy and specificity of the selected target genes and dsRNA sequences [73]. This validation can be

performed through various methods, such as quantitative real-time PCR (qRT-PCR), western blotting, or phenotypic assays [74].

The qRT-PCR is commonly used to measure the reduction in target gene expression following dsRNA treatment [75]. By comparing the mRNA levels of the target gene in treated and untreated insects, the efficiency of RNAi can be assessed [76]. Western blotting can be employed to evaluate the reduction in target protein levels, providing further confirmation of the RNAi effect [77].

Phenotypic assays are essential to determine the impact of gene silencing on insect survival, development, and reproduction [78]. These assays can involve monitoring parameters such as mortality, growth rate, fecundity, and fertility in treated insects [79]. The results of these assays provide valuable information on the potential of the selected target genes for pest control applications [80].

3.5 Resistance Management

The development of resistance to RNAi-based pest control strategies is a potential concern that must be addressed during the design and selection of target genes [81]. Insects may evolve resistance to RNAi through various mechanisms, such as mutations in the target gene, enhanced degradation of dsRNA, or reduced uptake of dsRNA [82,83].

To mitigate the risk of resistance, several strategies can be employed in the selection of target genes [84]. One approach is to target multiple genes simultaneously, either through the use of a single dsRNA that targets conserved regions across different genes or through the application of multiple dsRNAs targeting distinct genes [85]. This strategy reduces the likelihood of resistance developing, as insects would need to evolve resistance to multiple genes concurrently [86].

Another approach is to target genes that are essential for insect survival and have a low tolerance for mutations [87]. Mutations in such genes are likely to be lethal or have severe fitness costs, reducing the probability of resistance development [88]. Additionally, targeting genes that are not under strong selection pressure in the absence of the RNAi-based control can also reduce the risk of resistance [89].

Monitoring and early detection of resistance development are crucial for the successful implementation of RNAi-based pest management [90]. Regular surveillance of target pest populations and assessment of their susceptibility to RNAi can help identify potential resistance issues early on [91]. This information can guide the adaptation of RNAi-based strategies, such as rotating target genes or combining RNAi with other pest control methods to maintain their effectiveness over time [92].

4. DELIVERY METHODS FOR DSRNA IN INSECT PEST MANAGEMENT

The efficient delivery of dsRNA to the target insect is a critical factor in the success of RNAi-based pest management [93]. Various delivery methods have been explored, each with its own advantages and limitations [94]. The choice of delivery method depends on factors such as the target insect species, the crop system, and the desired mode of action [95].

4.1. Oral Delivery

Oral delivery is the most common and straightforward method of dsRNA administration in insects [96]. This method involves the ingestion of dsRNA by the target insect, either through feeding on dsRNA-expressing transgenic plants or through the application of dsRNA-containing baits or sprays [97].

4.1.1 Transgenic plants

The development of transgenic plants that express dsRNA targeting specific insect genes has shown great promise for RNAi-based pest control [98]. By engineering plants to produce dsRNA, a continuous supply of the RNAi trigger can be provided to the target insects as they feed on the plant tissues [99]. This approach offers a sustainable and targeted method for pest management, reducing the need for external dsRNA application [100].

The generation of dsRNA-expressing transgenic plants involves the introduction of a construct containing the target gene sequence in an inverted repeat orientation, driven by a strong promoter [101]. When transcribed, the inverted repeat sequence forms a hairpin RNA (hpRNA) structure, which is processed by the plant's RNAi machinery into siRNAs [102]. These siRNAs are

then ingested by the insect and trigger the RNAi response, leading to the silencing of the target gene [103].

Several studies have demonstrated the effectiveness of transgenic plants expressing dsRNA in controlling various insect pests. For example, transgenic corn expressing dsRNA targeting the V-ATPase gene of the western corn rootworm (*Diabrotica virgifera virgifera*) has shown significant reduction in root damage and insect survival [104]. Similarly, transgenic cotton expressing dsRNA against the CYP6AE14 gene of the cotton bollworm (*Helicoverpa armigera*) has exhibited increased resistance to the pest [105].

