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REVIEW

RNA interference for hemipteran pest control: mechanisms, resistance factors, and advances in delivery systems

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Abstract

RNA interference (RNAi) represents a species-specific and environmentally sustainable strategy for the control of hemipteran pests, including aphids, whiteflies, psyllids, and stinkbugs. This review integrates current knowledge on the molecular mechanisms of RNAi in insects and its translational applications in crop protection, with particular focus on host-induced gene silencing (HIGS, transgenic expression of dsRNA in planta) and spray-induced gene silencing (SIGS). The biological effects of gene knockdown – such as reduced survival, impaired development, and disrupted feeding – are discussed alongside physiological, genetic, and epigenetic factors limiting RNAi efficacy in Hemiptera. Key challenges include dsRNA degradation in the digestive tract, inefficient cellular uptake, and limited systemic spread. Recent innovations – including nanocarrier-based delivery, biodegradable formulations, and transplastomic expression – are critically evaluated. Similarly, advances in target gene identification using transcriptomic and proteomic approaches, as well as risk assessment tools for minimizing non-target effects, support the rational design of RNAi-based biopesticides. As RNAi technologies advance, their incorporation into integrated pest management strategies may facilitate species-specific, residue-free control of hemipteran pests while supporting the conservation of agroecosystem biodiversity.

Keywords: biopesticides, dsRNA delivery, Hemiptera, non-target effects, RNA interference

Introduction

Protecting crops from herbivorous insect pests remains a critical challenge in modern agriculture due to significant reductions in yield and quality resulting from direct feeding damage and pathogen transmission. Among the most economically detrimental insect groups are hemipterans (Insecta: Hemiptera), whose piercing-sucking mouthparts facilitate the extraction of plant sap and the introduction of phytopathogenic viruses and bacteria. This feeding behavior leads to tissue necrosis, chlorosis, fruit malformation, and an overall physiological decline in crops (Schaefer and Panizzi 2000). Furthermore, hemipterans such as aphids, whiteflies, planthoppers, and stink bugs exhibit high reproductive potential and adaptability, making chemical control both inefficient and environmentally problematic.

Conventional pest management strategies have predominantly relied on synthetic insecticides with broad-spectrum activity. However, their continued use is constrained by the rapid evolution of resistance in pest populations, growing regulatory restrictions, and mounting concerns over environmental and human health impacts. This context has intensified the search for selective and sustainable alternatives that minimize off-target effects and ecological disruption.

RNA interference (RNAi) has emerged as a highly specific and programmable gene-silencing mechanism with significant potential for insect pest control. The exogenous application or expression of double-stranded RNA (dsRNA) targeting essential genes in pests enables precise knockdown of mRNA transcripts, reducing gene expression and inducing deleterious

physiological effects. The process involves cellular uptake of dsRNA, its processing by Dicer enzymes into small interfering RNAs (siRNAs), and subsequent targeting of complementary mRNAs by the RNA-induced silencing complex (RISC) (Germing *et al.* 2025).

Two primary implementation models of RNAi have been developed for crop protection: (1) host-induced gene silencing (HIGS), wherein transgenic plants express pest-specific dsRNA, and (2) spray-induced gene silencing (SIGS), which involves exogenous application of formulated dsRNA onto plant surfaces or into the root zone (Mezzetti *et al.* 2020; Elbana and Saad-Allah 2024). While HIGS offers continuous in planta dsRNA production, SIGS allows flexible, non-transgenic deployment. However, both approaches face specific limitations in field efficacy, especially when targeting Hemiptera, due to factors such as dsRNA degradation by salivary nucleases, low uptake efficiency, and poor systemic spread (Jain *et al.* 2020a; Hunter and Wintermantel 2021).

Notably, Hemipteran insects are considered relatively recalcitrant to RNAi compared to coleopteran pests, largely due to biological barriers such as gut nuclease activity and limited expression of dsRNA transporters (Christiaens and Smagghe 2014). Consequently, recent advances have focused on optimizing dsRNA design, improving delivery formulations using nanocarriers, and enhancing target specificity using bioinformatic pipelines (e.g., *dsRNA-Engineer*, see Dalakouras *et al.* 2024).

This review synthesizes current progress in RNAi-based control of Hemipteran pests by analyzing molecular mechanisms, delivery strategies, and translational outcomes. The review focuses on studies published between 2000 and 2025 and retrieved from Web of Science, Scopus, PubMed, and Google Scholar using keywords such as RNAi, dsRNA, Hemiptera, HIGS, SIGS, nanocarrier, and biopesticide. In addition to consolidating the existing body of knowledge, this article highlights delivery-specific challenges unique to Hemiptera, discusses documented RNAi responses across taxa, and outlines emerging directions in formulation technology, resistance management, and regulatory integration. Special emphasis is placed on identifying barriers specific to hemipterans and summarizing validated applications targeting aphids, whiteflies, stink bugs, and leafhoppers.

Mechanism of RNA Interference

RNA interference is a conserved, sequence-specific gene silencing mechanism mediated by small RNAs associated with Argonaute-family protein complexes. First described in the nematode *Caenorhabditis elegans* (Maupas, 1900) (Rhabditida: Rhabditidae) (Fire *et al.* 1998), RNAi has since been documented in a wide range of eukaryotes, including insects, where it plays essential roles in antiviral defense, transposon suppression, and post-transcriptional gene regulation (Agrawal *et al.* 2003).

In insects, three main RNAi pathways have been characterized: the small interfering RNA (siRNA) pathway, the microRNA (miRNA) pathway, and the Piwi-interacting RNA (piRNA) pathway. Each pathway is mediated by distinct classes of small RNAs and specific Argonaute (AGO) proteins, which regulate gene expression through mRNA cleavage or translational repression (Zhu and Palli 2020).

siRNA pathway – exogenous response and pest control relevance

The siRNA pathway is directly activated by exogenous double-stranded RNA, making it the most relevant mechanism for pest management. Upon cellular entry, dsRNA is processed by the RNase III enzyme Dicer-2 (Dcr2) into ~21 nucleotide-long siRNAs. These duplexes are subsequently loaded into the RNA-induced silencing complex (RISC) with the assistance of double-stranded RNA-binding proteins (dsRBPs), notably R2D2. Within RISC, the antisense strand of siRNA guides AGO2 to complementary mRNA sequences, leading to target cleavage and suppression of gene expression (Marques *et al.* 2010; Svoboda 2020).

miRNA pathway – regulation of endogenous genes

The miRNA pathway modulates endogenous gene expression, particularly during development and cellular differentiation. It is initiated in the nucleus by the Drosha-Pasha complex, which processes primary miRNA transcripts (pri-miRNAs) into ~70-nucleotide precursors (pre-miRNAs). These hairpin structures are exported to the cytoplasm, where Dicer-1 cleaves them into mature miRNAs. Incorporated into AGO1-containing RISCs, miRNAs typically direct translational repression or transcript degradation, depending on the degree of base pairing with target mRNAs (Zhu and Palli 2020).

piRNA pathway – transposon silencing in germline cells

The piRNA pathway functions predominantly in the germline, where it defends genome integrity by silencing transposable elements. Unlike the other pathways, it is Dicer-independent and relies on the endonuclease Zucchini for the primary processing of long single-stranded precursor RNAs. The resulting piRNAs associate with Piwi subfamily proteins (e.g., Aubergine, AGO3) and engage in the "ping-pong" amplification cycle that reinforces transposon silencing (Mondal *et al.* 2018; Zhu and Palli 2020).

