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RNA interference (RNAi) technology for sustainable insect pest management: Recent advances, challenges, and future perspectives

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Abstract

The increasing demand for sustainable agricultural production has accelerated the development of environmentally responsible pest management strategies capable of reducing dependence on conventional chemical insecticides. RNA interference (RNAi) has emerged as a promising molecular technology for next-generation insect pest control due to its high target specificity, environmentally friendly nature, and compatibility with integrated pest management (IPM) systems. RNAi-mediated pest suppression relies on the introduction of sequence-specific double-stranded RNA (dsRNA), which activates endogenous gene silencing pathways and subsequently disrupts the expression of essential genes associated with insect survival, development, reproduction, and metabolism. This review provides a comprehensive overview of recent advances in RNAi-based insect pest management, including the molecular mechanisms underlying dsRNA processing, target gene identification approaches, and emerging delivery platforms. Various dsRNA delivery strategies, such as host-induced gene silencing (HIGS), spray-induced gene silencing (SIGS), nanoparticle-assisted delivery, symbiont-mediated RNAi, and viral-based approaches, are discussed with emphasis on their advantages and limitations under field conditions. Recent progress toward commercialization demonstrates the increasing potential of RNAi-based biopesticides; however, challenges including variable RNAi efficiency among insect taxa, rapid dsRNA degradation, inefficient cellular uptake, off-target effects, resistance evolution, regulatory uncertainty, and production costs continue to restrict widespread adoption. Future advancements integrating RNAi with nanotechnology, functional genomics, artificial intelligence-driven target prediction, and IPM-based approaches may enhance the reliability, scalability, and sustainability of RNAi applications. Therefore, RNAi represents a transformative approach in modern pest management, providing opportunities for precise insect suppression while reducing ecological risks associated with conventional pesticides.

Keywords: RNA interference; dsRNA; insect pest management; biopesticides; gene silencing; nanocarriers; sustainable agriculture; integrated pest management

1. Introduction

Insect pests remain one of the major constraints affecting global agricultural productivity and food security. Approximately 20-30% of global crop production is lost annually due to insect pests, plant diseases, and weeds, with insect-related damage accounting for a substantial proportion of these losses (Oerke, 2006; Savary *et al.*, 2019) ^[22, 26]. The increasing demand for food production driven by population growth and climate change has intensified the need for effective pest management strategies that maintain agricultural productivity while minimizing environmental consequences.

For several decades, synthetic chemical insecticides have represented the primary approach for controlling economically important insect pests. Although these compounds have contributed significantly to agricultural intensification, their excessive and repeated application has resulted in several ecological and agronomic challenges, including insecticide resistance development, disruption of beneficial insect communities, contamination of soil and aquatic ecosystems, and potential risks to human health (Sparks and Nauen, 2015; Bass *et al.*, 2014) ^[2, 27]. The rapid evolution of resistance in major pest species, including *Helicoverpa armigera*, *Plutella xylostella*, *Myzus persicae*, and *Bemisia tabaci*, has reduced the effectiveness of many commercially available insecticides and highlighted the urgent

requirement for alternative pest control technologies (Gould *et al.*, 2018) ^[11].

The concept of sustainable pest management has therefore shifted toward highly selective approaches that target pest species while preserving beneficial organisms and ecosystem functions. Among emerging technologies, RNA interference (RNAi) has gained considerable attention as a precision-based pest management strategy due to its ability to selectively suppress specific genes through sequence-dependent mechanisms (Fire *et al.*, 1998; Huvenne and Smagghe, 2010) ^[8, 14]. Unlike conventional insecticides that generally interfere with physiological processes across multiple organisms, RNAi technology enables species-specific regulation of gene expression by introducing double-stranded RNA (dsRNA) molecules complementary to target messenger RNA (mRNA) sequences.

RNA interference is a naturally occurring post-transcriptional gene regulation mechanism conserved among eukaryotic organisms. In insects, externally supplied dsRNA is processed into small interfering RNAs (siRNAs), which subsequently guide the RNA-induced silencing complex (RISC) toward complementary mRNA molecules, resulting in transcript degradation and suppression of target gene expression (Hammond *et al.*, 2001; Gordon and Waterhouse, 2007) ^[10, 12]. When essential genes involved in development, metabolism, reproduction, immunity, or cellular regulation are targeted, RNAi can induce mortality, developmental abnormalities, reduced fertility, or increased susceptibility to environmental stresses (Katoch *et al.*, 2013) ^[16].