The use of transgenic plants for RNAi-based pest control offers several advantages, such as the continuous production of dsRNA, the potential for long-term pest suppression, and the reduced need for external dsRNA application [106]. However, there are also challenges and concerns associated with this approach, including the potential for off-target effects on non-target organisms, the development of resistance in target pests, and the public acceptance of genetically modified crops [107,108].

4.1.2 dsRNA-containing baits and sprays

Another approach for oral delivery of dsRNA involves the application of dsRNA-containing baits or sprays directly onto the plant surface or into the soil [109]. This method allows for the targeted delivery of dsRNA to the insect pest without the need for transgenic plants [110].

dsRNA can be formulated into baits or sprays using various carriers, such as nanoparticles, liposomes, or polymers, which protect the dsRNA from degradation and facilitate its uptake by the insect [111,112]. These formulations can be applied to the crop using conventional spraying equipment or through irrigation systems [113].

The effectiveness of dsRNA-containing baits and sprays has been demonstrated in several studies. For instance, the application of dsRNA targeting the beta-tubulin gene of the Colorado potato beetle (*Leptinotarsa decemlineata*) on potato leaves resulted in significant mortality and reduced feeding damage [114]. Similarly, the use of dsRNA-containing baits targeting the carboxylesterase gene of the Asian citrus psyllid (*Diaphorina citri*) led to a decrease in insect populations and reduced transmission of the citrus greening disease [115].

The main advantages of using dsRNA-containing baits and sprays include the flexibility in application timing, the ability to target specific insect stages or populations, and the potential for integration with other pest management strategies [116]. However, challenges such as the stability of dsRNA in the environment, the efficiency of uptake by the target insects, and the potential for off-target effects need to be addressed for the successful implementation of this approach [117,118].

4.2 Injection and Soaking

Injection and soaking are two common methods used for the direct delivery of dsRNA into insects, particularly in laboratory studies [119]. While these methods are not practical for large-scale field applications, they provide valuable insights into the RNAi process and aid in the identification of potential target genes [120].

4.2.1 Injection

Injection involves the direct introduction of dsRNA into the insect's body cavity using a fine needle or a microinjector [121]. This method allows for the precise delivery of a known quantity of dsRNA to the target tissue or organ [122]. Injection has been widely used to study the effects of RNAi on insect physiology, development, and behavior [123].

The effectiveness of RNAi through injection has been demonstrated in various insect species, including the fruit fly (*Drosophila melanogaster*) [124], the red flour beetle (*Tribolium castaneum*) [125], and the tobacco hornworm (*Manduca sexta*) [126]. Injection of dsRNA has been used to investigate the functions of genes involved in processes such as metamorphosis, reproduction, and immunity [127,128].

However, injection is a labor-intensive and invasive method that is not suitable for large-scale pest management applications [129]. It also requires specialized equipment and skilled personnel, limiting its practicality in field settings [130].

4.2.2 Soaking

Soaking involves the immersion of insects or insect tissues in a solution containing dsRNA

[131]. This method is particularly useful for studying RNAi in smaller insects or in specific tissues, such as the midgut or the salivary glands [132].

Soaking has been successfully used to deliver dsRNA into various insect species, including the whitefly (*Bemisia tabaci*) [133], the oriental fruit fly (*Bactrocera dorsalis*) [134], and the silkworm (*Bombyx mori*) [135]. In these studies, soaking has been employed to investigate the roles of genes involved in processes such as digestion, detoxification, and virus transmission [136,137].

Like injection, soaking is primarily used in laboratory settings and is not practical for large-scale field applications [138]. However, it provides a useful tool for studying the RNAi process and identifying potential target genes for pest management [139].

4.3 Topical Application

Topical application involves the direct application of dsRNA onto the insect's cuticle or surface [140]. This method relies on the absorption of dsRNA through the insect's integument and its subsequent transport to the target tissues [141].

Topical application of dsRNA has been demonstrated in several insect species, such as the tobacco budworm (*Heliothis virescens*) [142], the diamondback moth (*Plutella xylostella*) [143], and the green peach aphid (*Myzus persicae*) [144]. In these studies, dsRNA was applied to the insect's cuticle using droplets or sprays, resulting in gene silencing and various phenotypic effects [145,146].

