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RNA interference in the insect ATPase gene

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A B S T R A C T

ATPases are crucial in insect physiology, regulating ion transport, energy metabolism, and cellular homeostasis. Among them, P-type and V-ATPase are of particular interest due to their essential functions in insect survival and their potential as novel targets for pest management. RNA interference (RNAi) has emerged as a promising genetic tool for silencing key insect genes, including ATPase-encoding genes, offering an alternative to conventional chemical insecticides. This review discusses RNAi delivery strategies, target gene selection, and validation methodologies, highlighting recent breakthroughs in genome-wide RNAi screens and high-throughput functional genomics. Furthermore, we examine the integration of RNAi with other pest control technologies, such as CRISPR-based gene editing, to enhance insecticidal efficacy and mitigate insecticide resistance. The environmental, ecological, and regulatory implications of ATPase RNAi in pest control are also considered, focusing on potential risks, ethical considerations, and sustainable agricultural applications. Despite its promise, RNAi-based pest control faces challenges, including variability in RNAi efficiency across insect species, the development of RNAi resistance, and the need for long-term ecological assessments. Future research should focus on improving RNAi delivery systems, optimizing gene silencing specificity, and integrating multi-omics approaches to identify novel ATPase targets. This review underscores the potential of ATPase RNAi as a transformative strategy in insect pest management. It highlights key areas for further investigation to achieve effective and sustainable pest control solutions.

Introduction

ATPases, particularly V-ATPases, play crucial roles in insect physiology by regulating proton transport across membranes. ATPases are essential for maintaining pH homeostasis in various cellular compartments, such as the midgut and epithelial tissues of insects, energy production, and nutrient absorption. These proton pumps are essential for acidifying intracellular organelles and nutrient absorption and removing waste in the midgut (Mohammed, 2016). These multi-subunit pumps are involved in vital processes, including endosomal trafficking, lysosomal degradation, neurotransmission, and fluid secretion, thereby influencing insect growth, development, and reproduction (Mo et al., 2020; Bai et al., 2023; Rakesh et al., 2024). The presence of multiple isoforms of ATPases allows for tissue-specific functions, highlighting their adaptability and importance in meeting the diverse physiological demands of insects (Yao et al., 2013; Mo et al., 2020; Zhang et al., 2022).

Insects utilize various ATPases, including V-ATPase and P-type Na+/K+-ATPase, to regulate ion transport across their epithelial membranes. They maintain homeostasis and facilitate nutrient absorption. V-ATPase plays a significant role in generating a high transmembrane electrical

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potential, essential for creating an alkaline environment in the midgut, which is vital for the function of many proteins (Bai et al., 2023). V-ATPases utilize ATP hydrolysis to transport protons across membranes, thereby maintaining pH homeostasis and enabling various cellular functions, including receptor-mediated endocytosis and neurotransmission (Mo et al., 2020). V-ATPases are multi-subunit proton pumps that utilize ATP hydrolysis to transport protons across membranes, thereby regulating pH in intracellular compartments such as endosomes, lysosomes, and synaptic vesicles (Mo et al., 2020).

Both ATPases are integral to the physiological processes in insects, influencing growth, development, and overall metabolic functions (D'Silva et al., 2017; Bai et al., 2023). Their activities are tightly regulated and can be affected by various inhibitors, highlighting their importance in insect physiology and potential as targets for pest control strategies (D'Silva et al., 2017). Disruption of V-ATPase activity can lead to significant physiological abnormalities, such as electrolyte imbalances and metabolic disorders, ultimately resulting in insect mortality (Bai et al., 2023). Furthermore, V-ATPases are recognized as potential targets for environmentally friendly insecticides, as their silencing has been shown to impact insect survival and reproductive fitness significantly (Mohammed, 2016; Zhang et al., 2022; Guo et al., 2023; Rakesh et al., 2024). This highlights the importance of ATPases in insect development and their potential utility in pest management strategies.

Silencing V-ATPase subunits through RNA interference has been shown to affect insect growth, development, and survival, indicating their critical function in maintaining cellular homeostasis (Bai et al., 2023).

RNA interference (RNAi) is a biological process that silences gene expression through the degradation of specific mRNA sequences, primarily initiated by double-stranded RNA (dsRNA) molecules that match the target gene sequence (Thakur et al., 2014; Zeng et al., 2021). RNAi operates in various eukaryotic organisms, including insects, and can induce significant phenotypic changes, such as growth reduction or mortality, by targeting critical genes (Thakur et al., 2014; Kaur et al., 2023). The efficiency of RNAi can be influenced by factors such as the delivery method of dsRNA and the specific tissues involved in the uptake (Zeng et al., 2021; Zhang et al., 2022).

RNAi presents several advantages for some insect pest control, primarily its specificity and reduced environmental impact. By targeting specific genes, RNAi can effectively suppress pest populations without harming non-target species, as demonstrated by the narrow activity spectrum of dsRNA (Guo et al., 2023). This specificity is crucial for minimizing unintended effects on beneficial insects, such as ladybeetles (Guo et al., 2021). RNAi offers a novel mode of action that can complement existing pest control strategies while addressing the challenges of pesticide resistance (Zeng et al., 2021).

This review aims to evaluate the role of ATPase RNAi in insect pest management. The review focuses on applying RNA interference targeting ATPase genes in insects, highlighting its potential for pest control and gene function studies. V-ATPase is crucial for maintaining ion balance and nutrient uptake in insect cells. It is a promising target for RNAi strategies to reduce insect populations or alter their reproductive capabilities. We aim to provide a critical resource for researchers and practitioners, ultimately contributing to developing effective and environmentally responsible pest management solutions.

ATPase Structure and Function

Insects possess both P-type and V-ATPase, crucial for various physiological processes. The V-ATPase, particularly noted in insect epithelia, is responsible for proton transport across membranes, facilitating functions such as luminal alkalinization in the midgut and driving ion transport (Mohammed, 2016; O'Donnell, 2017). Insect ATPases, particularly the vacuolar-type H+-ATPase (V-ATPase), play crucial roles in ion transport and maintaining electrochemical gradients across epithelial membranes. The V-ATPase is a multi-subunit enzyme composed of a peripheral catalytic cytosolic V1 domain responsible for ATP hydrolysis and a membrane-bound V0 domain that forms the proton channel, with subunits A and B playing key roles in ATP hydrolysis and structural integrity (Bai et al., 2023). Subunits A and B of the V1 domain are crucial for ATP hydrolysis and are highly conserved across insect taxa, making them

common targets for RNA interference studies aimed at pest control (Xie et al., 2022; Bai et al., 2023). The V-ATPase complex consists of multiple subunits, which have been shown to exist in different isoforms that perform specific functions in tissues, such as Drosophila wing development, where Vha100-2 and Vha100-4 play distinct roles in cuticle maturation and signaling pathways, respectively (Mo et al., 2020).

In addition to V-ATPase, P-type ATPases are another significant family. P-type ATPases typically transport ions across cellular membranes, maintaining essential gradients of ions such as Na+, K+, and Ca2+. P-type ATPases, such as the Na+/K+-ATPase, are involved in driving K+ absorption and maintaining ion gradients across membranes, particularly in the anterior midgut and Malpighian tubules (D'Silva et al., 2017; O'Donnell, 2017). The Na+, K+-ATPase regulates ionic balance and membrane potential, critical for cellular signaling and overall physiological stability in insects (Ding et al., 2020). This enzyme facilitates the transport of protons, which is coupled with the exchange of cations like K+ and Na+ through antiporters, thereby generating a positive transmembrane electrical potential that is vital for various physiological processes (D'Silva et al., 2017; O'Donnell, 2017). Additionally, the Na+/K+-ATPase, found in some insect tissues, contributes to ion homeostasis by pumping Na+ out and K+ into cells, highlighting the complexity and functional diversity of ATPases in insect physiology (D'Silva et al., 2017; O'Donnell, 2017). Specifically, Na+/K+-ATPases are responsible for maintaining the concentration gradients of sodium (Na+) and potassium (K+) ions in animal cells, a critical function for cellular homeostasis and signaling (D'Silva et al., 2017). The interplay between these ATPases is essential for maintaining cellular homeostasis and supporting metabolic activities in insect tissues. The functional diversity of these ATPases underscores their importance in insect development and adaptation to environmental challenges (Bai et al., 2023).

Mechanisms of RNAi in Insects

RNAi has emerged as a significant tool in insect research, particularly for pest management, since its discovery in the late 1990s. Initially identified in *Caenorhabditis elegans*, RNAi has since been applied across various insect orders, including Coleoptera, Hemiptera, Diptera, and Lepidoptera, demonstrating its potential for gene silencing and pest control strategies (Mohammed et al., 2015; Zhang et al., 2022). The technology has facilitated the development of transgenic plants expressing double-stranded RNA (dsRNA) targeting vital insect genes, leading to effective pest resistance (Thakur et al., 2014). Insecticide resistance is a growing concern in pest management, particularly among mosquito populations, where resistance to chemical insecticides has been documented extensively (Coy et al., 2012). This resistance complicates control efforts, necessitating alternative strategies. RNAi offers a promising approach to overcome this challenge by utilizing double-stranded RNA (dsRNA) to specifically target and silence genes associated with resistance mechanisms (Zeng et al., 2021; Zhang et al., 2022). This historical context underscores RNAi's evolution from a basic biological mechanism to a practical application in agricultural biotechnology. By exploiting the sequence-specific nature of RNAi, it is possible to develop bio-insecticides that minimize environmental impact and reduce the risk to non-target species, thereby enhancing the sustainability of pest control measures (Coy et al., 2012; Zeng et al., 2021).

Insects utilize RNAi as a crucial mechanism for gene regulation and defense against pathogens, primarily involving processing double-stranded RNA (dsRNA) into small interfering RNAs (siRNAs) by Dicer enzymes. These siRNAs are then incorporated into the RNA-induced silencing complex (RISC), guiding the cleavage of complementary mRNA, leading to gene silencing (Mao et al., 2015). In other words, RNAi is a crucial molecular mechanism for gene silencing in insects, involving the degradation of specific mRNA sequences through small interfering RNAs (siRNAs) and microRNAs (miRNAs). The process begins with the introduction of double-stranded RNA (dsRNA), which is processed by Dicer enzymes into siRNAs, leading to the formation of the RNA-induced silencing complex (RISC) that targets complementary mRNA for degradation, thus inhibiting gene expression (Fig. 1 and Fig. 2; Thakur et al., 2014; Adeyinka et al., 2020; Zeng et al., 2021; Niu et al., 2024). The stability of dsRNA in feeding solutions is critical for effective RNAi, as it influences the uptake and subsequent gene silencing efficiency (Zhang et al., 2022).