Species-specific efficiency and implications for Hemiptera

Among the three, only the siRNA pathway is responsive to externally applied dsRNA, which is central to RNAi-based pest control. However, the efficiency of this pathway is highly variable across insect taxa. In Hemiptera, the inconsistent RNAi responses are often attributed to differential expression of core components (e.g., Dcr2, AGO2, SID-like transporters), dsRNA degradation by salivary or gut nucleases, and limited systemic spread of the silencing signal (Yang *et al.* 2014; Ylla *et al.* 2016; Pacheco *et al.* 2022). Understanding these mechanistic differences is crucial for designing effective, species-specific RNAi strategies in sap-feeding pests.

Applications of RNAi in crop protection

The implementation of RNA interference in crop protection has introduced a novel paradigm for targeted pest control and genetic regulation in plants. RNAi offers high sequence specificity and functional modularity, enabling the selective silencing of essential genes in phytophagous insects, plant pathogens, or the host plants themselves. This molecular precision facilitates pest management strategies with reduced environmental impact and lower risk of resistance evolution, particularly when compared to broad-spectrum chemical insecticides (Dalakouras *et al.* 2024; Qi *et al.* 2024).

Host-induced gene silencing (HIGS)

Host-induced gene silencing (HIGS) involves the stable integration of a transgene into the plant genome that encodes a double-stranded RNA fragment targeting a specific gene in the pest or pathogen. Upon transcription in planta, dsRNA accumulates in plant tissues and is ingested by herbivorous insects during feeding. This triggers gene silencing in the pest via the siRNA pathway (Mezzetti *et al.* 2020; Vélez *et al.* 2024). Transgene delivery is commonly achieved through *Agrobacterium tumefaciens*-mediated transformation or biolistic particle bombardment. The transgenic plants thus act as autonomous biopesticide producers, allowing sustained pest suppression without the need for repeated treatments (Zhao *et al.* 2024).

HIGS has demonstrated efficacy against several hemipteran species, especially in aphids and whiteflies, which exhibit higher sensitivity to ingested dsRNA. However, successful application is often limited by dsRNA degradation in the insect gut and insufficient systemic spread of the silencing signal, particularly in taxa such as leafhoppers and stink bugs (Li *et al.* 2013; Christiaens and Smagghe 2014; Jain *et al.* 2020a).

Spray-induced gene silencing (SIGS)

Spray-induced gene silencing (SIGS) represents a non-transgenic alternative, relying on the exogenous application of dsRNA to plant surfaces via foliar spraying, soil drenching, or root uptake. This approach offers greater flexibility and regulatory acceptability, especially in regions with strict GMO restrictions (Elbana and Saad-Allah 2024). Nevertheless, SIGS faces major technical challenges associated with environmental instability of naked dsRNA. Degradation can occur due to nucleases, ultraviolet radiation, and microbial activity in the phyllosphere and rhizosphere (Dalakouras *et al.* 2024).

Efficient uptake of exogenous dsRNA by plant tissues – and its subsequent ingestion by hemipteran pests – is also influenced by several factors, including droplet size, leaf surface properties, and the physicochemical formulation of dsRNA. Advances in formulation, such as the use of nanocarriers, liposomes, or chitosan matrices, have been developed to address these constraints and improve delivery efficiency (Christiaens and Smagghe 2014; Hunter and Wintermantel 2021; Dalakouras *et al.* 2024).

Beyond pest control: endogenous gene modulation

Beyond direct pest suppression, RNAi technologies can also be used to manipulate endogenous gene expression in crops. Silencing of negative regulators of growth or stress-responsive genes has been shown to enhance tolerance to drought, salinity, or pathogen attack, thereby contributing to crop resilience and productivity under adverse conditions (Elbana and Saad-Allah 2024).

Strategic relevance for Hemiptera

Both HIGS and SIGS have been applied to various hemipteran pests, although success rates vary across species. Compared to coleopterans, hemipterans generally require higher dsRNA doses due to the presence of salivary nucleases and poor cellular uptake mechanisms (Christiaens and Smagghe 2014). Moreover, the method of dsRNA application to plants can influence its distribution within the vascular system. Since hemipterans primarily feed on phloem sap, the localization of dsRNA in phloem tissue is critical for successful ingestion. Importantly, the distribution of dsRNA within vascular tissues depends strongly on the delivery method. Foliar spraying has been shown to facilitate dsRNA accumulation in the phloem, whereas trunk injection may preferentially direct dsRNA into the xylem (Dalakouras *et al.* 2020). Consequently, the choice of delivery strategy directly influences RNAi efficiency in sap-feeding insects. This highlights the necessity of optimizing application methods to ensure that ingested dsRNA is effectively delivered to the feeding sites of hemipteran pests.

To fully harness the potential of RNAi in crop protection, future research must address species-specific barriers to uptake, improve formulation technologies, and refine sequence design using bioinformatics-guided tools. The successful translation of RNAi from controlled laboratory settings to field-scale applications will depend on multidisciplinary efforts in plant biotechnology, entomology, and regulatory science (Hunter and Wintermantel 2021).

Hemipteran insects as crop pests

Hemipterans (Insecta: Hemiptera) constitute a diverse and ecologically versatile order of insects, encompassing numerous species that significantly impact global agriculture. Their piercing-sucking mouthparts, adapted for extracting phloem or xylem sap, cause extensive physiological damage to host plants. Feeding activity leads to chlorosis, necrosis, stunted growth, deformation of reproductive organs, and premature senescence. In addition to direct tissue damage, many hemipteran species act as vectors of phytopathogenic viruses, bacteria, and phytoplasmas, thereby facilitating the spread of severe crop diseases (Nault 1997; Schaefer and Panizzi 2000).