The application of RNAi for insect pest management has progressed significantly since the first demonstration of RNA-mediated gene silencing in insects. Research has successfully applied RNAi against several agricultural pests, including coleopterans, hemipterans, dipterans, and lepidopterans (Christiaens *et al.*, 2020) ^[6]. The commercial development of RNAi-based insecticides, such as dsRNA products targeting the western corn rootworm (*Diabrotica virgifera virgifera*), demonstrates the practical feasibility of translating laboratory discoveries into agricultural applications (Bachman *et al.*, 2013) ^[11].

Despite substantial progress, RNAi-based pest management still faces significant biological and technological limitations. RNAi efficiency varies considerably among insect species due to differences in dsRNA uptake mechanisms, digestive system characteristics, nuclease activity, RNA transport pathways, and immune responses (Christiaens *et al.*, 2020; Prentice *et al.*, 2021) ^[6, 25]. Particularly, many lepidopteran pests exhibit limited sensitivity to orally delivered dsRNA, restricting field-level effectiveness and requiring innovative delivery strategies (Terenius *et al.*, 2011) ^[29]. Furthermore, environmental instability of naked dsRNA, potential off-target effects, resistance evolution, and regulatory challenges remain important barriers to commercialization.

Recent advances in nanotechnology, molecular biology, genome editing, and bioinformatics have created new opportunities to overcome these limitations. Nanoparticle-based formulations can improve dsRNA stability and cellular uptake, while transcriptomic and genomic approaches enable identification of highly specific pest targets with reduced ecological risks (Joga *et al.*, 2016; Nandety *et al.*, 2024) ^[15, 21]. Integration of RNAi with integrated pest management programs may further enhance

sustainability by combining molecular precision with ecological pest suppression strategies.

This review discusses the current status of RNAi technology for insect pest management, focusing on molecular mechanisms, target gene discovery, dsRNA delivery approaches, recent commercialization progress, major challenges, and future perspectives toward establishing RNAi as a sustainable component of modern agriculture.

2. Molecular mechanisms of RNA interference in insects

RNA interference (RNAi) is an evolutionarily conserved post-transcriptional gene regulation mechanism that plays a fundamental role in controlling gene expression, antiviral defense, and genome stability in eukaryotic organisms. Since its discovery in *Caenorhabditis elegans* by Fire *et al.* (1998) ^[8], RNAi has become an important molecular tool for functional genomics and has emerged as a promising strategy for insect pest management. In insects, RNAi-mediated gene silencing is primarily initiated through the introduction of exogenous double-stranded RNA (dsRNA), which activates intracellular pathways leading to sequence-specific degradation of complementary messenger RNA (mRNA) transcripts (Hammond *et al.*, 2001; Huvenne and Smagghe, 2010) ^[12, 14].

The efficiency of RNAi-based pest control depends on multiple sequential biological events, including dsRNA uptake, intracellular processing, small interfering RNA (siRNA) generation, assembly of the RNA-induced silencing complex (RISC), target recognition, mRNA degradation, and systemic spread of the silencing signal. Understanding these molecular processes is essential for improving RNAi effectiveness and designing efficient delivery systems for agricultural applications.

2.1 Initiation of RNAi pathway and dsRNA processing

The RNAi pathway in insects is initiated when long dsRNA molecules enter insect cells through environmental exposure, feeding, injection, or plant-mediated expression systems. Once internalized, dsRNA molecules are recognized by the RNase III family enzyme Dicer-2 (Dcr-2), which cleaves them into short interfering RNAs (siRNAs) approximately 21-22 nucleotides in length (Bernstein *et al.*, 2001; Hammond *et al.*, 2001) ^[4, 12].

Dicer-2 activity is facilitated by accessory proteins such as Loquacious-PD (Loqs-PD), which enhance substrate recognition and promote efficient siRNA production in insects (Förstemann *et al.*, 2005) ^[9]. The generated siRNA duplexes contain a guide strand complementary to the target mRNA and a passenger strand that is subsequently degraded. The guide strand is incorporated into the RNA-induced silencing complex (RISC), where it directs sequence-specific recognition of target transcripts.

The core catalytic component of RISC is the Argonaute-2 (Ago2) protein, which possesses endonuclease activity. Upon binding of siRNA-loaded RISC to complementary mRNA, Ago2 cleaves the transcript, resulting in rapid degradation and suppression of gene expression (Okamura *et al.*, 2004) ^[23]. This precise sequence-based mechanism provides RNAi with its characteristic specificity, making it highly attractive for selective pest control.