The efficiency of topical application depends on factors such as the permeability of the insect's cuticle, the stability of dsRNA on the surface, and the ability of the insect to absorb and transport the dsRNA to the target tissues [147]. To enhance the efficacy of topical application, various formulations and delivery vehicles have been explored, such as nanoparticles, liposomes, and penetration enhancers [148,149].

Topical application offers a non-invasive and relatively simple method for dsRNA delivery, making it a potential option for field-based pest management [150]. However, further research is needed to optimize the formulations and application techniques to improve the efficiency and practicality of this approach [151].

5. RNAI-BASED CONTROL OF MAJOR INSECT PESTS

RNAi has been explored as a potential tool for the control of various major insect pests that cause significant damage to agricultural crops worldwide [152]. In this section, we will discuss the application of RNAi-based strategies for the management of key insect pests from different orders, including Lepidoptera, Coleoptera, Hemiptera, and Diptera.

5.1 Lepidopteran Pests

Lepidopteran insects, which include moths and butterflies, are among the most destructive pests of agricultural crops [153]. Many lepidopteran species, such as the cotton bollworm (*Helicoverpa armigera*), the tobacco cutworm (*Spodoptera litura*), and the diamondback moth(Plutella xylostella), cause significant yield losses in various crops worldwide [154,155].

Table 1. Examples of RNAi-based control of lepidopteran pests

Insect Pest	Target Gene	Delivery Method	Effect	Reference
Helicoverpa armigera	Chitinase	Transgenic tobacco	Disrupted molting, increased mortality	[156]
Spodoptera litura	Ecdysone receptor	dsRNA injection	Developmental abnormalities, reduced survival	[157]
Plutella xylostella	Acetylcholinesterase	Oral delivery (dsRNA-containing diet)	Reduced survival, impaired neural transmission	[163]
Heliothis virescens	Juvenile hormone acid methyltransferase	Topical application	Disrupted metamorphosis, reduced survival	[164]

Table 2. Examples of RNAi-based control of coleopteran pests

Insect Pest	Target Gene	Delivery Method	Effect	Reference
Leptinotarsa decemlineata	Actin	Oral delivery (dsRNA-containing bait)	Reduced survival, impaired locomotion	[168]
Diabrotica virgifera virgifera	V-ATPase	Transgenic corn	Decreased feeding, increased mortality	[169]
Tribolium castaneum	Ribosomal protein S6	dsRNA injection	Developmental arrest, reduced survival	[173]
Cylas puncticollis	Cathepsin L	Oral delivery (dsRNA-containing diet)	Reduced feeding, increased mortality	[174]

Table 3. Examples of RNAi-based control of hemipteran pests

Insect Pest	Target Gene	Delivery Method	Effect	Reference
Myzus persicae	Salivary protein	Oral delivery (dsRNA-containing diet)	Reduced survival, decreased fecundity	[178]
Aphis gossypii	Acetylcholinesterase	dsRNA injection	Impaired neural transmission, increased mortality	[179]
Bemisia tabaci	Heat shock protein 70	Oral delivery (dsRNA-containing diet)	Reduced virus transmission	[182]
Nilaparvata lugens	Trehalose-6-phosphate synthase	Transgenic rice	Impaired trehalose metabolism, reduced survival	[186]

RNAi has shown promise as a potential control strategy for lepidopteran pests. Several studies have demonstrated the effectiveness of RNAi in silencing essential genes and inducing mortality in these insects. For example, the silencing of the chitinase gene in the cotton bollworm resulted in disrupted molting and increased mortality [156]. Similarly, RNAi-mediated knockdown of the ecdysone receptor gene in the tobacco cutworm led to developmental abnormalities and reduced survival [157].

The delivery of dsRNA to lepidopteran pests has been achieved through various methods, including oral delivery via transgenic plants or dsRNA-containing baits, and topical application [158,159]. Transgenic plants expressing dsRNA targeting essential genes have been developed for several lepidopteran pests, such as the cotton bollworm and the tobacco budworm [160,161]. These transgenic plants have shown increased resistance to the target pests and reduced crop damage [162].