The suborder Heteroptera includes both phytophagous and predatory taxa. Among phytophagous true bugs, *Nezara viridula* (Linnaeus, 1758) (Hemiptera: Pentatomidae) and *Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae) are economically important pests of legumes, vegetables, and cereals. Their feeding causes seed necrosis, malformation of fruits, and contamination with digestive secretions. Salivary enzymes produced by these species aid in the breakdown of plant cell walls and may interfere with host defense signaling pathways (Zielińska and Lis 2025). In contrast, predatory heteropterans such as *Orius* spp. (Hemiptera: Anthocoridae) and *Podisus maculiventris* (Say, 1832) (Hemiptera: Pentatomidae) serve as beneficial agents in biological control, targeting lepidopteran larvae and dipteran pests (Perdikis *et al.* 2011). Consequently, RNAi-based control measures targeting Hemiptera must account for these divergent ecological roles to avoid unintended effects on beneficial species.

The suborders Sternorrhyncha and Auchenorrhyncha encompass additional highly damaging taxa, including aphids (Aphididae), whiteflies (Aleyrodidae), scale insects (Coccoidea), and leafhoppers (Cicadellidae). These sap-feeding insects attack a broad range of host plants and are notorious for their high reproductive rates, short generation times, and capacity for parthenogenesis. Their role as vectors of plant viruses (such as luteoviruses, geminiviruses) and phytoplasmas – renders them particularly threatening to both annual and perennial crops (Nault 1997; Miles 1999; Blackman and Eastop 2000).

Many of these species excrete large volumes of honeydew, which supports the growth of sooty molds and reduces photosynthetic efficiency. This indirect damage further degrades crop quality and reduces commercial value. In perennial systems such as orchards, scale insects establish persistent infestations on woody tissues, undermining plant vigor and increasing susceptibility to abiotic stress factors (Miller *et al.* 2014).

Owing to their feeding strategy, cryptic behavior, and role in pathogen dissemination, hemipterans pose considerable challenges to traditional pest control approaches. The efficacy of chemical insecticides is often limited by resistance development, the insects' concealed feeding sites, and collateral effects on beneficial organisms. In this context, RNA interference represents a promising alternative, offering species-specific gene silencing with minimal off-target impact. Experimental studies have demonstrated that RNAi can selectively disrupt vital physiological functions in hemipteran pests, thereby reducing fitness, fecundity, and vector competence (Baum *et al.* 2007; Gu and Knipple 2013).

Current knowledge on RNAi in Hemipteran pest control

Over the past decade, RNA interference has gained considerable attention as a promising experimental strategy for the control of hemipteran pests, particularly in the context of phloem- and xylem-feeding insects. Numerous laboratory studies have demonstrated that silencing genes involved in essential physiological processes – such as digestion, neural signaling, osmoregulation, and detoxification – can lead to impaired survival, reproduction, and feeding efficiency. For instance, knockdown of genes encoding aminopeptidases, aquaporins, or salivary effectors has been shown to impair feeding, reduce fecundity, delay development, and increase mortality in various hemipteran species (Galdeano *et al.* 2018; Germing *et al.* 2025).

Despite these advances, RNAi efficiency in Hemiptera remains highly variable and is influenced by a complex interplay of intrinsic biological factors and environmental conditions. Key determinants include midgut pH, the activity of extracellular and intracellular nucleases that degrade dsRNA, and interspecific differences in the expression of core RNAi components such as Dicer-2, Argonaute-2, and SID-like (Systemic RNA interference-defective) transporters. While such variability can be observed at the species level, it also reflects deeper physiological and molecular differences among hemipteran families and suborders (Christiaens and Smagghe 2014; Ghosh *et al.* 2018; Jain *et al.* 2020a).

Aphids (Aphididae) and whiteflies (Aleyrodidae), both belonging to the suborder Sternorrhyncha, typically exhibit higher responsiveness to orally delivered dsRNA. These insects are characterized by a relatively

neutral gut pH and lower nuclease activity in their digestive fluids, which favor the persistence and uptake of exogenous RNA molecules (Christiaens and Smagghe 2014; Ghosh *et al.* 2018). In contrast, hemipterans from the suborders Auchenorrhyncha and Heteroptera – such as leafhoppers (Cicadellidae), planthoppers (Delphacidae), and stink bugs (Pentatomidae) – tend to display significantly lower RNAi sensitivity. Their digestive tract exhibits a strongly alkaline environment and high levels of dsRNase activity, which together contribute to the rapid degradation of ingested dsRNA before it can initiate effective gene silencing (Galdeano *et al.* 2018; Sharma *et al.* 2021). These differences underscore the importance of taxon-specific optimization of RNAi delivery and design strategies when targeting diverse hemipteran pests.

Moreover, transcriptomic analyses have revealed interfamily differences in the expression of core RNAi machinery genes such as *Dicer-2*, *Argonaute-2*, and *R2D2*, which may affect the intracellular processing of dsRNA (Jain *et al.* 2020a). SID-like dsRNA transporters, which contribute to systemic RNAi in some insects, are often poorly expressed or entirely absent in hemipterans, especially in Auchenorrhyncha and Heteroptera representatives (Ghosh *et al.* 2018). These molecular and physiological disparities underscore the need for family-specific delivery strategies and bioinformatic target optimization when designing RNAi--based pest control for hemipteran taxa.

Among the delivery strategies explored to date, host-induced gene silencing via transgenic expression of pest-specific dsRNA has shown potential in reducing hemipteran fitness. In this system, dsRNA is constitutively expressed in plant tissues and ingested during feeding. However, the efficacy of HIGS is often constrained by the hostile conditions of the hemipteran digestive tract, where alkaline pH and potent nucleolytic enzymes rapidly degrade dsRNA before it can trigger the RNAi machinery (Li *et al.* 2013; Jain *et al.* 2020b).

Alternative methods, such as direct injection of dsRNA into the hemolymph, have been used to circumvent gut-associated barriers and to validate gene function under controlled conditions. Although this approach demonstrates the physiological capacity of certain hemipterans to mount an RNAi response, it is unsuitable for field application due to its technical complexity and impracticality at scale (Li *et al.* 2013; Christiaens and Smagghe 2014; Jain *et al.* 2020b).

To address these challenges, considerable efforts have been directed toward enhancing dsRNA stability, uptake, and cellular delivery. Chemical modifications – such as 2′-fluoro or 3′-methyl substitutions – can improve dsRNA resistance to enzymatic degradation. Similarly, protective nanocarriers, including chitosan-based particles, lipid vesicles, carbon nanotubes, and layered double hydroxide (LDH) nanoclays, have been developed to encapsulate and stabilize dsRNA molecules. For example, LDH-dsRNA hybrids have shown efficacy against *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae), while star polycation-based carriers have improved silencing efficiency in *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) (Jain *et al.* 2022; Ma *et al.* 2023). These technologies improve dsRNA persistence on plant surfaces, facilitate internalization by plant tissues and hemipteran gut cells, and enable more robust silencing responses.