2.2 Systemic RNAi and dsRNA transport mechanisms

For RNAi to function effectively as an agricultural pest management technology, dsRNA must not only trigger local

gene silencing but also spread throughout insect tissues. This phenomenon, known as systemic RNAi, enables orally delivered dsRNA to generate physiological effects beyond the initial site of uptake.

Systemic RNAi responses differ considerably among insect species due to variations in dsRNA uptake pathways and intracellular transport mechanisms. In some insects, specialized membrane proteins facilitate dsRNA internalization. For example, the scavenger receptor-mediated uptake pathway has been implicated in dsRNA absorption in several insect species, allowing extracellular dsRNA molecules to enter epithelial cells and initiate gene silencing responses (Xu and Han, 2008) [33].

In *Caenorhabditis elegans*, systemic RNAi depends on SID-1-mediated dsRNA transport; however, the presence and functional significance of SID-1 homologues in insects remain variable (Winston *et al.*, 2002). Some insects possess SID-1-like proteins, whereas others rely primarily on endocytosis-based mechanisms for dsRNA uptake (Xu *et al.*, 2013) [34]. These differences contribute significantly to the variation in RNAi sensitivity observed among insect orders.

Recent studies have suggested that extracellular vesicles, particularly exosomes, may contribute to intercellular transport of RNA molecules and facilitate systemic propagation of RNAi signals (Tassetto *et al.*, 2017) [28]. However, the molecular basis of systemic RNAi movement in insects remains incompletely understood and represents a major research priority for improving RNAi-based pest control.

2.3 Taxonomic variation in RNAi efficiency among insects

One of the major limitations affecting the practical implementation of RNAi technology is the highly variable response among insect species. RNAi efficiency is strongly influenced by evolutionary differences in dsRNA uptake, degradation, processing, and amplification mechanisms.

Coleopteran insects are generally considered highly responsive to RNAi. The western corn rootworm (*Diabrotica virgifera virgifera*) represents one of the most successful examples, where ingestion of dsRNA targeting essential genes resulted in significant mortality and ultimately contributed to the development of commercial RNAi-based insect protection technologies (Baum *et al.*, 2007; Bachman *et al.*, 2013) [1, 3].

In contrast, many lepidopteran pests exhibit poor RNAi responses, particularly following oral dsRNA delivery. Species such as *Spodoptera frugiperda*, *Helicoverpa armigera*, and *Plutella xylostella* often possess high levels of dsRNA-degrading nucleases in the gut, inefficient cellular uptake mechanisms, and limited systemic RNAi capability (Terenius *et al.*, 2011; Christiaens *et al.*, 2020) [6, 29]. These physiological barriers reduce dsRNA stability and prevent sufficient activation of intracellular silencing pathways.

Hemipteran insects, including aphids and whiteflies, generally exhibit moderate to high RNAi sensitivity. In *Myzus persicae*, RNAi targeting genes associated with development and reproduction has successfully reduced population growth and reproductive performance (Pitino *et al.*, 2011) [24]. Similarly, RNAi approaches targeting essential genes in *Bemisia tabaci* have demonstrated

potential for controlling insecticide-resistant populations (Cai *et al.*, 2017) [5].

Therefore, understanding species-specific RNAi biology is essential for selecting suitable targets and developing effective delivery strategies tailored to different pest groups.

3. Target gene identification and functional validation for RNAi-based pest management

The selection of appropriate target genes represents a critical step determining the success of RNAi-based insect control strategies. Unlike conventional insecticides that generally affect broad physiological processes, RNAi relies on precise suppression of individual genes. Therefore, target genes must fulfil several criteria, including essentiality for insect survival, limited similarity with non-target organisms, efficient silencing response, and the ability to induce measurable fitness costs upon suppression.

Advances in genome sequencing, transcriptomics, proteomics, and functional genomics have significantly expanded the discovery of potential RNAi targets. High-throughput RNA sequencing (RNA-seq) and comparative genomic analyses have enabled researchers to identify genes involved in critical biological processes, including development, metabolism, reproduction, immunity, and detoxification pathways.

3.1 Essential genes as RNAi targets

Genes associated with fundamental cellular processes are among the most effective RNAi targets because their suppression often results in lethal or severe developmental consequences. Several studies have demonstrated successful RNAi-mediated disruption of genes involved in molting, hormone regulation, cytoskeletal organization, and cellular metabolism.