In vivo, RNAi delivery methods for insects primarily include microinjection, feeding, and soaking. Microinjection allows for direct delivery of double-stranded RNA (dsRNA) into the insect's body. However, it is impractical for field applications due to the mechanical damage it can cause and the small size of many insects (Zhang et al., 2022). Feeding methods incorporating dsRNA into artificial diets or natural food sources have shown promise, particularly with solutions like sucrose and honey, which can enhance dsRNA stability and uptake (Zhang et al., 2022; Guo et al., 2023). Additionally, the use of recombinant baculoviruses to deliver dsRNA represents a novel approach, allowing for sustained gene silencing through viral infection (Bai et al., 2023).

The efficiency of RNAi varies among insect orders, with Coleoptera showing robust responses compared to Lepidoptera, which often require higher dsRNA concentrations for effective silencing (Bally et al., 2018). Additionally, the lack of canonical RNA-dependent RNA polymerases in insects suggests that amplification of RNAi may not occur as it does in other eukaryotes, indicating a reliance on direct dsRNA uptake and processing (Mao et al., 2015; Bally et al., 2018). Understanding these pathways is essential for developing RNAi-based pest control strategies.

Effects of ATPase knockdown on insect physiology

Silencing V-ATPase genes through RNAi has been shown to induce high mortality rates across various insect species, including thrips, locusts, and weevils, by disrupting essential functions such as feeding, development, and reproduction (Liu et al., 2022; Rakesh et al., 2024). The effectiveness of RNAi in targeting V-ATPases relies on the efficient delivery of double-stranded RNA (dsRNA) via methods such as oral feeding and foliar application, minimizing impacts on non-target species and enhancing sustainable pest control strategies (Zhang et al., 2022; Guo et al., 2023).

Knockdown of ATPase subunits significantly affects insect physiology by impairing proton transport and pH regulation, leading to metabolic disorders and electrolyte imbalances (Bai et al., 2023). Targeting V-ATPase subunits has proven lethal across multiple insect orders, including Lepidoptera, Diptera, and Coleoptera, reinforcing their importance in physiological processes (Chang et al., 2021; Ramkumar et al., 2021; Liu et al., 2022). For instance, silencing V-ATPase subunits A and B in *Spodoptera frugiperda* resulted in reduced body weight, developmental delays, and increased larval mortality, emphasizing their role in maintaining physiological stability and energy balance (Bai et al., 2023). Similarly, RNAi targeting V-ATPase subunits A and C in *Hyphantria cunea* caused severe developmental defects and high mortality, reinforcing the importance of these genes in larval survival (Wang et al., 2024).

Studies have demonstrated that silencing V-ATPase subunit genes in pests such as *Diabrotica virgifera virgifera* and *Henosepilachna vigintioctopunctata* results in significant mortality (Yao et al., 2013; Zeng et al., 2021; Liu et al., 2022). In Sri Lanka, weevils and *corn planthoppers*, dsRNA targeting V-ATPase genes led to mortality rates of up to 92.7% and impaired reproductive capacity, respectively, underscoring the widespread applicability of this approach (Pinheiro et al., 2020; Guo et al., 2023). Likewise, knockdown of subunits B and D in pests such as *Peregrinus maidis*, *Tuta absoluta*, and *Frankliniella occidentalis* resulted in reduced fecundity and survival, demonstrating the specificity and efficiency of RNAi-based interventions (Yao et al., 2013; Ramkumar et al., 2021; Zhang et al., 2022; Rakesh et al., 2024).

RNAi-mediated knockdown in *Drosophila melanogaster* resulted in 100% mortality, with similar effects observed in *Manduca sexta*, *Acyrthosiphon pisum*, *Liriomyza trifolii*, and *Tribolium castaneum* (Yao et al., 2013; Chang et al., 2021). The specificity of RNAi allows for targeted pest control while minimizing harm to beneficial insects, making V-ATPases promising candidates for environmentally friendly biopesticides (Ramkumar et al., 2021; Liu et al., 2022; Li et al., 2022; Bai et al., 2023).

Methodologies in ATPase RNAi Research

RNAi Delivery Systems

In ATPase RNAi research, various methodologies have been employed to silence target genes effectively. These methodologies illustrate the potential of RNAi as a tool for pest control and functional gene analysis in various agricultural contexts. Recent advancements in RNAi delivery systems have significantly enhanced the effectiveness of ATPase knockdown in various insect species. Additionally, RNAi can be delivered through various methods, including oral

feeding and spraying, which enhances its applicability in agricultural settings (Dalla et al., 2017; Zhang et al., 2022; Guo et al., 2023). The effectiveness of RNAi is influenced by the method of dsRNA delivery, with microinjection yielding higher knockdown efficiency than feeding (Yao et al., 2013). Also, the choice of feeding solution can impact the stability and efficacy of dsRNA, with honey and pollen solutions showing better results in reducing V-ATPase transcription levels and insect survival (Zhang et al., 2022). Bacterially expressed dsRNA has shown higher efficacy and lower production costs than in vitro synthesized dsRNA, making it a promising option for sustainable pest management (Guo et al., 2021). Table 1 summarizes RNAi delivery methods and their pros/cons.

While microinjection is confined to laboratory studies, feeding and topical application methods are among the most promising for large-scale agricultural pest management. Transgenic plants, bacterial systems, and baculoviruses also hold significant potential, each with unique advantages and challenges. Continued research and development in dsRNA formulation, stability, and delivery technology will be key to making these methods more cost-effective and feasible in real-world settings. Table 2 summarizes large-scale agricultural applications of different delivery methods.

Among the various RNAi delivery methods, transgenic plants expressing dsRNA have shown considerable promise for crop protection due to their potential scalability and sustained expression. This approach, exemplified by the success of SmartStax® PRO maize targeting *D. virgifera virgifera* (western corn rootworm), demonstrates the effectiveness of plant-mediated RNAi under field conditions (Reinders et al., 2023). However, the commercialization and deployment of such transgenic crops are constrained by high regulatory costs, lengthy approval processes, and public resistance to genetically modified organisms (GMOs). Moreover, developing plant lines with sufficient dsRNA expression levels in phloem or other feeding sites remains a technical challenge for pests with different feeding strategies. Bacterial and yeast-based expression systems have been proposed as cost-effective platforms for dsRNA production and oral delivery (Zotti et al., 2018). While promising for laboratory and greenhouse settings, concerns remain regarding the survival and stability of engineered microbes in diverse environmental conditions, potential impacts on soil microbiota, and the need for robust biocontainment strategies.

Topical application of dsRNA, including foliar sprays and trunk injections, is an attractive non-transgenic alternative due to its flexibility and ease of deployment. Recent advances in formulation technologies, such as nanocarriers, liposomes, or clay nanosheets (BioClay), have significantly enhanced dsRNA stability and uptake in field conditions (Mitter et al., 2017). Nonetheless, the large-scale implementation of topical RNAi faces critical hurdles, including environmental degradation of naked dsRNA by UV light and RNases, high production costs of stabilized formulations, and the need for repeated applications due to the transient nature of the effect. Furthermore, delivery efficiency is influenced by insect feeding behavior, cuticle permeability, and weather conditions. While promising field trials have been reported for Coleopteran and Lepidopteran pests, comprehensive cost-benefit analyses and regulatory frameworks are still required before these approaches become viable commercial alternatives to conventional pesticides. Therefore, despite substantial progress, the field-readiness of RNAi delivery systems remains context-dependent and demands continued interdisciplinary innovation for successful agricultural implementation.

Microinjection

Microinjection allows for the direct introduction of double-stranded RNA (dsRNA) into the organism, triggering a systemic RNAi response. However, it is impractical for field applications due to the invasive nature of the method (Zhang et al., 2022). Microinjection of dsRNA into insects has shown significant reductions in ATPase transcript levels, leading to observable phenotypes such as increased mortality and reduced fecundity (Yao et al., 2013). For instance, microinjection of double-stranded RNA (dsRNA) targeting V-ATPase subunits resulted in up to a 90-fold reduction in transcript levels, leading to increased mortality and reduced fecundity in insects like *Peregrinus maidis* (Yao et al., 2013).

Feeding

Various delivery systems have been explored to enhance RNAi efficiency and specificity in insect pest management, including nanoparticles and oral

feeding methods. Nanoparticle-mediated delivery has shown promise in silencing target genes effectively, as demonstrated in studies involving the African malaria mosquito, where larval feeding facilitated the uptake of dsRNA (Mohammed, 2016). Additionally, the oral delivery method, which allows for the continuous administration of dsRNA, has effectively overcome the limitations of short-term RNAi effects (Guo et al., 2023). Feeding dsRNA is a noninvasive technique that preserves the integrity of the treated insects and is effective in silencing target genes, as demonstrated in studies involving aphids and thrips (Rebijith et al., 2015; Zhang et al., 2022). Oral delivery of dsRNA has been explored, with varying efficiencies noted across different insect species, highlighting the importance of optimizing delivery methods for effective gene silencing (Wuriyanghan et al., 2011; Ortolá et al., 2024). Oral delivery of dsRNA has shown promise, with studies indicating that feeding insects dsRNA can effectively silence target genes, as demonstrated with V-ATPase-B in *Thrips palmi*, resulting in significant mortality and reduced reproductive fitness (Rakesh et al., 2024). The silencing of V-ATPase genes has been effective in various Coleopteran species, such as the western corn rootworm, where ingestion of dsRNA led to significant mortality and growth inhibition (Baum & Roberts, 2014; Zeng et al., 2021). Studies have demonstrated that silencing the V-ATPase genes in various pests, such as the South American fruit fly (*Anastrepha fraterculus*) and the ladybird beetle (*Henosepilachna vigintioctopunctata*), resulted in significant mortality and reduced feeding damage when dsRNA was administered through soaking or dietary methods (Dias et al., 2019; Zeng et al., 2021). These delivery methods improve RNAi efficiency and facilitate the development of environmentally friendly pest management strategies (Guo et al., 2023).