5.2 Coleopteran Pests

Coleopteran insects, or beetles, include many important agricultural pests, such as the Colorado potato beetle (*Leptinotarsa decemlineata*), the western corn rootworm (*Diabrotica virgifera virgifera*), and the red flour beetle (*Tribolium castaneum*) [165,166]. These pests cause significant damage to crops, leading to substantial economic losses [167].

RNAi has been successfully used to control coleopteran pests by targeting essential genes involved in various biological processes. For instance, the silencing of the actin gene in the Colorado potato beetle resulted in reduced survival and impaired locomotion [168]. The knockdown of the V-ATPase gene in the western corn rootworm led to decreased feeding and increased mortality [169].

Oral delivery of dsRNA through transgenic plants or dsRNA-containing baits has been the primary method for RNAi-based control of coleopteran pests [170]. Transgenic corn expressing dsRNA targeting the Snf7 gene of the western corn rootworm has been developed and has shown significant protection against the pest [171]. Additionally, dsRNA-containing baits have been used to control the Colorado potato beetle, resulting in reduced feeding damage and increased mortality [172].

Table 4. Examples of RNAi-based control of dipteran pests

Insect Pest	Target Gene	Delivery Method	Effect	Reference
Drosophila suzukii	Ribosomal protein L19	Oral delivery (dsRNA-containing diet)	Decreased survival, reduced fecundity	[189]
Aedes aegypti	Odorant binding protein	dsRNA injection	Reduced host-seeking behavior, decreased blood-feeding success	[193]
Anopheles gambiae	Salivary gland protein (SG1)	dsRNA injection	Impaired blood-feeding, reduced parasite transmission	[197]
Ceratitis capitata	Transformer-2	Oral delivery (dsRNA-containing diet)	Feminization of males, reduced fertility	[198]

5.3 Hemipteran Pests

Hemipteran insects, which include aphids, whiteflies, and planthoppers, are major pests of various crops worldwide [175]. These insects cause damage through direct feeding and by transmitting plant viruses [176].

The green peach aphid (*Myzus persicae*) is a significant pest of many crops and a vector of plant viruses [177]. RNAi-mediated silencing of the salivary protein gene in this aphid resulted in reduced survival and fecundity [178]. Similarly, the knockdown of the acetylcholinesterase gene in the cotton aphid (*Aphis gossypii*) led to impaired neural transmission and increased mortality [179].

In the whitefly (*Bemisia tabaci*), RNAi has been used to target genes involved in virus transmission and insecticide resistance [180,181]. The silencing of the heat shock protein 70 gene in this insect reduced its ability to transmit the tomato yellow leaf curl virus [182].

Oral delivery of dsRNA through transgenic plants and dsRNA-containing diets has been the primary approach for RNAi-based control of hemipteran pests [183,184]. Transgenic tobacco plants expressing dsRNA targeting the cytochrome P450 gene of the cotton aphid have shown increased resistance to the pest [185].

5.4 Dipteran Pests

Dipteran insects, such as flies and mosquitoes, include several important agricultural and medical pests [187]. The spotted wing drosophila (*Drosophila suzukii*) is a significant pest of soft-skinned fruits, causing substantial economic losses [188]. RNAi has been used to target essential genes in this pest, such as the ribosomal protein L19 gene, resulting in decreased survival and reduced fecundity [189].

Mosquitoes, such as *Aedes aegypti* and *Anopheles gambiae*, are major vectors of human diseases, including dengue fever and malaria [190]. RNAi has been explored as a potential tool for mosquito control by targeting genes involved in disease transmission and insecticide resistance [191,192]. The silencing of the odorant binding protein gene in *A. aegypti* resulted in reduced host-seeking behavior and decreased blood-feeding success [193].

The delivery of dsRNA to dipteran pests has been achieved through various methods, including injection, soaking, and topical application [194,195]. Oral delivery of dsRNA through dsRNA-containing diets has also been demonstrated in the spotted wing drosophila [196].