For example, targeting the vacuolar ATPase (V-ATPase) gene, which regulates cellular proton transport and energy metabolism, has produced strong mortality responses in several insect species, including *Tribolium castaneum* and *Diabrotica virgifera* (Whyard *et al.*, 2009) [31]. Similarly, genes encoding chitin synthase, juvenile hormone pathway enzymes, and ecdysone receptors have been investigated as promising targets because of their essential roles in insect growth and development (Katoch *et al.*, 2013) [16].

3.2 Targeting insect-specific genes for enhanced biosafety

A major advantage of RNAi technology is the possibility of designing highly species-specific sequences that minimize impacts on beneficial insects and other organisms. However, careful bioinformatic screening is required to identify regions of target genes that exhibit minimal sequence similarity with non-target species.

Mahanta *et al.* (2025) [18] emphasized that future RNAi-based biopesticides require systematic evaluation of target specificity through genomic databases and computational prediction tools to reduce unintended effects on pollinators, predators, and parasitoids.

Genes unique to insect physiology, such as species-specific digestive enzymes, reproductive regulators, and developmental proteins, represent attractive targets because they provide greater selectivity compared with conserved housekeeping genes.

3.3 Functional validation approaches

The validation of RNAi targets requires experimental confirmation through gene knockdown studies, phenotypic analysis, and molecular characterization. Common approaches include:

- Microinjection of synthetic dsRNA
- Feeding assays using dsRNA-treated diets
- Plant-mediated RNAi experiments
- Quantitative PCR analysis of transcript suppression
- Phenotypic evaluation of mortality, development, fertility, and behaviour

Recent integration of CRISPR-based gene editing with RNAi screening has further improved target validation by allowing direct confirmation of gene function and identification of synthetic lethal interactions (Joga *et al.*, 2016) [15].

High-throughput RNAi screening combined with artificial intelligence-assisted prediction models is expected to accelerate the discovery of novel pest-specific targets and improve the development of next-generation RNAi-based insecticides.

4. Delivery strategies for dsRNA in insect pest management

The successful application of RNA interference (RNAi) technology in agriculture depends largely on the efficient delivery of double-stranded RNA (dsRNA) molecules into target insects. Although RNAi provides exceptional sequence specificity, the practical effectiveness of this technology is strongly influenced by dsRNA stability, cellular uptake, degradation by nucleases, transport across insect tissues, and activation of the intracellular silencing machinery. Therefore, developing efficient delivery systems remains one of the most critical challenges for converting RNAi from a laboratory-based molecular tool into a commercially viable pest management technology (Christiaens *et al.*, 2020) [6].

Several dsRNA delivery approaches have been developed, including spray-induced gene silencing (SIGS), host-induced gene silencing (HIGS), nanoparticle-mediated delivery, symbiont-based delivery systems, and virus-induced gene silencing (VIGS). Each strategy offers unique advantages and limitations depending on pest biology, crop system, regulatory considerations, and field application requirements.

4.1 Spray-Induced Gene Silencing (SIGS)

Spray-induced gene silencing (SIGS) represents a non-transgenic approach in which externally synthesized dsRNA is directly applied onto plant surfaces through conventional spraying techniques. Following application, insect pests acquire dsRNA during feeding, resulting in sequence-specific suppression of target genes and subsequent disruption of essential biological processes (Mitter *et al.*, 2017) [20].

The major advantage of SIGS is its flexibility because it does not require genetic modification of crops. Farmers can apply dsRNA formulations similarly to conventional pesticides, allowing integration into existing crop management systems. Additionally, SIGS enables rapid modification of target sequences when pest populations evolve resistance or when new pest species emerge.

Successful examples of SIGS include suppression of essential genes in fungal pathogens and insect pests through topical RNA applications. For instance, topical dsRNA treatments targeting genes associated with development and survival have demonstrated significant mortality effects in several insect species, including *Diabrotica virgifera*, *Bemisia tabaci*, and *Myzus persicae* (Wang *et al.*, 2011; Pitino *et al.*, 2011) [24, 30].

However, the major limitation of SIGS is the poor environmental stability of naked dsRNA. Ultraviolet radiation, rainfall, temperature fluctuations, and microbial degradation can rapidly reduce dsRNA persistence on plant surfaces (Dubelman *et al.*, 2014) [7]. Furthermore, insect digestive fluids contain abundant nucleases capable of degrading dsRNA before it reaches target cells, particularly in lepidopteran species (Christiaens *et al.*, 2020) [6].