Topical application

Topical application of double-stranded RNA (dsRNA) is a promising method for inducing RNA interference (RNAi) in insects, allowing for gene silencing without ingestion. This method involves applying dsRNA directly onto the insect's exoskeleton, which can penetrate the body wall and circulate within the body cavity, as demonstrated in studies with adult *Diaphorina citri* and *Ostrinia nubilalis* larvae (Kunte et al., 2020). While topical application is less labor-intensive than microinjections and can facilitate high-throughput gene screening, challenges remain, such as the variability in dsRNA uptake and the need for further research to elucidate the exact mechanisms of entry (Kunte et al., 2020; Bai et al., 2023).

The ability to deliver double-stranded RNA (dsRNA) through various methods, such as topical application, enhances the practicality of these strategies in agricultural settings (Pan et al., 2017; Guo et al., 2023). For instance, studies on the South American pinworm *Tuta absoluta* demonstrated that topically applied dsRNA targeting juvenile hormone binding protein and V-ATPase genes resulted in increased larval mortality and reduced leaf damage (Ramkumar et al., 2021; Zeng et al., 2021). Despite these hurdles, topical dsRNA application holds potential for targeted pest management, particularly as part of integrated pest control strategies (Guo et al., 2021).

Transgenic plants

Additionally, transgenic and transplastomic plants expressing dsRNA have been developed as a pest control strategy, showcasing RNAi's potential in agricultural applications (Zeng et al., 2021). Transplastomic plants have been utilized to express dsRNA targeting ATPase, demonstrating stable expression and high RNAi activity, which is advantageous over traditional siRNA delivery methods due to reduced degradation in the digestive system (Jin et al., 2015; Kaur et al., 2023). The development of transgenic plants expressing specific dsRNAs has been demonstrated to effectively silence target genes in pests like whiteflies, leading to high mortality rates and reduced reproduction (Ibrahim et al., 2017). For instance, the development of transgenic maize expressing dsRNA against *D. virgifera virgifera* has shown significant efficacy in controlling this pest by inducing mortality and growth inhibition (Baum & Roberts, 2014).

Bacterial systems

RNA interference (RNAi) for pest control has been explored through various delivery systems, including bacterial systems. Bacterially expressed double-stranded RNA (dsRNA) has advantages over in vitro synthesized dsRNA, such as lower production costs and enhanced stability, which can lead to effective pest management strategies (Guo et al., 2021). RNAi can be integrated with bacterial systems that express dsRNA, enhancing the delivery and efficacy of gene silencing in target

insects (Kaur et al., 2023). For instance, ingesting dsRNA from bacteria can induce significant mortality in pests like *Henosepilachna vigintioctopunctata* (Guo et al., 2021). Additionally, transgenic plants expressing dsRNA have been developed to target specific insect genes, demonstrating the potential for RNAi to be integrated into crop management systems (Thakur et al., 2014). Optimizing dsRNA delivery methods and identifying effective target genes are crucial for enhancing RNAi efficacy in agricultural pest management (Guo et al., 2023).

Baculoviruses

Recombinant baculoviruses have been developed as a novel strategy for pest control, mainly targeting the fall armyworm (*S. frugiperda*) through RNA interference (RNAi). In this approach, two specific subunits of V-ATPase (SfvATPaseA and SfvATPaseB) were cloned to create recombinant baculoviruses capable of producing small interfering RNA, which significantly suppressed the expression of these target genes, leading to reduced larval growth and increased mortality rates (Bai et al., 2023). The engineered baculoviruses demonstrated excellent genetic stability over multiple passages and exhibited potent insecticidal activity, suggesting their potential as environmentally friendly biopesticides (Bai et al., 2023). However, challenges remain in achieving effective oral delivery of these viruses for field applications (Bai et al., 2023; Guo et al., 2023). Using recombinant baculoviruses to deliver dsRNA targeting V-ATPase subunits has demonstrated substantial growth inhibition and mortality in *S. frugiperda* (Bai et al., 2023).

Target Selection and Design of RNAi Constructs

Selecting target genes for RNAi constructs is crucial for effective pest control, focusing on genes that induce rapid lethality or feeding cessation. To improve RNAi efficiency and specificity, selecting appropriate target genes is crucial. For instance, the death-associated inhibitor of apoptosis protein 1 (diap1) was identified as a promising target, as its silencing led to acute feeding disorders in the pest *Henosepilachna vigintioctopunctata* within 24 hours, demonstrating a faster response compared to traditional targets like V-ATPase genes (Chikami et al., 2021). Studies have shown that targeting housekeeping genes like V-ATPase subunits can induce significant mortality in pests such as *Aphis gossypii*, with silencing efficiencies reaching up to 73.21% for juvenile hormone-binding protein (JHBP) and 66.1% for V-ATPase-H, correlating positively with aphid mortality (Rebijith et al., 2016).

Additionally, the design of species-specific double-stranded RNA (dsRNA) constructs is essential to minimize off-target effects, as shown by the lower conservation of diap1 sequences across different insect species (Chikami et al., 2021). This specificity was confirmed through injection studies, which indicated that the diap1 dsRNA did not affect non-target species, highlighting the importance of careful target selection in RNAi applications (Chikami et al., 2021; Kaur et al., 2023).

Furthermore, understanding the mechanisms of dsRNA uptake, such as clathrin-mediated endocytosis, can enhance the systemic spread of RNAi effects in various insect species (Pinheiro et al., 2018). These strategies collectively contribute to more effective RNAi-based pest management.

The selection of ATPase genes for RNAi is based on their essential roles in insect physiology and previous successful applications in pest control. Specifically, genes encoding V-ATPase B and juvenile hormone binding protein (JHBP) were chosen due to their involvement in critical processes such as growth and development in insects, as demonstrated in studies targeting *Tuta absoluta* (Ramkumar et al., 2021). Additionally, the effectiveness of RNAi targeting these genes has been supported by prior research indicating significant insect mortality upon silencing these targets (Pinheiro et al., 2020; Zeng et al., 2021). The criteria also include the availability of conserved sequences across related species, which aids in designing effective double-stranded RNA (dsRNA) for gene silencing (Ramkumar et al., 2021). The selection process emphasizes functional importance and empirical evidence of RNAi efficacy in pest management strategies.

Designing effective small interfering RNA (siRNA) for ATPase targets involves careful selection of target sequences to ensure high efficacy and minimal off-target effects. In the context of V-ATPase, specific genes such as V-ATPase B and D have been identified as promising targets for RNAi in various insect species, demonstrating significant mortality upon silencing (Chang et al., 2021; Zeng et al., 2021). The design process includes filtering candidate sequences based on thermodynamic properties and ensuring

low GC content to enhance functionality (Jin et al., 2015). Additionally, the delivery method, such as microinjection or transgenic expression, plays a crucial role in the effectiveness of siRNA, as demonstrated by the successful knockdown of V-ATPase genes leading to increased mortality in pests like *Liriomyza trifolii* (Chang et al., 2021; Zeng et al., 2021). Optimizing siRNA design and delivery is essential for effective pest control strategies.

Validation and Assessment of RNAi Efficiency

The assessment of RNAi efficiency targeting ATPase genes has been validated through various molecular and phenotypic assays. In Ceratitis capitata studies, significant knockdown of the V-ATPase and ribosomal protein RPS13 was achieved, reducing fecundity and fertility in treated females and indicating effective gene silencing (Ortolá et al., 2024). Additionally, the use of recombinant baculoviruses to mediate RNAi against SfvATPaseA and SfvATPaseB in *S. frugiperda* demonstrated substantial reductions in target gene expression, with larval body weight and length significantly affected, showcasing the phenotypic consequences of ATPase knockdown (Bai et al., 2023). Furthermore, the correlation between dsRNA dosage and RNAi effectiveness was highlighted, emphasizing the importance of optimizing delivery methods for enhanced silencing outcomes (Jin et al., 2015).

RNAi efficiency can be validated and assessed through various experimental designs, including time course and concentration experiments. For instance, in studies involving the microinjection of double-stranded RNA (dsRNA) targeting V-ATPase subunits, significant reductions in gene expression were observed, with silencing efficiencies exceeding 60% at specific time points post-injection (Chang et al., 2021). Quantifying gene silencing was performed using qRT-PCR, which allowed for the measurement of target genes' relative expression levels (RELs) compared to controls (Bai et al., 2023). Additionally, factors influencing RNAi efficiency include the effective dose of dsRNA, the life stage of the insect, and the persistence of gene silencing (Mohammed, 2016). Additionally, the effectiveness of RNAi varied with the method of dsRNA delivery, with microinjection generally yielding a more substantial and rapid response than oral administration (Yao et al., 2013; Rakesh et al., 2024). These methodologies provide a robust framework for assessing RNAi's effectiveness in gene silencing applications.

Optimizing siRNA design for improved silencing efficiency

Careful design and delivery of small interfering RNA (siRNA) are crucial to improve RNAi efficiency and specificity. Factors influencing RNAi efficiency include the target gene's function, tissue-specific expression, and the stability of the protein encoded by the gene, as more stable proteins tend to exhibit lower RNAi responses (Zeng et al., 2021). Additionally, optimizing the concentration of dsRNA is essential, as exceeding optimal levels does not necessarily enhance silencing (Chang et al., 2021). The timing of dsRNA treatment is also significant; younger larval instars show higher susceptibility to RNAi, suggesting that targeting these stages may yield better results (Zeng et al., 2021). Furthermore, ensuring that dsRNA escapes from endosomes into the cytoplasm is critical, as this can be a rate-limiting factor in RNAi efficiency (Shi et al., 2022). Thus, a multifaceted approach involving design, timing, and delivery mechanisms is necessary for optimizing siRNA effectiveness.

Genome-Wide RNAi Screens for ATPases

Genome-wide RNAi screens targeting ATPases have shown promise in elucidating gene functions and developing pest control strategies. For instance, studies on V-ATPases in insects, such as *Diaphorina citri* and *Peregrinus maidis*, have demonstrated that silencing specific V-ATPase subunits can lead to significant reductions in transcript levels and observable phenotypic changes, including increased mortality and reduced fecundity (Yao et al., 2013; Grace et al., 2022). Additionally, the structural and functional insights gained from recent advances in P-type ATPase research, including cryo-electron microscopy, have furthered our understanding of these enzymes, highlighting their potential as targets for RNAi-based interventions (Stock et al., 2023).