Another innovative delivery strategy is chloroplast-based (transplastomic) expression of dsRNA. Due to the absence of RNAi machinery in plastids, intact long dsRNA molecules can accumulate in high quantities and be ingested by herbivorous insects. Moreover, maternal inheritance of plastids limits transgene dissemination via pollen. This approach has demonstrated success in aphids and whiteflies but requires further optimization for broader hemipteran applicability (Dong *et al.* 2022; Kaplanoglu *et al.* 2022).

A critical factor determining RNAi success lies in dsRNA design. Recent bioinformatic tools, such as *dsRNA-Engineer*, enable precise *in silico* prediction of effective and specific target sites, incorporating considerations for thermodynamic stability, transcript accessibility, and off-target risk (Chen *et al.* 2025). Nonetheless, even seemingly innocuous sequences – such as dsRNA targeting GFP – have elicited RNAi effects in non-target organisms like bees (Nunes *et al.* 2013). Therefore, computational prediction must be validated through *in vivo* bioassays to confirm efficacy and safety. This is especially pertinent in RNAi-based pest control, where non-target exposure is possible via phyllosphere contact or trophic transfer.

Although resistance to RNAi has not yet been confirmed in hemipterans, it has been documented in coleopterans such as *Diabrotica virgifera virgifera* LeConte, 1868 (Coleoptera: Chrysomelidae) and *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae) (Khajuria *et al.* 2018; Mishra *et al.* 2021). Resistance mechanisms may involve single-locus mutations affecting dsRNA uptake, processing, or systemic transport. Hemipteran populations may similarly evolve resistance under selective pressure, necessitating the development of fusion dsRNA constructs targeting multiple essential genes to reduce the risk of adaptive evasion (Yang *et al*. 2023).

Altogether, the current state of knowledge highlights both the promise and the complexity of RNAi in hemipteran pest control. While proof-of-concept studies support the feasibility of RNAi-mediated suppression in species such as aphids, whiteflies, and planthoppers, substantial work remains to optimize delivery strategies, improve sequence specificity, and validate field-level efficacy. As illustrated in Table 1 below, selected RNAi applications targeting hemipteran

Table 1. Selected examples of RNAi applications against hemipteran pests. The table summarizes target species, host plants, dsRNA delivery methods, silenced genes, observed biological effects, and corresponding references. Data represent experimentally validated cases published in peer-reviewed literature between 2006 and 2024

Target species	Host plant	Delivery method †	Target gene	Observed effects	Reference
Acyrthosiphon pisum (Harris, 1776) (Hemiptera: Aphididae)	artificial diet (no plant)	artificial diet feeding (lab method)	*C002*	knockdown of *C002* transcript; salivary dysfunction and mortality	Mutti *et al.* (2006)
Aphis glycines (Matsumura, 1917) (Hemiptera: Aphididae)	*Glycine max*	foliar spray (SIGS; dsRNA + dendrimer + surfactant)	*ATPD* + *CHS1*	78.5% mortality; efficient gene silencing	Yan *et al.* (2020)
Diaphorina citri (Kuwayama, 1908) (Hemiptera: Psyllidae)	*Murraya odorifera*	in-plant delivery *via* hydroponic uptake of dsRNA by *Murraya odorifera* shoots	Vg4, VgR	transcript knockdown; reduced egg production and impaired ovary development	Li *et al.* (2023)
Euscelidius variegatus (Kirschbaum, 1858) (Hemiptera: Cicadellidae)	no plant (laboratory-reared adults)	microinjection (abdominal)	*ATP synthase β, muscle actin*	transcript knockdown and increased adult mortality	Abbà *et al.* (2019)
	artificial diet (no plant)	oral feeding (dsRNA + liposome carrier)	*ATP synthase β*	transcript knockdown; significant increase in mortality after 9 days	Rossi *et al.* (2024)
Euschistus heros (Fabricius, 1798) (Hemiptera: Pentatomidae)	artificial diet (no plant)	oral feeding (dsRNA + liposomes + EDTA)	*vATPase-A*, *αCop*, *snf7*	transcript knockdown; mortality up to 45% after 7 days post-ingestion	Castellanos *et al.* (2018)
Halyomorpha halys (Stål, 1855) (Hemiptera: Pentatomidae)	no plant (laboratory-reared adults)	topical thoracic application (dsRNA + surfactant)	*IAP-1-like*, *ATPase*, *ATPase N2B*	transcript knockdown and mortality (up to 45%)	Finetti *et al.* (2023)
Myzus persicae (Sulzer, 1776) (Hemiptera: Aphididae)	*Brassica napus* (leaf discs)	foliar spray (SIGS; SPc nanoparticle + bacteria-expressed hpRNA)	*V-ATPase* subunits D & G	transcript knockdown; growth inhibition; mortality up to 61% at day 3, 50% at day 6	Ma *et al.* (2023)
Nezara viridula (Linnaeus, 1758) (Hemiptera: Pentatomidae)	no plant (laboratory-reared adults)	oral feeding (artificial diet in Parafilm sachets)	*V-ATPase A, αCop*	transcript knockdown; mortality up to 45% after 14 days	Sharma *et al.* (2021)
	no plant (laboratory-reared adults)	topical thoracic application (dsRNA + surfactant)	*IAP-1-like*, *ATPase*, *ATPase N2B*	transcript knockdown and dose-dependent mortality (up to 38%)	Finetti *et al.* (2023)
Nilaparvata lugens (Stål, 1854) (Hemiptera: Delphacidae)	*Oryza sativa* (rice)	HIGS (transgenic plant expressing dsRNA)	*NlCDK1*	transcript knockdown; reduced ovary development, fecundity, and survival	Hao *et al.* (2018)
Scaphoideus titanus (Ball, 1932) (Hemiptera: Cicadellidae)	no plant (laboratory-reared adults)	microinjection (abdominal dsRNA)	*ATP synthase β*	female sterility due to impaired egg development; transcript knockdown	Ripamonti *et al.* (2022)
	artificial diet (no plant)	oral feeding (dsRNA + liposome carrier)	*ATP synthase β*	transcript knockdown; no significant mortality increase after 9 days	Rossi *et al.* (2024)
Sitobion avenae (Fabricius, 1775) (Hemiptera: Aphididae)	*Hordeum vulgare* (barley)	SIGS (semi-systemic foliar spray)	*Shp*	~60% transcript knockdown of *Shp* in aphids feeding on untreated leaf parts	Biedenkopf *et al.* (2020)

† Delivery method classification:
– HIGS: Host-induced gene silencing via transgenic plants
– SIGS: Spray-induced gene silencing using topical or nanoparticle formulations
– Artificial diet and microinjection/injection are considered experimental laboratory delivery systems

species provide a valuable reference for experimental design and delivery strategy optimization.