To overcome these limitations, researchers are developing stabilized dsRNA formulations using protective polymers, clay-based materials, and nanocarriers to enhance persistence and biological activity under field conditions.

4.2 Host-Induced Gene Silencing (HIGS)

Host-induced gene silencing (HIGS) is a genetically engineered approach where crop plants are modified to continuously produce dsRNA molecules targeting essential genes of insect pests. During feeding, insects ingest plant-derived dsRNA, which activates RNAi pathways and suppresses corresponding genes within the pest organism (Zhang *et al.*, 2017) [35].

HIGS represents one of the most extensively studied RNAi strategies because it provides continuous dsRNA production within plant tissues, eliminating the need for repeated external applications. This approach has shown considerable success in protecting crops against important pests.

One of the most notable examples is RNAi-mediated control of western corn rootworm (*Diabrotica virgifera virgifera*). Transgenic maize expressing dsRNA against the essential gene *DvSnf7* produced significant larval mortality and demonstrated commercial feasibility of RNAi-based crop protection (Bachman *et al.*, 2013) [1]. This technology contributed to the development of genetically engineered maize varieties expressing insect-specific dsRNA.

HIGS has also been investigated against hemipteran pests such as aphids and whiteflies. Expression of dsRNA targeting genes involved in insect development, reproduction, and metabolism has reduced pest populations and impaired insect fitness (Pitino *et al.*, 2011; Zhang *et al.*, 2017) [24, 35].

Despite its effectiveness, HIGS faces several challenges. Public acceptance of genetically modified crops, regulatory restrictions, variable dsRNA movement within plant tissues, and concerns regarding long-term ecological effects influence its adoption. Additionally, some insects exhibit limited uptake of plant-derived dsRNA, reducing the effectiveness of HIGS in certain pest groups.

4.3 Nanotechnology-based dsRNA delivery systems

Nanotechnology has emerged as a powerful approach to overcome the major limitations associated with conventional dsRNA delivery. Nanocarriers protect dsRNA from enzymatic degradation, improve cellular uptake, enhance tissue penetration, and facilitate controlled release of RNA molecules (Zhang *et al.*, 2020) [36].

Various nanomaterials have been investigated for RNAi delivery, including:

- Lipid nanoparticles
- Chitosan-based nanoparticles
- Polymeric nanoparticles
- Carbon-based nanomaterials
- Layered double hydroxide (LDH) nanoparticles
- Clay nanosheets

These materials improve dsRNA stability by protecting RNA molecules from environmental degradation and insect digestive enzymes.

A major advancement in this field is the development of BioClay technology, where dsRNA molecules are stabilized using layered double hydroxide nanosheets. Mitter *et al.* (2017) [20] demonstrated that BioClay-mediated delivery significantly prolonged dsRNA persistence on plant surfaces and enhanced gene silencing efficiency compared with naked dsRNA applications.

Nanocarrier systems are particularly valuable for lepidopteran pests, which frequently exhibit poor RNAi responses due to inefficient dsRNA uptake and degradation. Nanoparticle-assisted delivery has improved RNAi efficiency in species such as *Spodoptera frugiperda*, *Helicoverpa armigera*, and *Plutella xylostella* by increasing dsRNA protection and cellular internalization (Zhang *et al.*, 2020) [36].

Despite their potential, nanoparticle-based RNAi systems require further evaluation regarding production cost, environmental fate, nanoparticle accumulation, and regulatory acceptance before widespread agricultural application.

4.4 Symbiont-mediated RNAi delivery

Symbiont-mediated RNAi represents an innovative biological delivery strategy where engineered microorganisms associated with insects are modified to produce dsRNA molecules. These microorganisms continuously generate RNAi triggers within the insect host,

potentially providing long-term and self-sustaining gene silencing.

The approach exploits natural insect-microbe interactions, particularly gut-associated symbiotic bacteria, to overcome barriers associated with external dsRNA delivery. Engineered bacteria expressing dsRNA have demonstrated the ability to suppress essential insect genes and reduce pest fitness.

A notable example was demonstrated in the kissing bug *Rhodnius prolixus*, where genetically engineered symbiotic bacteria producing dsRNA successfully induced gene silencing after colonization of the insect gut (Whitten *et al.*, 2016) [32]. The study suggested that symbiont-based delivery could potentially allow horizontal transmission of RNAi signals among insect populations.