The identification of novel ATPase targets can be facilitated through high-throughput screening techniques, as demonstrated by using RNAi targeting specific V-ATPase subunits in various insect species (Chang et al., 2021). The study of V-ATPase subunits across different species, including their expression levels and interactions, can be enhanced through bioinformatics approaches such as BLAST for homology

screening (Liu et al., 2019). This method allows for identifying conserved sequences that can be targeted for gene silencing, thereby providing a pathway for discovering new ATPase targets that may have therapeutic implications in both agricultural and medical contexts (Oot et al., 2017).

Functional genomics approaches, particularly RNAi, have effectively studied ATPase-related pathways, specifically targeting V-ATPase subunits in various insect species. For instance, silencing V-ATPase subunits in *Liriomyza trifolii* and *D. melanogaster* resulted in significant mortality, demonstrating the critical role of these subunits in insect survival and development (Chang et al., 2021). The integration of functional genomics with proteomic analyses has also revealed the involvement of V-ATPase in responses to environmental stressors, such as Cry toxins in various insect species, suggesting a broader role in insect physiology and adaptation (Xie et al., 2022; Lovero et al., 2023). These findings underscore the importance of ATPase-related pathways in understanding insect biology and developing novel control strategies.

The integration of RNAi with CRISPR/Cas9-based gene editing presents a promising strategy for pest control, particularly in addressing the limitations of RNAi in particular insect species. While RNAi has shown potential in silencing genes essential for pest survival, its effectiveness can vary significantly among different insects, especially in Lepidoptera, where some species exhibit resistance to RNAi due to factors like dsRNA degradation and inefficient uptake mechanisms (Pan et al., 2017; Zeng et al., 2021). In contrast, CRISPR/Cas9 allows for precise genetic modifications, enabling targeted gene knockouts that can complement RNAi approaches (Pan et al., 2017; Mo et al., 2020). This dual strategy could enhance pest management by leveraging both technologies' strengths, potentially leading to more effective and sustainable control methods against agricultural pests (Pan et al., 2017; Bai et al., 2023).

Multi-Omics Approaches for RNAi Target Identification

Identifying novel RNAi targets, particularly ATPase genes, can benefit from multi-omics approaches integrating transcriptomics, proteomics, and metabolomics. Multi-omics approaches, including transcriptomic analysis, have been utilized to identify potential RNAi targets in pest species such as the South American fruit fly (*Anastrepha fraterculus*) and the Mediterranean fruit fly (*Ceratitis capitata*). In the case of *A. fraterculus*, a comprehensive transcriptome database was created to screen for RNAi pathway genes and potential target genes related to growth and reproduction, revealing 55 genes associated with the RNAi machinery and 143 target genes for dsRNA bioassays (Dias et al., 2019). Similarly, in *C. capitata*, candidate genes were selected based on previous reports of increased mortality in other insects, leading to the identification of effective targets for RNAi-mediated control (Ortolá et al., 2024).

Using transcriptomic data to identify conserved sequences across species can facilitate the selection of effective RNAi targets. At the same time, proteomic analyses can reveal the functional roles of these proteins in metabolic pathways (Liu et al., 2019). Additionally, metabolomic profiling can provide insights into the biochemical impacts of silencing these targets, thereby enhancing the understanding of their roles in insect physiology and aiding in developing targeted pest management strategies (Chang et al., 2021; Ramkumar et al., 2021). These studies highlight the importance of integrating multi-omics data to enhance the efficiency of RNAi strategies in pest management.

Implications of ATPase RNAi in Insect Pest Management

Environmental and Ecological Implications

Using RNAi targeting V-ATPase in insect pest management presents significant environmental and ecological implications, particularly regarding the persistence of dsRNA in the environment. Studies indicate that dsRNA can be effectively delivered through oral administration, leading to substantial reductions in target gene expression and increased mortality in pests like Thrips palmi and Peregrinus maidis (Yao et al., 2013; Rakesh et al., 2024). The stability of spray-applied dsRNA on plant foliage has been shown to last up to 24 hours, allowing for effective pest control while minimizing environmental accumulation (Rakesh et al., 2024).

Using RNAi targeting V-ATPase genes in insect pest management raises concerns regarding potential non-target effects and ecological risks. Studies have shown that while RNAi can effectively suppress target pests like the western corn rootworm, it may also impact non-target

organisms, such as the monarch butterfly, which exhibited no adverse effects from exposure to dsRNA targeting V-ATPase A, suggesting a low risk of ecological disruption (Pan et al., 2017). However, the specificity of RNAi is crucial, as shared sequences between target and non-target species could lead to unintended consequences (Liu et al., 2022). Additionally, the presence of nucleases in the gut and saliva of insects can degrade dsRNA, potentially limiting its effectiveness and reducing risks to non-target species (Dias et al., 2019).

While RNAi is often praised for its specificity, off-target effects remain a significant biosafety concern, especially in large-scale agricultural applications. Off-target silencing can occur when the dsRNA sequence shares partial complementarity with unintended transcripts in non-target organisms, leading to unintended gene knockdown. For instance, studies have reported that dsRNAs designed to target pest species can inadvertently affect closely related beneficial insects, such as predators or pollinators, if shared sequence motifs exist in conserved genes (Pan et al., 2016). In D. melanogaster, exposure to dsRNA targeting V-ATPase in pest species resulted in mild off-target gene suppression, emphasizing the need for rigorous bioinformatics screening and in vitro validation before field deployment (Christiaens et al., 2020). Furthermore, non-lethal or sublethal effects, such as changes in gene expression, development, or behavior, may not be immediately observable but could have long-term ecological consequences if overlooked during early risk assessments.

The environmental fate and stability of dsRNA molecules are equally important in assessing the safety and efficacy of RNAi-based technologies. The dsRNA introduced into the environment through topical sprays, transgenic plants, or microbial carriers can persist on plant surfaces, soil, or water, depending on environmental conditions such as UV radiation, pH, and microbial activity. Some studies suggest that naked dsRNA degrades rapidly under field conditions, reducing potential risks (Dubelman et al., 2014). However, stabilization strategies like nanoparticle encapsulation or chemical modifications (e.g., phosphorothioate backbones) are increasingly used to prolong dsRNA persistence and enhance uptake in target pests, which in turn raises concerns about prolonged exposure to non-target species (Zhang et al., 2017). These modifications may increase the probability of trophic transfer or unintended environmental accumulation, particularly in complex ecosystems. Therefore, a more comprehensive understanding of dsRNA environmental dynamics, combined with tailored delivery methods and target-specific design, is crucial for minimizing off-target and ecological risks.

Carefully assessing RNAi's ecological implications is essential for its safe application in pest management strategies. RNAi-based strategies utilizing V-ATPase as a target can provide a sustainable alternative to chemical insecticides, reducing their harmful environmental impacts.

Ethical challenges in using RNAi for pest control

The application of RNAi targeting ATPase genes in insect pest management presents opportunities and challenges, particularly regarding ethical and regulatory considerations and its impact on non-target organisms. RNAi-based insecticides, such as those targeting V-ATPase, are considered environmentally friendly alternatives to traditional chemical pesticides, potentially reducing harm to non-target organisms and promoting sustainable agriculture (Guo et al., 2021). However, the specificity of dsRNA to target pests while minimizing effects on beneficial species is crucial for regulatory approval (Rakesh et al., 2024).

One of the most pressing ecological concerns surrounding RNAi-based pest management is its potential impact on non-target organisms, particularly pollinators (e.g., *Apis mellifera*) and beneficial arthropods such as parasitoids and predators (e.g., Coccinellidae, Chrysopidae, and Trichogrammatidae). Although RNAi is considered a species-specific technology due to the requirement for sequence homology between the dsRNA and target mRNA, some studies have shown that off-target effects may occur when there is partial sequence complementarity in conserved genes of non-target organisms (Pan et al., 2016). Furthermore, many beneficial insects interact with plant surfaces or consume prey containing residual or degraded dsRNA, raising the possibility of unintended gene silencing. For example, *A. mellifera* exposed to dsRNA targeting V-ATPase in pests showed no immediate lethal effects. However, sublethal impacts on gene expression and behavior have been observed under certain conditions (Christiaens et al., 2020). These

findings underscore the importance of conducting bioinformatics screening and empirical testing of dsRNA sequences across a broad range of non-target species before field deployment.

Research on the impact of RNA interference (RNAi) on non-target organisms, particularly pollinators like the monarch butterfly (*Danaus plexippus*), indicates that these insects may not be significantly affected by dsRNA designed for pest control. In a study assessing the effects of V-ATPase A dsRNA from the western corn rootworm on *D. plexippus*, no adverse impacts on survival or development were observed, suggesting that this non-target organism is refractory to dietary RNAi (Pan et al., 2017). Additionally, the specificity of RNAi means that while it can effectively target pest species, it is less likely to affect beneficial insects due to the lack of significant sequence matches in their genomes (Zhang et al., 2022). This specificity is crucial for ensuring that RNAi applications do not unintentionally harm beneficial insects in agricultural ecosystems (Guo et al., 2023).

Some studies indicate that dsRNA can unintentionally affect related species, which may lead to ecological imbalances, as evidenced by the adverse effects observed in lady beetles when exposed to dsRNA targeting V-ATPase genes (Guo et al., 2021; Rakesh et al., 2024). Furthermore, the specificity of dsRNA is crucial; while designed to target specific pests, there remains a risk of off-target effects, necessitating careful design to minimize unintended consequences (Guo et al., 2021; Rakesh et al., 2024). Regulatory frameworks must address these ethical challenges, ensuring that RNAi applications do not compromise ecosystem integrity or harm beneficial species, thereby fostering public acceptance of RNAi-based pest control strategies (Guo et al., 2021). As RNAi technologies advance, ensuring public acceptance and compliance with safety standards will be essential for their successful integration into pest management strategies (Guo et al., 2021; Rakesh et al., 2024).

The potential for off-target effects and the need for optimized dsRNA constructs to enhance stability and uptake in various insect species are critical areas for further research (Coy et al., 2012; Rakesh et al., 2024).