Challenges and limitations of RNAi in Hemiptera

Despite its high molecular specificity and potential for integration into sustainable pest management programs, the practical deployment of RNA interference against hemipteran pests is constrained by several biological, environmental, and technical limitations. These factors contribute to the highly variable efficacy observed across different studies and insect taxa.

Barriers to dsRNA uptake and stability

A central limitation in the implementation of RNA interference for hemipteran pest control is the inefficient uptake and limited persistence of exogenously delivered dsRNA. The hemipteran cuticle functions as a robust barrier, poorly permeable to topically applied molecules, rendering dermal delivery approaches largely ineffective in most species. Consequently, oral delivery via feeding is the most commonly employed method; however, this route introduces additional biological challenges related to the hostile conditions of the digestive tract (Christiaens and Smagghe 2014).

The gut environment of many hemipterans, particularly phloem-feeding species such as some aphids and whiteflies, is characterized by strongly alkaline pH and elevated nuclease activity, which together promote the rapid degradation of ingested dsRNA (Li *et al.* 2013; Jain *et al.* 2020a). Experimental studies have shown that midgut extracts from *Nilaparvata lugens* (Stål, 1854) (Hemiptera: Delphacidae) degrade dsRNA within minutes, whereas similar extracts from *Acyrthosiphon pisum* Harris, 1776 (Hemiptera: Aphididae) exhibit significantly lower degradation rates, suggesting substantial interspecies variation in nuclease profiles (Christiaens *et al.* 2014; Sharma *et al.* 2020). Furthermore, the composition and expression levels of dsRNases can vary not only between species but also across developmental stages and environmental conditions, further complicating RNAi efficiency predictions (Galdeano *et al.* 2018).

Beyond extracellular stability, the intracellular uptake of dsRNA is another bottleneck. Hemipteran cells primarily rely on clathrin-mediated endocytosis for dsRNA internalization, but the efficiency of this process appears limited and is further hampered by the weak or absent expression of SID-like transmembrane proteins – a class of transporters critical for systemic RNAi in nematodes such as *Caenorhabditis elegans*. Although alternative mechanisms such as clathrin-independent pathways or scavenger receptor-mediated uptake have been proposed, their functional relevance in Hemiptera remains poorly characterized and likely insufficient to compensate for the lack of SID-like transporters (Christiaens and Smagghe 2014; Ghosh *et al.* 2018).

Additionally, even when dsRNA is successfully internalized by some cells, the systemic spread of the silencing signal to distant tissues is often inefficient or entirely absent. This results in localized gene knockdown effects and undermines the utility of RNAi for targeting genes expressed in internal or dispersed organs. The limited systemic response in hemipterans stands in contrast to the more efficient systemic RNAi observed in coleopteran insects such as *Diabrotica virgifera virgifera*, which exhibit strong RNAi responses following oral dsRNA delivery (Rangasamy and Siegfried 2012; Christiaens *et al.* 2020).

Taken together, the interplay between cuticular impermeability, gut-associated degradation, low transporter expression, and poor systemic dissemination presents a formidable barrier to the practical application of RNAi in Hemiptera. Overcoming these limitations requires a nuanced understanding of species-specific physiological and molecular traits, as well as the continued development of optimized delivery systems capable of protecting dsRNA and enhancing its uptake and intracellular routing.

Local versus systemic RNAi response in Hemipteran insects

The efficacy of RNAi in insects depends not only on the ability of individual cells to internalize and process dsRNA, but also on the capacity of the silencing signal to spread beyond the initial site of exposure. In this context, it is critical to distinguish between local RNAi effects, which are confined to cells directly exposed to dsRNA (e.g., the midgut epithelium following oral ingestion), and systemic RNAi responses, where siRNAs or silencing signals disseminate through the hemolymph and affect distal tissues.

Local RNAi is well documented in hemipteran insects and is typically observed in digestive tissues. Numerous studies have shown that ingestion of dsRNA leads to transcript knockdown and phenotypic effects in midgut cells of *Nilaparvata lugens* (Zha *et al.* 2011), *Acyrthosiphon pisum* (Mutti *et al.* 2006), and *Nezara viridula* (Sharma *et al.* 2021). However, in many hemipteran species, the silencing effect remains restricted to the site of dsRNA exposure, indicating limited or inefficient systemic propagation of the RNAi signal.

By contrast, systemic RNAi requires the intercellular transport of dsRNA or its processed siRNAs from the site of uptake to other tissues. In the nematode *Caenorhabditis elegans*, this process is mediated by SID-1 family transmembrane proteins that facilitate passive dsRNA uptake (Feinberg and Hunter 2003). While insects lack direct orthologs of SID-1, some possess SID-like (Sil) genes, which may support dsRNA transport

and systemic effects. However, these SID-like genes are not universally conserved, and their functionality remains unclear across most insect taxa (Christiaens and Smagghe 2014; Ghosh *et al.* 2018). In hemipterans such as *Myzus persicae* and *Bemisia tabaci*, systemic RNAi is typically weak or inconsistent, possibly due to low expression of dsRNA uptake and transport components (Younis *et al.* 2014).

Coleopteran insects, such as *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae), exhibit particularly strong systemic RNAi responses, as demonstrated by durable knockdown effects following dsRNA administration (Posnien *et al.* 2009). Although the molecular mechanisms underlying this phenomenon have not been fully elucidated, it is hypothesized that coleopterans possess more efficient uptake and intracellular transport systems than hemipterans, potentially involving clathrin-mediated endocytosis or other receptor-mediated pathways (Christiaens and Smagghe 2014).

In hemipterans, dsRNA internalization primarily relies on endocytic mechanisms, with clathrin-mediated endocytosis playing a central role (Saleh *et al.* 2006; Ghosh *et al.* 2018). However, this mode of uptake appears insufficient for enabling systemic spread, often limiting RNAi effects to the digestive tract. Consequently, genes expressed in tissues inaccessible to ingested dsRNA – such as salivary glands or reproductive organs – may remain unaffected. This restricted distribution of the RNAi effect represents a substantial bottleneck for practical pest control applications.

To address these limitations, researchers have developed delivery platforms – such as lipid-based nanoparticles, chitosan matrices, and layered nanoclays – that can facilitate hemolymph entry and promote systemic distribution (Jain *et al.* 2022; Ma *et al.* 2023). These systems may enhance the bioavailability of dsRNA in hemipteran insects and enable more consistent silencing across multiple tissues.

Overall, the available evidence suggests that local RNAi responses are consistently inducible in midgut tissues. As a result, systemic RNAi remains elusive, species-specific, and technically challenging. Overcoming this limitation is essential for achieving robust and long-lasting suppression of gene targets expressed in non-intestinal tissues, and for enabling the full potential of RNAi-based pest management in Hemiptera.