Advantages of symbiont-mediated RNAi include:

- Continuous dsRNA production
- Reduced application frequency
- Potential population-level effects
- Improved delivery efficiency

However, significant challenges remain, including ecological safety concerns, stability of engineered microorganisms, host specificity, and regulatory approval.

4.5 Virus-Induced Gene Silencing (VIGS)

Virus-induced gene silencing (VIGS) utilizes modified viral vectors to deliver RNA molecules capable of activating RNAi pathways. Although VIGS has been widely used in plant functional genomics, its direct application for insect pest management remains comparatively limited.

In insect systems, recombinant viruses have potential advantages because they naturally infect and replicate within insect hosts, allowing efficient delivery of RNAi triggers. Baculovirus-based vectors have been explored as possible RNAi delivery platforms due to their insect specificity and ability to spread within pest populations (Kolliopoulou and Swevers, 2014) [17].

However, biosafety concerns regarding viral evolution, host range expansion, and environmental persistence have restricted practical agricultural applications.

Table 1: Comparison of Major dsRNA Delivery Strategies for Insect Pest Management

Delivery Strategy	Principle	Advantages	Major Limitations
SIGS	External application of dsRNA sprays	Non-transgenic, flexible application, rapid deployment	UV degradation, low persistence, repeated application required
HIGS	Plants produce insect-targeting dsRNA	Continuous dsRNA production, strong protection	Regulatory concerns, GMO acceptance issues
Nanocarrier Delivery	Nanomaterials protect and transport dsRNA	Improved stability, uptake, and efficiency	Cost, environmental assessment required
Symbiont-Mediated RNAi	Engineered microbes produce dsRNA inside insects	Long-lasting delivery, possible population spread	Ecological and regulatory concerns
VIGS	Viral vectors deliver RNAi triggers	Efficient biological delivery	Biosafety and containment challenges

4.6 Future improvements in dsRNA delivery technology

The future success of RNAi-based pest management will depend heavily on the development of delivery systems that combine high efficiency, environmental stability, affordability, and regulatory acceptance. Emerging approaches include chemically modified dsRNA molecules, biodegradable nanoparticles, targeted delivery systems, and artificial intelligence-assisted formulation optimization.

The integration of nanotechnology with molecular genetics may enable precision delivery of RNA molecules

specifically to pest species while reducing impacts on beneficial organisms. Furthermore, combining RNAi delivery platforms with integrated pest management programs will provide a sustainable approach that minimizes resistance development and enhances long-term agricultural productivity.

5. Recent advances and commercial development of RNAi-based insect pest management

During the past two decades, RNA interference (RNAi) has progressed from a fundamental molecular biology discovery to a promising agricultural biotechnology platform. Advances in genome sequencing, transcriptomics, bioinformatics, nanotechnology, and synthetic biology have significantly improved the identification of RNAi targets, optimization of dsRNA molecules, and development of efficient delivery systems. These developments have accelerated the transition of RNAi from laboratory experiments toward practical field applications and commercialization.

The successful commercialization of RNAi-based insect control products represents a major milestone in sustainable pest management. Unlike conventional insecticides that generally target physiological pathways shared across multiple organisms, RNAi-based technologies provide sequence-specific pest suppression with improved ecological compatibility. The first commercial RNAi crop protection technology was developed against the western corn rootworm (*Diabrotica virgifera virgifera*), where genetically engineered maize expressing dsRNA targeting the essential gene *DvSnf7* provided effective larval control (Bachman *et al.*, 2013) ^[1].

The commercial success of RNAi maize demonstrated the feasibility of utilizing insect-specific gene silencing as an agricultural protection strategy. Subsequently, research efforts have expanded toward developing sprayable RNAi formulations, nanoparticle-based dsRNA products, and RNAi technologies targeting a broader range of insect pests, including hemipterans, lepidopterans, and coleopterans (Christiaens *et al.*, 2020) ^[6].

5.1 Commercial RNAi products and agricultural applications

The development of RNAi-based insecticides has primarily focused on economically important pests causing severe crop losses. One of the most successful examples is RNAi maize expressing dsRNA against the *Snf7* gene of western corn rootworm. Feeding on this maize resulted in reduced larval growth, impaired development, and increased mortality (Bachman *et al.*, 2013) ^[1].

Similarly, RNAi approaches have been investigated against several major agricultural pests

Helicoverpa armigera

The cotton bollworm (*Helicoverpa armigera*) is one of the most destructive polyphagous pests worldwide. RNAi targeting genes associated with development, digestion, and reproduction has demonstrated significant reductions in larval survival and reproductive fitness (Mao *et al.*, 2007) ^[19]. However, field application remains challenging because this species exhibits variable RNAi sensitivity.