However, challenges remain, including the effective delivery of double-stranded RNA (dsRNA) to target insects, as the efficiency of RNAi can vary based on the method of dsRNA administration (oral vs. injection) and the specific gene-targeted (Yao et al., 2013; Liu et al., 2022). The trophic transfer of dsRNA through ecological food webs remains a relatively unexplored risk area. Some beneficial insects, such as predatory beetles and parasitic wasps, may acquire dsRNA by feeding on herbivorous pests that have ingested RNAi-expressing plants or baits. Although the gut environment of many non-target insects can degrade dsRNA, particular species possess stable uptake and systemic RNAi responses, increasing the risk of unintentional gene silencing (Roberts et al., 2015). Moreover, variability in dsRNA stability, exposure duration, and feeding behavior across insect taxa complicates the assessment of ecological safety. Future research should adopt a multi-tiered risk assessment approach combining in silico off-target prediction, laboratory toxicity assays, and field-scale ecological monitoring to mitigate these risks. Developing pest-specific dsRNA constructs with minimal homology to beneficial species and employing spatial or temporal application strategies may further reduce the likelihood of non-target impacts and enhance the environmental safety profile of RNAi-based pest control. Addressing these challenges is essential for successfully applying RNAi-based biopesticides in sustainable agriculture.

Potential for Sustainable Agriculture

V-ATPase is crucial for various physiological processes in insects, and its silencing has been shown to significantly increase mortality rates in pests, thereby reducing their populations and reproductive fitness (Yao et al., 2013; Baum & Roberts, 2014; Ramkumar et al., 2021; Rakesh et al., 2024). The specificity of RNAi allows for targeted pest control while minimizing harm to non-target organisms, which is essential for maintaining ecological balance (Pan et al., 2017; Guo et al., 2021; Liu et al., 2022; Guo et al., 2023). Furthermore, the development of sprayable dsRNA formulations enhances the practicality of RNAi applications in field conditions, offering a non-chemical alternative to traditional insecticides and contributing to more sustainable pest management practices (Guo et al., 2021; Rakesh et al., 2024). Furthermore, applying dsRNA through various delivery methods, such as topical sprays or transgenic plants, enhances the feasibility of RNAi in real-world agricultural practices (Zhang et al.,

2022). As RNAi technology continues to evolve, its integration into pest management could contribute to long-term sustainability by reducing reliance on traditional chemical insecticides and addressing pest resistance issues (Baum & Roberts, 2014; Ramkumar et al., 2021).

Comparative Analysis

RNA interference (RNAi) offers a highly specific pest control method by silencing genes unique to target pests, minimizing unintended impacts on beneficial organisms. In contrast, traditional chemical insecticides deliver immediate pest suppression but pose significant risks, including broad-spectrum toxicity, rapid resistance development, and persistent environmental residues. Biological control methods, which rely on natural predators or pathogens, provide an eco-friendly alternative yet often exhibit variable efficacy that depends on local ecological conditions. CRISPR-based gene editing promises permanent, self-sustaining pest suppression through targeted genetic modifications, but high development costs and unresolved ethical and regulatory concerns challenge it. Table 3 compares these strategies' efficacy, cost/scalability, and environmental impact (Pan et al., 2017; Chikami et al., 2020). To better position RNA interference (RNAi) among current pest control strategies, a comparative analysis was conducted evaluating its performance against chemical insecticides, biological control, and CRISPR-based gene editing (Table 3). RNAi stands out for its high specificity, targeting pest species at the gene level with minimal off-target or non-target effects, unlike chemical insecticides, which often exhibit broad-spectrum activity and contribute to environmental contamination and resistance development. Although the cost and scalability of RNAi remain moderate due to formulation and delivery challenges, advances in nanoparticle carriers and plant-mediated RNAi are improving their field applicability. In contrast, chemical insecticides are highly scalable and inexpensive but pose significant ecological and health risks. Biological control offers environmentally friendly solutions, though it is often limited by ecological compatibility and slower action. Meanwhile, CRISPR-based technologies hold transformative potential but are still experimental and face substantial regulatory and public acceptance hurdles. Given these comparisons, RNAi emerges as a promising, environmentally sustainable, and precise approach for future pest management programs, particularly when integrated into an IPM (Integrated Pest Management) framework to minimize resistance evolution and enhance long-term efficacy.

RNA interference (RNAi) and CRISPR-Cas-based gene editing are two prominent molecular approaches for gene function studies and pest control, each with unique advantages and limitations. RNAi offers high target specificity, especially when designed against unique sequences in pest genomes, resulting in minimal off-target effects on non-target species (Christiaens et al., 2020). Its action is post-transcriptional and reversible, allowing for temporal gene silencing without permanent alterations to the genome, a feature particularly advantageous in ecological safety assessments. Furthermore, RNAi typically faces fewer regulatory and ethical hurdles compared to CRISPR-based technologies, making it more feasible for near-term application in pest management programs (Legros et al., 2021). In contrast, CRISPR-Cas systems enable permanent gene disruption or modification, which can provide long-term or even heritable control effects, especially when integrated into gene drive systems. However, the use of CRISPR is limited by delivery challenges, higher costs, stringent regulations, and public concerns regarding genetically modified organisms (Fitzgerald and Cunniffe, 2021). Additionally, while RNAi operates effectively in many insect orders such as Coleoptera, its efficiency can be inconsistent in others like Lepidoptera and Hemiptera due to barriers in dsRNA uptake and degradation (Christiaens et al., 2020). Unlike CRISPR, RNAi cannot induce permanent genomic changes or be used for precise gene insertions or deletions. Therefore, although both technologies have transformative potential, RNAi currently offers a more environmentally benign, reversible, and species-specific alternative for pest control, while CRISPR remains more suited to long-term population suppression or functional genomics under controlled conditions.

Challenges and Limitations

Technical Challenges in RNAi Delivery

Effective RNAi delivery in insects faces several technical challenges, primarily due to biological barriers that vary across species. The alkaline environment of the lepidopteran midgut and the

presence of nucleases in the midgut and salivary secretions significantly degrade dsRNA, limiting its efficacy when ingested, complicating its uptake by intestinal epithelial cells (Baum & Roberts, 2014; Rahmani & Bandani, 2021; Zhang et al., 2022). Factors such as nucleases in the midgut that degrade dsRNA and the alkaline environment can hinder effective RNA delivery. The presence of nucleases in the digestive systems of Lepidoptera may degrade dsRNA before it can trigger RNAi (Baum & Roberts, 2014; Bally et al., 2018).

The efficiency of RNAi varies significantly across different insect orders, particularly between Lepidoptera and Coleoptera, due to inherent differences in their RNAi machinery. Lepidoptera possess the RNAi hallmark genes Dcr-2 and AGO2, but their RNAi response is generally weaker compared to Coleoptera, which show a robust RNAi response. In Coleoptera, dsRNA is effectively processed into siRNA, leading to target mRNA degradation. In contrast, Lepidoptera often retains dsRNA in endocytic compartments, limiting its processing. Lepidopteran species require higher concentrations of dsRNA for effective gene silencing, while Coleopterans can respond to much lower doses (Bally et al., 2018).

Additionally, the uptake of dsRNA by midgut epithelial cells is inefficient, with mechanisms of cellular uptake not yet fully understood (Baum & Roberts, 2014). In hemipteran species, such as aphids, dietary concentrations of dsRNA required for effective gene silencing are often three orders of magnitude higher than those needed for sensitive Coleopteran species, indicating a disparity in RNAi responsiveness (Baum & Roberts, 2014; Rakesh et al., 2024).

Furthermore, the stability of dsRNA in various feeding solutions can impact its effectiveness, as seen in studies with different insect species (Hu & Xia, 2019; Rakesh et al., 2024). Studies indicate that dsRNA can be rapidly degraded by nucleases in the insect midgut, limiting its efficacy when delivered orally. For instance, dsRNA targeting the V-ATPase gene has shown variable stability across different insect species, with some reports highlighting that dsRNA remains intact in specific feeding solutions, such as honey. In contrast, it degrades in others, like Luria-Bertani broth (Rahmani & Bandani, 2021).

While injection methods have proven effective for systemic gene silencing, they are impractical for field applications (Badillo-Vargas et al., 2015). Oral delivery methods, although promising, often require high concentrations of dsRNA to achieve significant gene knockdown, as seen in various studies targeting the V-ATPase gene (Guo et al., 2023; Rakesh et al., 2024). Additionally, the stability of dsRNA in the environment and its uptake by target cells remain critical issues that need to be addressed to enhance the efficacy of RNAi-based pest management strategies (Hu & Xia, 2019). Thus, optimizing delivery methods and stabilizing formulations are crucial for enhancing RNAi efficiency in pest management.

Insect Resistance to RNAi

Insect resistance to RNAi is a significant challenge in pest management, as many species exhibit varying insensitivity to environmental RNAi. For instance, while Coleopteran species like the western corn rootworm show high sensitivity to ingested dsRNA, Lepidopteran and hemipteran pests often require much higher concentrations for effective gene silencing, indicating biological barriers to RNAi efficacy (Baum & Roberts, 2014; Guo et al., 2023). The alkaline environment of the Lepidopteran midgut further complicates dsRNA delivery (Baum & Roberts, 2014). Additionally, the uptake and processing of dsRNA in Lepidopteran cells are less efficient. While Coleopteran cells can process dsRNA into small interfering RNAs (siRNAs) and degrade target mRNA, Lepidopteran cells often retain dsRNA in endocytic compartments, limiting its exposure to the RNAi machinery (Bally et al., 2018).

One of the significant challenges limiting the universal application of RNAi in pest control is the significant variability in RNAi efficiency across different insect orders. Among these, Coleopterans (e.g., *Tribolium castaneum*, *D. virgifera virgifera*) are generally highly responsive to RNAi, often exhibiting strong and systemic gene knockdown after exposure to dsRNA via feeding, injection, or topical application. This efficiency is partly attributed to their robust dsRNA uptake and processing mechanisms, which are likely linked to their defense strategies against viruses (Bally et al., 2018; Pinheiro et al., 2018). In contrast, Lepidopterans (e.g., *S. frugiperda*, *Helicoverpa armigera*) generally display weaker and more variable RNAi responses, necessitating higher dsRNA concentrations to initiate silencing. This variability is influenced by factors such as rapid dsRNA degradation by midgut nucleases, inefficient cellular uptake mechanisms, the

retention of dsRNA in endocytic compartments, which limits its processing into siRNAs, and differences in core RNAi machinery components such as SID-like (Systemic RNA interference Defective) proteins (Bally et al., 2018; Chikami et al., 2020). Differences in the efficiency of dsRNA uptake and processing, particularly reduced endosomal escape and weaker expression or functionality of key RNAi pathway components such as Dicer and Argonaute, contribute to the limited response in Lepidopterans. For instance, Lepidopterans often lack functional SID-1 homologs that facilitate dsRNA uptake and systemic spread, resulting in localized and transient RNAi responses. The presence of SID-1-like genes involved in dsRNA uptake varies among these orders and influences their RNAi responses. For instance, while sid-1-like genes are absent in Diptera, they are present in Coleoptera and some Lepidoptera. However, their presence does not always correlate with a robust RNAi response, as seen in the silkworm, *Bombyx mori* (Pinheiro et al., 2018). Additionally, the effectiveness of RNAi can be influenced by the choice of target gene, dsRNA length, and delivery method, which are critical factors in determining the success of RNAi across these insect orders (Bally et al., 2018; Zeng et al., 2021).