6. CHALLENGES AND LIMITATIONS OF RNAI-BASED INSECT PEST MANAGEMENT

Despite the promising potential of RNAi as a tool for insect pest management, several challenges and limitations need to be addressed for its successful implementation in agricultural systems [199]. These challenges include variable RNAi efficiency across insect species, potential off-target effects, environmental stability of dsRNA, and the development of resistance in target pests [200,201].

6.1 Variable RNAi Efficiency

The efficiency of RNAi varies significantly among different insect species and even among different populations of the same species [202]. This variability can be attributed to factors such as differences in the uptake and processing of dsRNA, the presence of RNAi-inhibiting enzymes, and the specificity of the RNAi machinery [203,204].

Lepidopteran insects, for example, have been reported to exhibit lower RNAi efficiency compared to coleopteran insects [205]. This difference has been attributed to the presence of dsRNA-degrading enzymes in the gut of lepidopteran larvae, which can limit the efficacy of orally delivered dsRNA [206]. In contrast, coleopteran insects generally show a more robust RNAi response, possibly due to the absence or lower activity of such enzymes [207].

To overcome the variable RNAi efficiency across insect species, researchers have explored various strategies, such as the use of different dsRNA delivery methods, the optimization of dsRNA design, and the identification of more susceptible target genes [208,209]. Additionally, the development of formulations that protect dsRNA from degradation and enhance its uptake by the insect gut has shown promise in improving RNAi efficiency [210].

6.2 Off-Target Effects

Another challenge associated with RNAi-based pest management is the potential for off-target effects, where the dsRNA intended to silence a specific gene in the target insect also affects unintended genes or non-target organisms [211]. Off-target effects can occur due to sequence similarities between the dsRNA and non-target genes, leading to the unintended silencing of these genes [212].

To minimize off-target effects, careful design and selection of dsRNA sequences are crucial [213]. In silico tools can be used to identify potential off-target genes and design dsRNA sequences that are specific to the target gene [214]. Additionally, the use of multiple dsRNAs targeting different regions of the same gene can reduce the risk of off-target effects, as it is less likely that all dsRNAs will have significant sequence similarities with non-target genes [215].

The potential impact of RNAi-based pest control on non-target organisms, such as beneficial insects, pollinators, and soil microorganisms, is another important consideration [216]. Studies have shown that the ingestion of dsRNA by non-target organisms can lead to gene silencing and adverse effects on their fitness and survival [217,218]. Therefore, it is essential to conduct comprehensive risk assessments and develop strategies to minimize the exposure of non-target organisms to dsRNA [219].

6.3 Environmental Stability of dsRNA

The environmental stability of dsRNA is a critical factor in the success of RNAi-based pest management [220]. When dsRNA is delivered to the target insects through transgenic plants or dsRNA-containing baits and sprays, it is exposed to various environmental factors that can affect its stability and persistence [221].

Factors such as UV radiation, temperature, humidity, and the presence of microorganisms can degrade dsRNA in the environment [222,223]. The degradation of dsRNA can reduce its effectiveness in inducing RNAi in the target insects and may require frequent applications to maintain pest control [224].

To enhance the environmental stability of dsRNA, various formulations and delivery systems have been developed [225]. These include the use of nanoparticles, liposomes, and polymers that can protect dsRNA from degradation and improve its delivery to the target insects [226,227]. Additionally, the expression of dsRNA in chloroplasts of transgenic plants has been shown to increase its stability and persistence in the environment [228].

6.4 Development of Resistance

The development of resistance to RNAi-based pest control is a potential long-term challenge that needs to be addressed [229]. Insects may evolve resistance to dsRNA through various mechanisms, such as mutations in the target gene, enhanced degradation of dsRNA, or reduced uptake of dsRNA [230,231].

To mitigate the risk of resistance development, several strategies can be employed. One approach is to target multiple genes simultaneously, either through the use of a single dsRNA that targets conserved regions across different genes or through the application of

multiple dsRNAs targeting distinct genes [232,233]. This strategy reduces the likelihood of resistance developing, as insects would need to evolve resistance to multiple genes concurrently [234].

Another approach is to use RNAi as part of an integrated pest management (IPM) program, where it is combined with other pest control methods, such as biological control, cultural practices, and selective use of insecticides [235]. By using multiple control strategies, the selection pressure on the target pest is reduced, decreasing the risk of resistance development [236].