Genetic and potential epigenetic resistance mechanisms

The variability of RNAi efficacy in hemipteran insects is influenced not only by physiological barriers and environmental conditions but also by intrinsic genetic traits that modulate responsiveness to dsRNA exposure. One documented mechanism involves the differential expression or mutation of core RNAi components such as Dicer and Argonaute proteins. For example, significant interspecies and intraspecies differences in the expression of Ago2, Dcr2, and R2D2 have been observed in hemipterans, which may influence their baseline capacity for initiating a robust RNAi response (Younis *et al.* 2014; Sharma *et al.* 2021). In *Nezara viridula*, oral delivery of dsRNA targeting essential genes such as *V-ATPase-A* and *αCop* demonstrated partial silencing and moderate mortality, highlighting that limited systemic spread or gene-specific resistance may constrain efficiency even in target tissues (Sharma *et al.* 2021).

Another factor contributing to resistance is the degradation of dsRNA by endogenous nucleases. Insects from various hemipteran families, such as Aphididae and Pentatomidae, have been shown to possess gut-associated and hemolymph nucleases that rapidly degrade exogenous dsRNA before it can be internalized by cells or processed into small interfering RNAs (Christiaens *et al.* 2014; Jain *et al.* 2020a). For instance, *Nezara viridula* displays high levels of dsRNase activity in the midgut, and gene silencing efficiency was markedly improved when dsRNA was administered via injection instead of feeding, further implicating nuclease-mediated degradation as a major limitation (Sharma *et al.* 2021). A promising strategy to mitigate this barrier involves the co-delivery of dsRNAs targeting endogenous RNases, which was shown to reduce nuclease activity and enhance silencing efficiency when applied together with a vital target gene in insects (Volpe *et al.* 2024).

Beyond the degradation and delivery challenges, recent transcriptomic studies suggest that hemipteran species may mount transcriptional responses that downregulate or functionally suppress RNAi-related pathways upon exposure to dsRNA. In *Myzus persicae*, for example, dietary dsRNA led to a rapid transcriptional shift involving immune response genes and potential regulators of gene silencing pathways (Christiaens *et al.* 2020). While these changes do not directly confirm adaptive resistance mechanisms, they raise the possibility that insects might develop inducible regulatory strategies that diminish the impact of repeated dsRNA exposure.

The potential involvement of epigenetic factors in modulating RNAi efficacy remains speculative, as direct experimental evidence in hemipterans is currently lacking. However, given that RNAi pathway gene expression is tightly regulated and can respond dynamically to environmental stimuli, it is plausible that epigenetic mechanisms – such as histone modifications or DNA methylation – could contribute to observed variability in gene silencing outcomes. While RNA-directed DNA methylation (RdDM) is a well-established mechanism in plants, there is currently no evidence for an equivalent pathway in insects.

Nevertheless, insect genomes do exhibit DNA methylation and chromatin remodeling processes, which may indirectly influence RNAi efficiency by modulating gene accessibility or stability of the silencing machinery (Glastad *et al.* 2019; Lewis *et al.* 2020). This hypothesis warrants future investigation, particularly in populations subjected to repeated or prolonged exposure to RNA-based pest control agents.

Collectively, these findings underscore the need for integrated strategies that can counteract or bypass resistance mechanisms. The use of multi-target dsRNA constructs, suppression of nucleases through co-delivery approaches, and the refinement of delivery technologies may all contribute to achieving more consistent and durable RNAi effects in hemipteran pest species.

Sequence specificity and non-target effects

RNA interference is widely regarded as a highly selective strategy for pest control; however, the potential for unintended gene silencing in non-target organisms requires careful consideration. This risk arises when exogenous dsRNA sequences display sufficient homology to endogenous genes of beneficial species, particularly entomophagous or pollinator species occupying the same agroecosystems as the target pests (EFSA GMO Panel, 2020). Homologous regions of 21 nucleotides or more are generally considered sufficient to induce off-target silencing, depending on the susceptibility of the organism and the efficiency of its RNAi machinery. However, experimental evidence from *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) cell-based assays demonstrated that even shorter regions – as small as 19 nucleotides – can cause unintended knockdown of transcripts, thereby contributing to false positive results (Kulkarni *et al.* 2006). These findings emphasize the need for more stringent bioinformatic criteria during dsRNA design to proactively identify potential off-targets and minimize unintended silencing effects.

RNAi safety assessments must carefully distinguish between sequence-dependent and sequence-independent effects. Sequence-dependent effects arise when dsRNAs share sufficient homology with non-target genes, leading to unintended silencing. In contrast, sequence-independent effects are unrelated to base-pair complementarity and instead result from the intrinsic recognition of long dsRNA molecules by cellular defense systems, including immune stimulation and stress responses (Niu *et al.* 2024). Both categories of effects require consideration during dsRNA design and biosafety evaluation, since they may influence both the efficacy and safety of RNAi-based pest control strategies.

The likelihood of such effects is influenced by several factors, including the degree of sequence similarity, the presence and expression levels of dsRNA uptake channels, and the stability of dsRNA in the gut. In some beneficial insects, such as pollinators or natural predators, reduced sensitivity may result from high nuclease activity or limited internalization of dsRNA molecules. For instance, studies indicate that honey bees exhibit low systemic response to dietary dsRNA, partly due to degradation in the digestive tract and weak systemic uptake (Tan *et al.* 2016).

To minimize ecological risks, it is essential to apply sequence alignment and silencing prediction tools during the design phase of RNAi constructs. Region-specific dsRNA targeting ensures high specificity and reduces the likelihood of cross-reactivity with genes in non-target species. Demonstrations of this approach in pest-predator systems, such as *Nilaparvata lugens* and its predator *Cyrtorhinus lividipennis* Reuter, 1885 (Hemiptera: Miridae), support the feasibility of safe implementation when appropriate design parameters are followed (Dang *et al.* 2022).

Effective risk assessment protocols and homology screening against genomes and transcriptomes of non-target species remain essential steps in the regulatory evaluation of RNAi-based pest management tools. The integration of bioinformatic predictions with laboratory bioassays will further support the ecological safety of future applications.

Environmental instability of dsRNA

Under field conditions, unprotected dsRNA is highly susceptible to degradation by a combination of abiotic and biotic factors. Ultraviolet radiation, high temperatures, desiccation, and microbial nuclease activity in soil or on leaf surfaces all contribute to the rapid breakdown of RNA molecules. Quantitative degradation studies confirm this instability. In agricultural soils, DT50 (time to 50% dissipation) values were consistently below 30 h and DT90 (time to 90% dissipation) values below 35 h (Dubelman *et al.* 2014; Bachman *et al.* 2020). In aquatic microcosms, dsRNA typically degraded within three days (Fischer *et al.* 2017). Field trials with foliarly applied dsRNA on soybean plants showed even faster dissipation, with DT50 of 0.5-0.7 days and DT90 of 1.9-2.3 days (Bachman *et al.* 2020). Such rapid degradation underscores the importance of formulation strategies to extend dsRNA persistence under field conditions.