Hemipterans, including species like the brown planthopper (*Nilaparvata lugens*), also show variability in RNAi efficiency. While some hemipteran species can effectively utilize RNAi, others, such as specific plant bugs, are more recalcitrant, possibly due to their saliva containing nucleases that degrade dsRNA before it can be absorbed (Bally et al., 2018).

This variability has profound implications for the development of RNAi-based pest management strategies. While RNAi has shown promise as a targeted, environmentally friendly alternative to chemical insecticides in Coleopteran pests, its limited efficacy in Lepidopteran species necessitates the development of species-specific delivery systems and stability-enhancing strategies. Approaches such as dsRNA encapsulation in nanoparticles, liposomes, or chitosan-based carriers have been explored to protect dsRNA from enzymatic degradation and enhance cellular uptake in RNAi-recalcitrant insects (Christiaens et al., 2020). Furthermore, optimizing dsRNA length and sequence specificity and using RNAi enhancers (e.g., calyx fluid proteins or synergistic chemical adjuvants) could improve knockdown efficiency in less-responsive species. Understanding the molecular basis of inter-order RNAi variability is therefore critical for expanding the applicability of RNAi across pest taxa and informing regulatory frameworks and resistance management strategies.

Inefficient uptake by midgut epithelial cells also contributes to the recalcitrance observed in many insect species, as the mechanisms for dsRNA absorption remain poorly defined (Baum & Roberts, 2014; Zhang et al., 2022). Factors such as nucleases in the insect gut, which degrade dsRNA, and inefficient uptake by midgut epithelial cells contribute to this resistance (Baum & Roberts, 2014; Zhang et al., 2022). Additionally, the expression of dsRNA-degrading enzymes in certain insect species further complicates the effectiveness of RNAi-based strategies (Baum & Roberts, 2014; Rakesh et al., 2024). The presence of double-stranded RNA-degrading enzymes (dsRNases) in the midgut and salivary secretions poses a significant challenge, as these nucleases can rapidly degrade dsRNA, limiting its effectiveness (Baum & Roberts, 2014; Mohammed et al., 2015). The presence of nucleases in the digestive tract and hemolymph of Lepidopteran insects degrades double-stranded RNA (dsRNA) before it can trigger an RNAi response. This enzymatic degradation is a significant barrier, as seen in the saliva of the tarnished plant bug and the gut of the pea aphid, which contain dsRNases that break down dsRNA, preventing effective RNAi (Pan et al., 2017).

Furthermore, variations in the expression of RNAi machinery components, such as Dicer and Argonaute proteins, across different insect species may influence the efficiency of the RNAi response (Baum & Roberts, 2014).

The variability in RNAi response is also linked to the choice of target genes and the length of dsRNA, with longer dsRNAs generally being more effective in Coleopterans but not necessarily in Lepidopterans (Bally et al., 2018). Moreover, the systemic spread of RNAi, which is crucial for effective gene silencing, is less pronounced in Lepidoptera due to the absence of RNA-dependent RNA polymerase (RdRp) that could amplify the RNAi signal, a feature present in other organisms like plants and nematodes (Bally et al., 2018). These factors collectively contribute to the observed variability in RNAi efficacy across insect

orders, with Lepidoptera being notably less responsive than Coleoptera.

This interspecific variation poses a significant challenge for the widespread adoption of RNAi as a universal pest control tool. It underscores the necessity for species-specific optimization of delivery methods, dsRNA design, and formulation strategies. For Lepidoptera, efforts to improve RNAi efficiency have included co-application with dsRNase inhibitors, encapsulation of dsRNA in nanoparticles for gut protection, and the use of cell-penetrating peptides to enhance cellular uptake (Christiaens et al., 2020; Silver et al., 2021). Moreover, transcriptomic studies that characterize RNAi pathway gene expression in different species may offer insights for selecting more responsive developmental stages or target tissues. Understanding the mechanistic basis of RNAi variability will be essential for improving efficacy in recalcitrant species and designing pest-specific RNAi approaches that minimize non-target effects. Therefore, addressing RNAi variability remains a key research priority for advancing its implementation in integrated pest management (IPM) programs.

Understanding these resistance mechanisms is crucial for developing practical RNAi applications in agriculture. The development of dual-effect RNAi systems, such as those utilizing baculovirus-mediated gene silencing, can enhance the efficacy of RNAi by continuously delivering dsRNA to target insects, thereby improving the chances of overcoming resistance (Bai et al., 2023). Furthermore, employing cocktail RNAi approaches, which involve using multiple dsRNAs targeting different genes, may also help mitigate resistance by reducing the likelihood of insects developing resistance to a single target (Mohan et al., 2021). These strategies highlight the importance of innovative gene-targeting methods in enhancing the effectiveness of RNAi in pest management.

Lack of Long-Term Data

Assessing the long-term effects of RNAi on insect populations and ecosystems presents several challenges. One significant concern is the potential for non-target effects, as dsRNA can unintentionally impact beneficial insects sharing the same environment, leading to ecological imbalances (Coy et al., 2012). There is a risk that dsRNA may inadvertently silence genes in non-target species, particularly if the target sequences are conserved across species. RNAi-based insecticidal crops and pesticides can inadvertently affect non-target organisms through off-target gene silencing, immune stimulation, and saturation of the RNAi machinery, with exposure to insecticidal small RNAs occurring on a large scale. Suppose non-target species possess homologous genes (high similarity in DNA sequence) to the target genes in the pest species. In that case, there is a risk that dsRNA designed to silence insect pests could unintentionally affect those genes in non-target organisms. This cross-reactivity could lead to alterations in non-target species, potentially causing negative ecological impacts (Liu et al., 2019).

In hemipteran insects, sequence-non-specific effects have been observed, such as the suppression of aphid feeding by dsRNA, indicating that even small sequence differences can lead to unintended impacts on non-target species (Arora et al., 2021). Arora et al. demonstrated that dsRNA targeting specific genes in aphids could inadvertently suppress feeding in non-target hemipterans like whiteflies and mealybugs. However, heterologous dsRNA showed limited effects, indicating a need for sequence specificity (Arora et al., 2021). However, research on dsRNAs targeting the inhibitor of apoptosis gene in various insect species showed no significant cross-species activity, suggesting that the risk of non-target effects may be minimal when dsRNAs are highly sequence-specific (Chereddy et al., 2020). Conversely, Chen et al. reported that dsRNA targeting rpl19 from *Bactrocera dorsalis* significantly affected the expression of this gene in non-target species like *Bactrocera minax* and *Diachasmimorpha longicaudata*. However, it did not impact *A. mellifera* (Chen et al., 2015). Additionally, Rougeot et al. highlighted potential off-target effects when using GFP as a control, with differential gene expression observed in *Nasonia vitripennis*, underscoring the importance of careful experimental design in RNAi studies (Rougeot et al., 2021). While RNAi presents a promising pest management tool, its implications for non-target species require thorough investigation to mitigate unintended consequences. Furthermore, the environmental risk assessment for dsRNA-based products emphasizes the need to evaluate potential impacts on non-target arthropods, which are crucial for ecosystem services like biological pest control. This

assessment involves selecting suitable test species and conducting robust laboratory-based toxicity studies to understand the exposure and hazards posed by dsRNA applications (Romeis & Widmer, 2020).

While laboratory and field studies suggest that risks to non-target organisms are generally low, continued research is necessary to refine dsRNA design, assess long-term environmental effects, and develop targeted delivery mechanisms. Future work should focus on enhancing bioinformatics screening to eliminate potential off-target effects, investigating sub-lethal effects of RNAi on pollinators and natural enemies, and developing field-scale monitoring programs to track RNAi persistence and exposure in real-world ecosystems. Additionally, the stability of dsRNA in the environment is a critical factor; while dsRNA is readily degraded by bacterial enzymes, its persistence and efficacy in field applications remain uncertain (Baum & Roberts, 2014). The environmental stability of double-stranded RNA (dsRNA) is crucial for its effectiveness in pest control applications, particularly in the context of RNA interference (RNAi) technology. dsRNA stability can be influenced by the presence of microorganisms, which may degrade dsRNA through RNases. Honey solutions have been shown to inhibit such degradation, enhancing dsRNA stability compared to pollen solutions, which lack similar protective substances (Zhang et al., 2022).

Furthermore, the variability in RNAi sensitivity among different insect species complicates predictions about population dynamics and resistance development (Zhang et al., 2022). The slow speed-to-kill associated with RNAi may also affect its effectiveness in managing pest populations, as insects may reproduce before the effects manifest (Baum & Roberts, 2014). These factors necessitate careful evaluation and monitoring to understand the broader ecological implications of RNAi-based pest control strategies.

Ethical concerns

Using genetically modified organisms (GMOs) for RNAi delivery, particularly transgenic plants expressing dsRNA, raises significant ethical considerations that must be addressed alongside their scientific and environmental implications. One major concern is the potential unintended effects on non-target organisms and ecosystems, as the persistence and mobility of RNAi molecules in the environment remain active research areas. Additionally, the public perception and acceptance of GMOs continue to be contentious, with debates surrounding food safety, labeling, and consumer rights. Ethical discussions also extend to the intellectual property and socioeconomic impact of RNAi-based GM crops, as biotechnology patents held by large corporations may limit access for smallholder farmers and influence global food security. Furthermore, regulatory disparities between countries create challenges in international trade and agricultural policy, necessitating harmonized frameworks for assessing risks and benefits. Given these ethical complexities, it is essential to implement transparent risk assessment protocols, engage stakeholders in decision-making, and promote interdisciplinary dialogue to ensure that RNAi-based GMOs are developed and deployed responsibly (Zeng et al., 2021; Bai et al., 2023).