Monitoring and early detection of resistance development are also crucial for the successful implementation of RNAi-based pest management [237]. Regular surveillance of target pest populations and assessment of their susceptibility to RNAi can help identify potential resistance issues early on [238]. This information can guide the adaptation of RNAi-based strategies, such as rotating target genes or adjusting the frequency and timing of dsRNA applications, to maintain their effectiveness over time [239].

7. ENVIRONMENTAL AND ECOLOGICAL CONSIDERATIONS

The use of RNAi-based strategies for insect pest management raises important environmental and ecological considerations that need to be addressed to ensure their safe and sustainable application [240]. These considerations include the potential impact on non-target organisms, the fate of dsRNA in the environment, and the need for appropriate risk assessment and regulation [241].

7.1 Impact on Non-Target Organisms

One of the main environmental concerns associated with RNAi-based pest control is the potential impact on non-target organisms [242]. While RNAi is generally considered to be a highly specific approach, there is a risk that dsRNA intended to silence genes in the target pest may also affect closely related species or other organisms that share sequence similarities [243].

Studies have shown that the ingestion of dsRNA by non-target organisms, such as beneficial insects, pollinators, and soil microorganisms, can lead to gene silencing and adverse effects on their fitness and survival [244,245]. For example, the ingestion of dsRNA targeting the V-ATPase gene of the western corn rootworm by the ladybird beetle (*Coleomegilla maculata*), a non-target organism, resulted in reduced fertility and survival [246].

7.2 Fate of dsRNA in the Environment

Another important consideration is the fate of dsRNA in the environment, including its persistence, degradation, and potential for horizontal transfer [250]. When dsRNA is introduced into the environment through transgenic plants or dsRNA-containing sprays, it can persist in the soil, water, and plant tissues [251].

The persistence of dsRNA in the environment raises concerns about its potential long-term effects on non-target organisms and the possibility of horizontal gene transfer to other species [252]. Studies have shown that dsRNA can be detected in the soil and water for extended periods after application, and it can be taken up by non-target plants and soil microorganisms [253].

To address these concerns, it is important to understand the factors that influence the persistence and degradation of dsRNA in the environment, such as soil type, temperature, and microbial activity [236]. Additionally, the development of biodegradable dsRNA formulations and delivery systems that minimize the persistence of dsRNA in the environment can help mitigate potential risks [240].

7.3 Risk Assessment and Regulation

Given the potential environmental and ecological impacts of RNAi-based pest control, appropriate risk assessment and regulation are essential to ensure its safe and responsible use [241]. Regulatory frameworks need to be established to evaluate the risks and benefits of RNAi-based technologies and to guide their development and application [251].

Risk assessment should consider the potential effects of RNAi-based pest control on non-target organisms, the fate of dsRNA in the environment, and the likelihood of resistance development in target pests [247,248,249]. This requires a comprehensive understanding of the molecular mechanisms of RNAi, the ecology of the target pest and its interactions with other

species, and the environmental factors that influence the effectiveness and persistence of dsRNA [235].

Regulatory oversight should ensure that RNAi-based pest control products are thoroughly tested for their safety and efficacy before they are released into the environment [261]. This may involve the establishment of standardized testing protocols, the setting of appropriate thresholds for non-target effects, and the implementation of post-release monitoring and surveillance programs [230].

Effective communication and engagement with stakeholders, including farmers, environmental organizations, and the general public, are also crucial for the successful adoption and regulation of RNAi-based pest control [250]. Transparency about the potential risks and benefits of these technologies, as well as the measures taken to mitigate any adverse effects, can help build public trust and support for their responsible use [264].

8. CONCLUSION AND FUTURE PERSPECTIVES

RNAi-based strategies hold great promise for revolutionizing insect pest management and contributing to the development of sustainable and resilient agricultural systems. By harnessing the power of gene silencing, these approaches can provide targeted and eco-friendly solutions to the complex challenges posed by insect pests. With continued research, innovation, and responsible implementation, RNAi-based pest control can play a vital role in ensuring food security and environmental sustainability for future generations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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