To address the environmental instability of exogenously applied dsRNA, several stabilization strategies have been developed. One widely adopted approach involves chemical modifications of nucleotide residues, such as the incorporation of 2′-fluoro or 3′-methyl groups, which enhance the resistance of dsRNA to nuclease-mediated degradation. These chemical modifications can significantly prolong the half-life of dsRNA under field conditions, without impairing its

silencing activity in the target organism (Christiaens and Smagghe 2014; Hunter and Wintermantel 2021).

Another promising strategy is the encapsulation of dsRNA within protective nanocarriers. Liposomes, dendrimers, silica nanoparticles, and chitosan-based matrices have been successfully used to shield dsRNA molecules from UV radiation, enzymatic hydrolysis, and microbial degradation. Such formulations enhance dsRNA stability and facilitate its transport across plant and insect cellular barriers through enhanced adhesion, penetration, and endosomal release (Christiaens and Smagghe 2014; Hunter and Wintermantel 2021).

Moreover, controlled-release formulations based on biodegradable polymers – such as alginate or poly(lactic-co-glycolic acid) – have been explored to provide sustained and spatially targeted dsRNA delivery. These systems may release dsRNA gradually over time, ensuring prolonged bioactivity and reducing the need for repeated field applications. When integrated with advanced foliar or root application technologies, these strategies collectively represent a critical step toward the practical deployment of RNAi as a next-generation pest management tool in agriculture (Christiaens and Smagghe 2014; Hunter and Wintermantel 2021).

Cost and production constraints

The scalability of RNAi-based pest control depends not only on biological efficacy but also on the development of economically viable production and formulation methods. A major limitation is the high cost of chemically synthesized dsRNA, with laboratory-scale production typically ranging from approximately 100 to over 1000 USD per milligram, depending on sequence length, chemical modifications, and purification standards. Such prices are reported by commercial providers including AgroRNA (Korea) and Synbio Technologies (USA/China), and are consistent with independent market analyses of dsRNA synthesis for agricultural use (Zotti *et al.* 2018; Parker *et al.* 2019). These costs currently render broad-acre agricultural applications financially prohibitive, highlighting the need for innovations in large-scale microbial or cell-free dsRNA production systems.

To overcome these limitations, microbial expression systems have been explored as low-cost alternatives. Engineered strains of *Escherichia coli*, such as HT115(DE3), have been used to produce high quantities of dsRNA by expressing complementary sequences under dual T7 promoters. Crude bacterial lysates containing dsRNA can then be applied directly to plant surfaces or used in feeding assays, simplifying formulation, and reducing purification costs. This approach has demonstrated promising results in multiple hemipteran species, although large-scale field validation remains limited (Zha *et al.* 2011; Sharma *et al.* 2021).

Another route to cost-effective delivery involves transgenic host plants engineered to express pest-specific dsRNA (HIGS). While effective under experimental and greenhouse conditions, the adoption of HIGS-based technologies faces considerable regulatory hurdles and public resistance, especially in regions with strict GMO policies, such as the European Union. Furthermore, environmental risk assessments of such plants require demonstration of minimal off-target effects and horizontal gene transfer, which adds regulatory complexity and financial burden to product development.

Insect-specific viruses such as Flock House virus (FHV) are being investigated as novel RNAi delivery platforms capable of bypassing physiological barriers such as gut nucleases and limited cellular uptake. A proof-of-concept study demonstrated that recombinant FHV – engineered to produce dsRNA targeting *Drosophila melanogaster* genes – achieved substantial gene knockdown and increased mortality in both cell cultures and live flies (Taning *et al.* 2018). Although its application has so far been limited to *Drosophila*, this virus-induced gene silencing (VIGS) approach has potential as a highly species-specific delivery method for other pest insects, including hemipterans that are recalcitrant to conventional RNAi.

Additionally, efforts are underway to integrate dsRNA production with delivery platforms, such as biodegradable nanocarriers or sprayable formulations. These systems not only stabilize dsRNA under field conditions but also allow for precise targeting, dose control, and potentially repeated application throughout the growing season. This convergence of biotechnology and nanomaterials may facilitate the development of modular RNAi systems tailored to crop protection, with reduced costs and improved operational flexibility.

Ultimately, the transition from laboratory research to field-level implementation will require continued optimization of dsRNA production, regulatory alignment, and user-friendly application systems. Sustainable deployment of RNAi technology hinges on its ability to compete with conventional chemical pesticides not only in efficacy but also in economic accessibility.

Despite the multifaceted limitations outlined above, RNA interference remains a promising platform for species-specific pest control in Hemiptera. Continued refinement of delivery methods, improved understanding of interspecies variability, and the development of resistant-evading strategies will be essential to overcome current bottlenecks. Moreover, interdisciplinary innovations at the interface of entomology, molecular biology, nanotechnology, and systems ecology may help unlock the full potential of RNAi. The following section explores emerging areas of research that could address these challenges and enable

the broader deployment of RNAi-based tools in sustainable crop protection programs.

Future areas of research

Realizing the full potential of RNAi for controlling hemipteran pests will depend on continued interdisciplinary progress in molecular biology, bioinformatics, nanotechnology, and crop protection systems. One of the most pressing challenges remains the rational selection of effective genetic targets. Although early RNAi applications largely focused on silencing genes involved in essential physiological processes such as digestion, development, or detoxification, recent multi-omics approaches have substantially expanded the target space (Jain *et al.* 2020a; Yang *et al.* 2023). Novel targets now include salivary effectors involved in plant-insect interactions, components of the neuroendocrine axis regulating molting and feeding, and conserved elements of signal transduction pathways. Integrating transcriptomic, proteomic, and metabolomic data enables the identification of gene networks rather than single loci, facilitating the design of multi-target constructs that may enhance phenotypic impact while mitigating the emergence of resistance (Kaplanoglu *et al*. 2022).

Another central research priority involves the development of advanced dsRNA delivery platforms that protect RNA molecules from degradation and facilitate their transport across physiological barriers. The susceptibility of naked dsRNA to nucleolytic degradation in the insect gut, combined with its rapid breakdown in the external environment, severely limits the effectiveness of conventional delivery methods (Christiaens and Smagghe 2014; Hunter and Wintermantel 2021). To address this, nanoparticle-mediated delivery systems are being actively explored. Chitosan- and lipid-based nanocarriers have demonstrated improved protection of dsRNA molecules and enhanced their uptake by insect midgut cells (Li *et al.* 2013; Jain *et al.* 2020a). These systems can be functionalized with ligands that facilitate tissue-specific binding or endosomal escape. Furthermore, the use of stimuli-responsive materials – capable of releasing their cargo in response to pH, enzymatic activity, or redox potential – represents a promising strategy for spatially and temporally controlled gene silencing (Zotti *et al.* 2018).