Myzus persicae

The green peach aphid (*Myzus persicae*) causes direct feeding damage and transmits numerous plant viruses. RNAi targeting genes involved in cuticle formation and metabolism has reduced aphid growth and reproduction, demonstrating the potential of RNAi-based aphid management (Pitino *et al.*, 2011) ^[24].

Bemisia tabaci

The whitefly (*Bemisia tabaci*) represents another important target due to its ability to transmit economically significant plant viruses. RNAi-mediated suppression of genes involved

in development, immunity, and detoxification pathways has successfully reduced whitefly survival and reproductive performance (Cai *et al.*, 2017) ^[5].

These examples demonstrate the broad applicability of RNAi technology; however, successful commercialization requires overcoming species-specific biological barriers and developing cost-effective delivery systems.

5.2 Integration of RNAi with omics technologies

The identification of effective RNAi targets has been accelerated by advances in functional genomics and multi-omics approaches. Genome sequencing, transcriptomics, proteomics, and metabolomics provide comprehensive information about insect biological pathways and enable systematic identification of essential genes.

RNA sequencing (RNA-seq) has become particularly valuable for discovering genes differentially expressed during insect development, pesticide exposure, or host adaptation. Combining transcriptomic data with RNAi screening allows researchers to identify genes whose suppression produces strong lethal or sublethal effects (Nandety *et al.*, 2024) ^[21].

Furthermore, comparative genomics helps identify insect-specific sequences that minimize risks to beneficial organisms. This approach is critical for developing environmentally safe RNAi products because target sequences must be carefully selected to avoid unintended effects on pollinators, natural enemies, and other non-target organisms.

Artificial intelligence (AI)-based prediction models are also emerging as useful tools for RNAi development. Machine learning algorithms can analyze genomic datasets to predict optimal dsRNA sequences, improve target selection, and estimate potential off-target interactions (Mahanta *et al.*, 2025) ^[18].

6. Major challenges limiting RNAi-based pest management

Although RNAi offers substantial advantages over traditional pesticides, several biological, technological, economic, and regulatory challenges continue to restrict widespread adoption. Addressing these limitations is essential for achieving reliable field performance and commercial success.

6.1 Variable RNAi efficiency among insect species

One of the most significant limitations of RNAi technology is the variation in response among insect species. While some insects exhibit strong and consistent gene silencing responses, others show weak or inconsistent effects despite efficient dsRNA delivery.

Coleopteran insects generally demonstrate high RNAi sensitivity because of efficient dsRNA uptake and systemic spread mechanisms. In contrast, many lepidopteran species exhibit reduced sensitivity due to rapid dsRNA degradation, limited cellular uptake, and inefficient RNA transport pathways (Terenius *et al.*, 2011; Christiaens *et al.*, 2020) ^[6, 29].

Several factors influence RNAi efficiency, including:

- dsRNA length and concentration
- Target gene expression level
- Presence of dsRNA-degrading nucleases
- Midgut physiological conditions
- Cellular uptake mechanisms

- Activity of RNAi pathway proteins

Understanding these species-specific differences remains essential for designing effective RNAi strategies.

6.2 dsRNA stability and environmental degradation

Environmental instability represents a major barrier for field application of RNAi technology. Naked dsRNA molecules are susceptible to degradation by ultraviolet radiation, rainfall, temperature fluctuations, soil microorganisms, and plant-associated nucleases (Dubelman *et al.*, 2014)^[7].

Following ingestion, dsRNA must also survive the insect digestive system. Many insect species possess abundant extracellular nucleases that rapidly degrade dsRNA before it reaches epithelial cells. This limitation is particularly important in lepidopteran pests, where digestive degradation significantly reduces RNAi efficiency.

Current strategies to improve dsRNA stability include:

- Nanoparticle encapsulation
- Polymer-based protection
- Chemical modification of RNA molecules
- Clay-based formulations
- Controlled-release delivery systems

These approaches aim to increase persistence, improve uptake, and reduce application frequency.

6.3 Off-target effects and environmental safety concerns

The high specificity of RNAi is considered its major advantage; however, unintended effects remain an important biosafety concern. Sequence similarity between dsRNA molecules and genes present in non-target organisms may potentially cause unintended gene silencing.