The use of genetically modified organisms (GMOs) for RNA interference (RNAi) delivery, mainly through transgenic plants, faces significant public hesitance due to concerns about safety and environmental impact. While RNAi technology is considered environmentally friendly and has shown potential in pest control, the acceptance of transgenic plants remains low among the public, primarily due to fears surrounding genetic modification and its implications for food safety (Bai et al., 2023). Regulatory challenges also complicate the commercialization of these technologies, as evidenced by the approval processes for transgenic crops like SmartStax PRO, which target specific pests (Zeng et al., 2021). Furthermore, ethical frameworks surrounding the use of GMOs must address public concerns and the need for transparent communication regarding the benefits and risks associated with RNAi applications in agriculture (Mohammed et al., 2015).

Despite the promising potential of RNAi-based pest control strategies, particularly those utilizing transgenic plants for in planta expression of double-stranded RNA (dsRNA), significant ecological and ethical concerns remain. One of the foremost ethical issues revolves around using genetically modified organisms (GMOs) for environmental release. While transgenic crops expressing dsRNA offer species-specific targeting and reduce reliance on chemical insecticides, they raise questions about unintended ecological

effects, such as horizontal gene transfer, off-target impacts on non-target organisms (e.g., beneficial insects, soil microbes), and the long-term evolutionary consequences on pest populations (Christiaens et al., 2020). Additionally, the persistence of dsRNA in the environment and its potential accumulation through trophic levels have yet to be comprehensively assessed. These concerns underscore the importance of rigorous ecological risk assessments prior to the widespread adoption of transgenic RNAi crops in open agricultural systems.

Equally critical are the societal and ethical dimensions associated with public perception and regulatory oversight of RNAi-based GMOs. The deployment of RNAi through transgenic plants is often met with skepticism by the public, especially in regions with strong anti-GMO sentiment or where transparent regulatory frameworks are lacking. Ethical considerations include the right to informed consumer choice, intellectual property restrictions that may affect smallholder farmers, and potential economic monopolization by biotech companies. Regulatory agencies across different countries vary in their assessment protocols and definitions of genetic modification, with some classifying RNAi crops under existing GMO legislation. In contrast, others propose RNAi-specific regulatory pathways (Roberts et al., 2015). To foster responsible development and societal acceptance, future work must engage stakeholders through inclusive dialogue, transparent communication of risks and benefits, and developing context-sensitive ethical frameworks that balance innovation with biosafety and public trust.

Future Directions

Despite promising advances in insect RNAi, several key challenges must be addressed to enhance its field applicability. One major focus should be on improving dsRNA stability. Currently, dsRNA is prone to degradation by environmental nucleases and adverse weather conditions, which limits its persistence and efficacy in open-field applications. Research efforts are increasingly turning to nanoparticle-based delivery systems, such as liposomes, polymeric nanoparticles, or clay-based carriers, that can encapsulate dsRNA and protect it from rapid degradation. In parallel, chemical modifications like 2'-O-methylation or the incorporation of phosphorothioate linkages are being explored to enhance molecular stability without impairing gene-silencing efficiency. Both strategies warrant systematic evaluation under controlled laboratory conditions and subsequent field trials to determine their effectiveness and scalability.

In addition, enhancing the overall RNAi efficiency in insects remains a critical goal. Variability in RNAi responses among insect orders suggests that intrinsic factors, such as dsRNA uptake mechanisms and the activity of dsRNases, may limit the success of RNAi interventions. Future studies should therefore investigate RNAi enhancers or synergists that could improve cellular uptake and protect dsRNA from enzymatic degradation. Methodologies may include the co-delivery of uptake-promoting peptides or small-molecule inhibitors of dsRNases and high-throughput screening to identify novel enhancers. Complementary transcriptomic and proteomic analyses could elucidate the underlying molecular pathways that govern RNAi efficacy, providing a foundation for the rational design of more potent RNAi formulations.

Future research in insect RNAi must prioritize enhancing dsRNA stability and delivery efficiency under field conditions. One promising avenue is the development of nanoparticle-based delivery systems that protect dsRNA molecules from environmental degradation by UV radiation, nucleases, and extreme pH conditions. Nanocarriers such as layered double hydroxide (LDH) nanosheets (e.g., BioClay), liposomes, chitosan nanoparticles, and carbon quantum dots have been shown to significantly improve dsRNA stability and facilitate cellular uptake in both plants and insects (Mitter et al., 2017; Christiaens et al., 2020). Additionally, chemical modifications of dsRNA, such as incorporating phosphorothioate linkages, 2′-O-methyl RNA, or locked nucleic acids (LNAs), can improve nuclease resistance and enhance persistence without compromising RNAi efficacy. These strategies offer promising tools for optimizing topical and systemic delivery methods suitable for large-scale agricultural use. However, their biosafety, cost-effectiveness, and regulatory acceptance require comprehensive evaluation before field deployment.

Another critical area for future research is the identification and application of RNAi enhancers

and synergists that can potentiate gene silencing, especially in insect orders less responsive to RNAi, such as Lepidoptera and Hemiptera. Co-application of RNAi with nanoparticles, endosomal escape agents (e.g., cationic polymers, cell-penetrating peptides), or dsRNase inhibitors may enhance intracellular dsRNA stability and endosomal release (Shukla et al., 2016). Exploring host-pathogen interactions and natural RNAi regulators, such as SID-like (Systemic RNA Interference Defective) proteins and RNA transporters, could improve systemic RNAi efficacy. Moreover, transcriptomic and proteomic profiling of RNAi-resistant insect strains may help uncover genetic and epigenetic barriers to RNAi, enabling targeted solutions to overcome them. Future studies should also integrate CRISPR-based tools for functional screening of RNAi pathway components, potentially allowing for the genetic enhancement of RNAi susceptibility in pest populations. Together, these directions will pave the way toward more reliable, efficient, and field-ready RNAi technologies for sustainable pest management.

Together, these future research directions aim to overcome current limitations, thereby facilitating the development of robust, sustainable RNAi-based pest management strategies. Such advances are expected to improve RNAi's reliability and cost-effectiveness, ultimately supporting its integration into integrated pest management programs.

Conclusions

The future of ATPase RNAi in insect pest control appears promising, particularly in identifying specific ATPase subunits as effective targets. Studies have shown that silencing genes encoding V-ATPase can lead to significant mortality in various insect pests, highlighting the potential for developing RNAi-based biopesticides that are both effective and environmentally safe. Furthermore, the ability to deliver double-stranded RNA (dsRNA) through various methods, such as oral feeding and topical application, enhances the practicality of these strategies in agricultural settings. As research continues to refine delivery systems and target gene selection, ATPase RNAi could become a cornerstone of integrated pest management, offering a targeted approach that minimizes harm to non-target species. However, challenges remain, such as ensuring effective delivery systems and overcoming transient RNAi effects.

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Table 1. RNAi delivery methods and their pros/cons

Delivery Method	Description	Advantages	Disadvantages

Microinjection	Direct injection of dsRNA into the insect's body.	- High knockdown efficiency - Precise dosage control - Rapid gene silencing	- Labor-intensive - Invasive, may cause injury - Impractical for large-scale/field use
Feeding (Oral Delivery)	Incorporation of dsRNA into diets or feeding solutions.	- Non-invasive - Scalable and field-applicable - Cost-effective	- Lower efficiency - dsRNA degradation by digestive enzymes - Requires higher doses
Topical Application	Direct application of dsRNA onto the insect's exoskeleton.	- Non-invasive - Suitable for high-throughput screening - Field applicability	- Variable uptake and penetration - Generally lower and inconsistent efficiency
Transgenic Plants	Plants engineered to produce dsRNA targeting pest genes.	- Continuous, in situ delivery - Integrated into pest management systems - Sustainable	- Regulatory hurdles - Potential off-target effects - Public acceptance issues
Bacterial Systems	dsRNA produced in bacteria that is then applied or ingested by insects.	- Cost-effective production - Enhanced stability of dsRNA - Mass production potential	- Variable uptake - Environmental persistence/regulatory concerns
Recombinant Baculoviruses	Viral vectors engineered to express and deliver dsRNA to insects.	- High specificity - Sustained gene silencing - Systemic delivery	- Limited host range - Challenges in field (oral) delivery - Biosafety concerns
Nanoparticle-mediated	dsRNA complexed with nanoparticles for improved delivery.	- Protects dsRNA from degradation - Enhances cellular uptake - Potential for targeted delivery	- Complex formulation - Possible toxicity - Increased production cost

Table 2. Comparison of dsRNA delivery methods for insect pest management in large-scale agricultural applications

Delivery Method	Scalability	Cost-Effectiveness	Feasibility in Real-World Settings	Key Challenges/Considerations
Microinjection	Very low – highly labor-intensive and impractical for field-scale application	Low – high labor and operational costs due to the need for individual handling	Impractical – excellent for lab studies but not deployable on a large scale	Not scalable; requires technical precision; invasive; unsuitable for small and numerous insect populations
Feeding (Oral Delivery)	Moderate to high – can be integrated with existing spraying systems or bait stations; adaptable via transgenic approaches	Moderate – cost savings possible with in planta or bacterially produced dsRNA; however, higher doses/formulations may be needed to overcome degradation	Promising – widely applicable via foliar sprays or bait; environmental factors (UV, rain, gut enzymes) can affect dsRNA stability	dsRNA degradation in the digestive system; formulation improvements needed to protect dsRNA; variable efficacy across species
Topical Application	High – utilizes existing agricultural spraying equipment to cover large areas	Potentially high – non-invasive and lower labor costs; however, protective carriers (e.g., nanoparticles) may increase costs	Encouraging – with optimized formulations that improve dsRNA stability and cuticular penetration, this method can be field-applied	Variable uptake due to differences in insect cuticle; environmental degradation (UV, rainfall) may reduce dsRNA persistence and efficacy
Transgenic Plants	High – once developed, plants continuously produce dsRNA and can cover extensive acreage	Potentially cost-effective over time – initial R&D and regulatory costs are high, but ongoing production is self-sustaining	Promising – integration into crop systems offers continuous protection; however, regulatory hurdles and public acceptance issues must be managed	High initial development costs; regulatory and biosafety concerns; potential off-target effects; gene flow risks and public acceptance issues
Bacterial Systems	Moderate to high – bacteria can be mass-produced in fermentation systems and formulated for field application	High – low production costs and scalable fermentation; can be an economical method for dsRNA synthesis	Feasible – can be applied as sprays or incorporated into bait formulations; however, formulation and environmental persistence need optimization	Consistency of dsRNA expression; stability of dsRNA in the environment; potential environmental persistence and off-target effects
Baculoviruses	Moderate – production is scalable in cell culture but the host range is limited	Moderate – production costs are higher than bacterial systems; however, efficient gene silencing can offset some costs	Promising for targeted applications – offers sustained gene silencing; challenges remain in achieving effective field (oral) delivery and biosafety considerations	Limited host range; field delivery challenges; biosafety and regulatory concerns; stability and genetic consistency during mass production