Plastid-based (transplastomic) expression of dsRNA provides abundant and stable in planta production, ensuring continuous exposure for feeding insects. Separately, chemical modifications of dsRNA – such as 2′-fluoro or methylated nucleotides, pseudouridine, or phosphorothioate linkages – have been shown to enhance molecular stability and resistance to degradation without impairing silencing efficiency (Hunter and Wintermantel 2021; Germing *et al.* 2025). Both strategies, although mechanistically distinct, converge on the common goal of improving dsRNA stability and efficacy in crop protection.

At the same time, formulation technologies are being adapted to agricultural needs. Dry powders, encapsulated granules, hydrogels, and biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA) are being developed as delivery systems compatible with foliar sprays or soil applications (Mezzetti *et al.* 2020; Elbana and Saad-Allah 2024). These materials offer improved protection of dsRNA under field conditions and may enable controlled release over time. The integration of RNAi formulations into existing integrated pest management (IPM) frameworks is particularly promising, as RNAi can be combined with beneficial biocontrol agents (e.g., *Beauveria bassiana*, *Bacillus thuringiensis*) without disrupting ecological balances (Zotti *et al.* 2018; Vélez *et al.* 2024). Moreover, the combination of RNAi with CRISPR-Cas13 systems presents an opportunity to unify transient knockdown and transcript disruption, providing complementary approaches to pest suppression (Pacheco *et al.* 2022).

Finally, research must increasingly focus on the ecological safety and regulatory governance of RNAi--based technologies. Although the sequence specificity of RNAi reduces off-target risks compared to conventional pesticides, there remains a need for rigorous homology-based screening and empirical assessment of non-target impacts (EFSA GMO Panel, 2020). This includes *in vitro* cytotoxicity tests, acute and chronic feeding assays with beneficial insects, and long-term monitoring of species diversity in treated ecosystems. Importantly, current research highlights the low susceptibility of most pollinators and natural enemies to dietary dsRNA due to gut-mediated degradation and limited systemic uptake. However, interspecies differences must be evaluated case by case (Pan *et al.* 2016; Tan *et al.* 2016). Clear stakeholder engagement and public dialogue, the development of standardized regulatory protocols, and compliance with environmental risk assessment guidelines are essential for gaining public trust and enabling the responsible adoption of RNAi-based crop protection (Mezzetti *et al.* 2020; Dalakouras *et al.* 2024).

Concurrently, regulatory attention has increasingly turned to spray-induced gene silencing (SIGS), particularly formulations based on exogenous application of dsRNA. While host-induced gene silencing (HIGS) products fall under genetically modified organism (GMO) legislation in the European Union, the regulatory status of SIGS remains under discussion. According to recent EFSA guidance, risk assessment of RNAi-based genetically modified plants must consider the molecular characteristics of the dsRNA construct, potential off-target effects, and the likelihood

of unintended gene silencing in non-target organisms (EFSA, 2025). Although these recommendations do not directly apply to non-GMO applications such as SIGS, they provide a conceptual basis for evaluating the safety and environmental fate of exogenously applied RNA molecules. Recent reviews have emphasized the importance of standardized exposure testing, homology-based screening, and persistence studies to support future authorization of RNA-based biopesticides in the EU (Dalakouras *et al.* 2024).

In summary, the successful translation of RNAi from experimental systems to field-scale applications will require synergistic progress across multiple domains: target validation, formulation engineering, delivery optimization, ecological safety, and regulatory alignment. As these efforts converge, RNAi holds significant promise as a next-generation, species-selective tool in sustainable agricultural pest management.

Conclusions

RNA interference represents a sophisticated, sequence--specific gene-silencing mechanism that holds great promise as an environmentally sustainable alternative to broad-spectrum insecticides in the control of hemipteran pests. Over the past two decades, considerable advances have been made in elucidating the molecular underpinnings of RNAi pathways, identifying physiologically relevant gene targets, and achieving proof-of-concept knockdown effects in multiple hemipteran species under laboratory conditions. However, the practical deployment of RNAi in field scenarios remains challenging due to the inconsistent efficacy observed across taxa, delivery systems, and environmental settings.

Among the major obstacles limiting RNAi efficacy in Hemiptera are physiological barriers, including rapid degradation of dsRNA by gut and hemolymph nucleases, low transmembrane uptake owing to absent or weak expression of SID-like transporters, and inefficient systemic dissemination of the silencing signal. These factors are further compounded by genetic and putative epigenetic traits that modulate RNAi responsiveness – such as variable expression of core RNAi components and inducible defense mechanisms. Moreover, the anatomical and immunological heterogeneity observed across hemipteran families complicates the development of generalized RNAi-based interventions.

Nevertheless, recent developments in molecular biology and bioengineering offer promising solutions to these bottlenecks. Engineered delivery platforms – including chitosan nanoparticles, liposomes, and virus-like particles – enhance dsRNA stability and facilitate transcellular transport. Transplastomic expression of dsRNA in chloroplasts provides a potentially scalable and field-adaptable delivery method, particularly when coupled with chemical modifications that confer increased resistance to degradation. In the same way, multi-omics approaches have expanded the pool of viable targets to include salivary effectors, neuroendocrine regulators, and signaling molecules, thereby enhancing the potential for durable and synergistic pest suppression.

The intrinsic specificity of RNAi not only supports its ecological safety but also enables highly selective pest control that preserves beneficial arthropods and promotes agroecosystem resilience. However, this specificity also necessitates rigorous homology screening, robust target selection workflows, and long-term ecological risk assessments to mitigate the possibility of off-target effects. While these safeguards are essential, they contribute to the regulatory and technical complexity of RNAi product development.

In addition, economic and logistical factors – particularly those related to large-scale dsRNA production, formulation, and deployment – pose significant challenges. Microbial and in planta expression systems offer promising cost-reduction strategies, but their adoption depends on regulatory acceptance, manufacturing scalability, and compatibility with prevailing agronomic practices.

In conclusion, the effective implementation of RNAi for hemipteran pest management will require a multi-pronged strategy that addresses physiological, ecological, technical, and socio-economic dimensions. Continued investment in both fundamental research and translational innovation – supported by interdisciplinary collaboration among entomologists, molecular biologists, agronomists, and regulators – will be essential for unlocking the full potential of RNAi. If these efforts are realized, RNAi-based technologies may become a cornerstone of species-selective, environmentally sound crop protection in twenty-first-century agriculture.

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