Risk assessment is therefore required for:

- Pollinators such as honeybees (*Apis mellifera*)
- Predatory insects
- Parasitoids
- Soil organisms
- Aquatic organisms

Bioinformatic screening tools are commonly used to identify potential off-target interactions before commercial development. Additionally, laboratory toxicity studies and ecological field assessments are necessary to confirm environmental safety (Hanamasagar *et al.*, 2024)^[13].

Current evidence indicates that properly designed RNAi molecules generally show limited effects on non-target organisms; however, long-term ecological monitoring remains essential.

6.4 Evolution of RNAi resistance

Similar to chemical insecticides, intensive use of RNAi-based pest control may impose selective pressure leading to resistance evolution. Although RNAi resistance mechanisms are still being investigated, several potential pathways have been identified.

Possible resistance mechanisms include:

Reduced dsRNA uptake

Mutations affecting dsRNA transport proteins may reduce cellular internalization and prevent activation of RNAi pathways.

Increased dsRNA degradation

Enhanced production of nucleases capable of degrading dsRNA may reduce the amount of active RNA molecules reaching target cells.

Alterations in RNAi machinery

Changes in Dicer, Argonaute, or other RNAi pathway components may decrease silencing efficiency.

Target gene modification

Mutations in target sequences may prevent effective recognition by siRNAs.

Resistance management strategies should therefore include:

- Rotation of different RNAi targets
- Combination with biological control agents
- Integration with conventional IPM practices
- Use of multiple dsRNA targets simultaneously

Combining RNAi with existing pest management approaches will reduce selection pressure and improve long-term sustainability.

6.5 Regulatory and economic challenges

Despite technological progress, regulatory approval remains a major challenge for RNAi-based agricultural products. Regulatory frameworks differ among countries, creating uncertainty regarding classification, environmental assessment, and commercialization pathways.

Important regulatory considerations include:

- Environmental persistence of dsRNA
- Effects on non-target organisms
- Food safety evaluation
- Gene sequence characterization
- Production quality standards

Economic factors also influence adoption. Although dsRNA production costs have decreased significantly through advances in microbial and enzymatic synthesis, large-scale manufacturing remains more expensive compared with conventional chemical insecticides (Christiaens *et al.*, 2020)^[6]. Development of affordable production platforms and efficient formulation technologies will be essential for widespread farmer adoption.

7. Future perspectives and integration with sustainable pest management

The future of RNAi-based insect management will depend on multidisciplinary innovations combining molecular biology, nanotechnology, computational biology, and ecological pest management.

7.1 Advanced delivery technologies

Future research will focus on improving dsRNA delivery through:

- Biodegradable nanoparticles
- Targeted delivery systems
- Self-amplifying RNA platforms
- Improved spray formulations
- Controlled-release technologies

These innovations will enhance field stability and enable RNAi application against currently resistant insect groups.

7.2 Integration with Integrated Pest Management (IPM)

RNAi should not be considered a replacement for all existing pest management approaches but rather as a complementary tool within integrated pest management programs.

Combining RNAi with:

- Biological control agents
- Semiochemicals
- Resistant crop varieties

- Cultural practices
- Precision agriculture technologies can improve pest suppression while reducing resistance risks.

The combination of RNAi with digital agriculture, remote sensing, and predictive modeling may enable more precise timing and location-specific application of RNAi products.

7.3 Role of artificial intelligence and functional genomics

Future RNAi development will increasingly rely on computational approaches. AI-driven models can accelerate:

- Target gene prediction
- dsRNA sequence optimization
- Off-target risk assessment
- Resistance prediction
- Formulation development

Integration of AI with genome editing and high-throughput screening will significantly improve the efficiency of RNAi discovery pipelines.

8. Conclusion

RNA interference represents one of the most promising molecular technologies for sustainable insect pest management. By exploiting natural gene regulatory mechanisms, RNAi enables highly specific suppression of insect pests while reducing dependence on broad-spectrum chemical insecticides.

Significant advances have been achieved in target gene identification, dsRNA delivery technologies, nanoparticle formulations, and commercialization of RNAi-based products. However, challenges related to species-specific efficiency, environmental stability, resistance evolution, regulatory approval, and economic feasibility must still be addressed.

Future success will depend on integrating RNAi with nanotechnology, functional genomics, artificial intelligence, and integrated pest management strategies. With continued innovation, RNAi has the potential to become a cornerstone technology for environmentally sustainable agriculture and contribute significantly to global food security.

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