Table 3. Comparative Analysis of Pest Control Strategies

Feature / Parameter	RNA Interference (RNAi)	Chemical Insecticides	Biological Control	CRISPR-based Gene Editing
Efficacy	High target-specific efficacy if dsRNA delivery is optimized.	Generally high, but prone to resistance development.	Variable; depends on ecological compatibility and agent success.	Potentially high; gene knockout can cause sterility or death.
Cost	Moderate to high; production and formulation of dsRNA is costly but decreasing with new technologies.	Low to moderate; cheap production, but recurring applications needed.	Moderate; one-time cost for agent introduction but long-term monitoring required.	High; lab-based genome editing and delivery are expensive.
Scalability	Emerging field; scalability improving with nanoparticle and plant-based dsRNA delivery.	Highly scalable and widely used.	Limited; often region- and crop-specific.	Limited in field use; still in research and early trial stages.
Specificity	Very high; gene- and species-specific with minimal off-target effects.	Low; broad-spectrum, affects non-target organisms.	Moderate to high; depends on host specificity of biocontrol agents.	High, but off-target effects are still a concern.
Environmental Impact	Low; biodegradable and non-toxic to non-targets when well designed.	High; residues persist in the environment and affect biodiversity.	Low; eco-friendly, but ecological balance may be disturbed.	Unknown; potential unintended ecological consequences.
Resistance Development	Low to moderate; can be mitigated with gene rotation strategies.	High; rapid evolution of resistance observed in many pests.	Low; less common due to biological adaptation.	Unknown; long-term resistance patterns are still being studied.
Regulatory Challenges	Moderate; varies by country, with increasing acceptance.	Low; well-established regulatory frameworks.	Moderate; import and release of non-native species are regulated.	High; strict regulations and public concerns over GMOs.

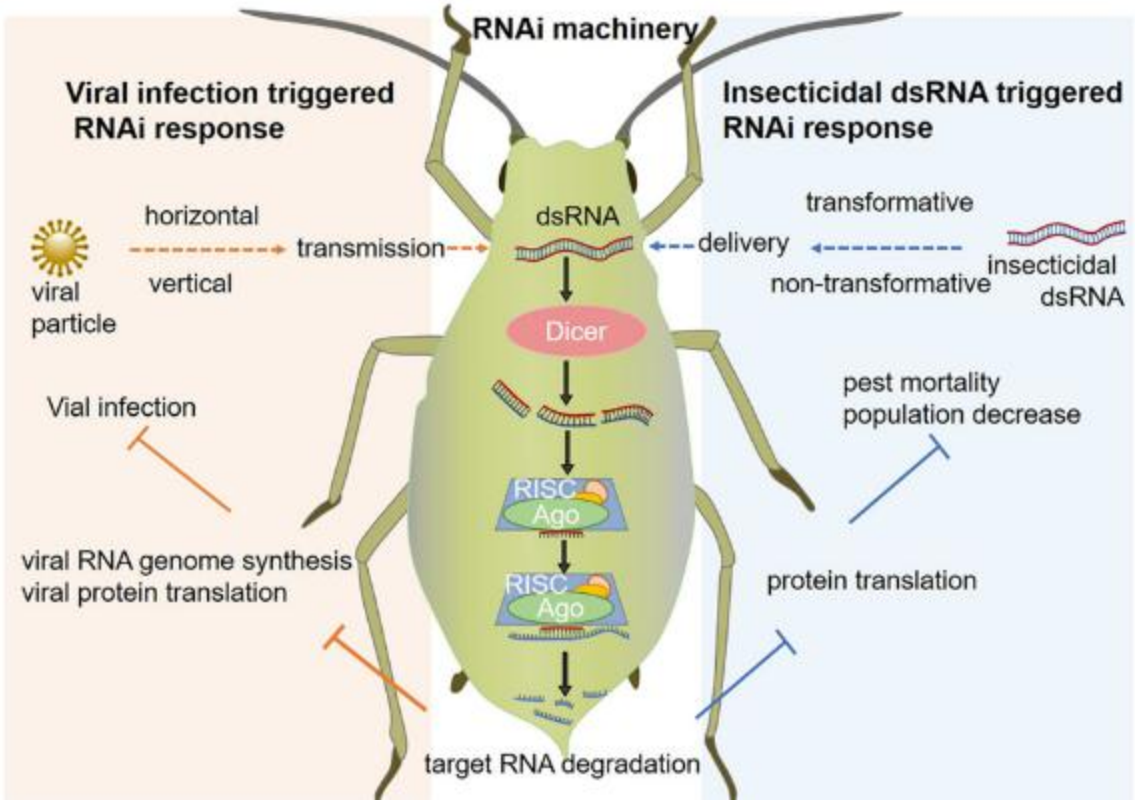

Fig 1. RNAi in antiviral defense mechanisms and its application as a pest control strategy (Niu et al., 2024).

The RNAi system is a natural defense against viral infections, activated upon detecting double-stranded RNA (dsRNA) signatures associated with viruses, such as those generated during viral genome replication. The enzyme Dicer processes These viral dsRNAs into small interfering RNAs (siRNAs). The resulting single-stranded siRNAs then combine with Argonaute (Ago) proteins to form the RNA-induced silencing complex (RISC), facilitating the specific degradation of viral genomic RNAs and their transcripts. Similarly, introducing insecticidal dsRNA externally engages the RNAi machinery, targeting mRNAs of genes critical for developing or surviving pests. The subsequent degradation of these target mRNAs leads to developmental stasis and increased mortality, thereby decreasing pest populations.

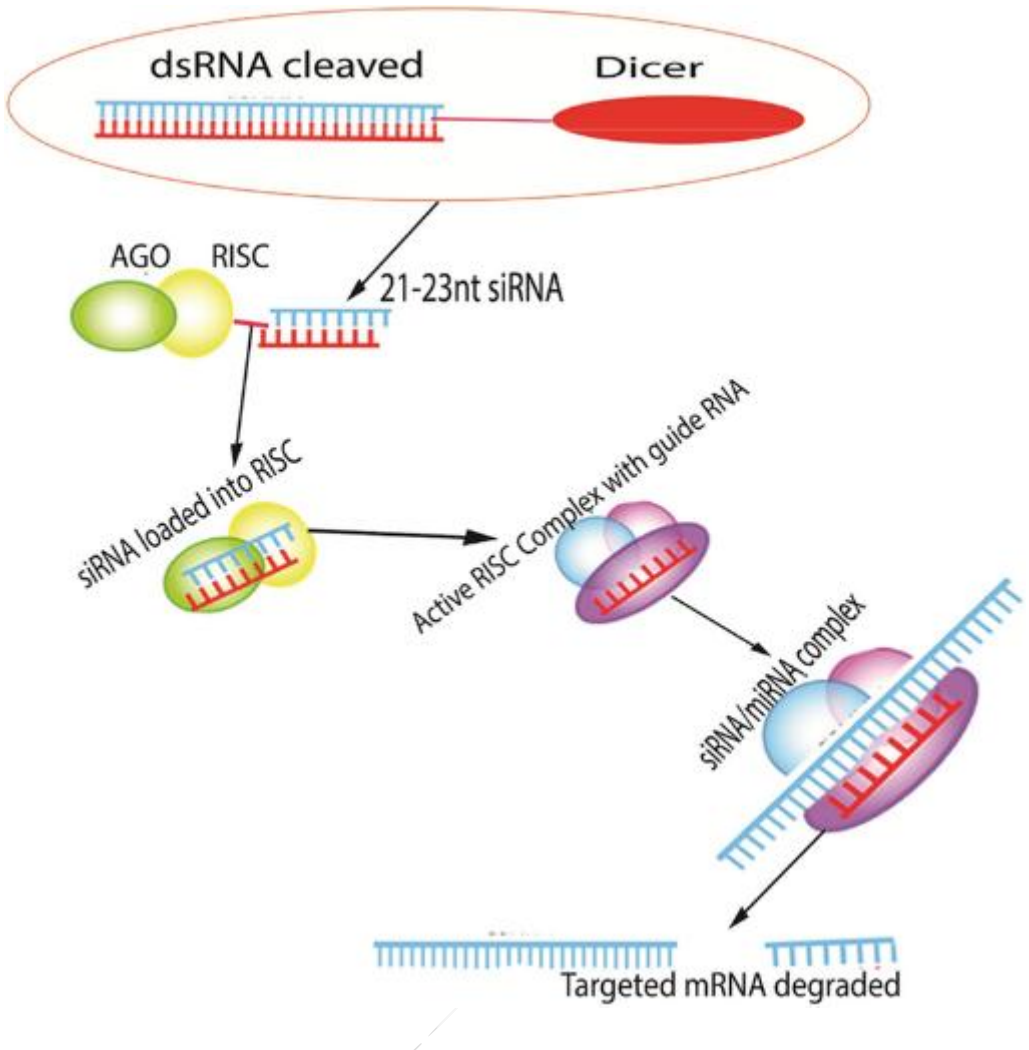

Fig 2. The RNAi mechanism in insects (Adeyinka et al., 2020).

The lengthy double-stranded RNA (dsRNA) undergoes cleavage by Dcr2. The small interfering RNA (siRNA) derived from the DICER-cleaved dsRNA is subsequently incorporated into the RNA-induced silencing complex (RISC) with the assistance of Argonaute (Ago) enzymes. The guide siRNA then hybridizes with complementary mRNA sequences, leading to the degradation of the mRNA and the suppression of protein expression.