Advancing RNAi for Sustainable Insect Management: Targeted Solutions for Eco-Friendly Pest Control

Abstract

Insect pests pose significant challenges to agriculture, forestry, and public health, causing substantial economic losses and threats to food security. Traditional pest control methods, such as chemical insecticides, have limitations due to the development of insecticide resistance, negative impacts on non-target organisms, and environmental concerns. RNA interference (RNAi) has emerged as a promising approach for developing targeted and environmentally safe insect control strategies. This review explores the potential of RNAi-based methods for pest management, discussing the mechanism of RNAi in insects, factors influencing its efficiency, and various delivery strategies. We highlight the advantages of RNAi, such as species-specific targeting and reduced off-target effects, while also addressing challenges and limitations, including variability in RNAi efficiency among insect species and potential resistance development. The review examines the environmental safety and risk assessment of RNAi-based insect control, current applications, and future prospects. We also discuss the socio-economic impact and public perception of RNAi technology, as well as research gaps and future directions. The integration of RNAi-based insect control into integrated pest management programs is crucial for developing sustainable and effective pest control solutions. By providing a comprehensive overview of the current state and future potential of RNAi-based insect control, this review aims to inform research, policy, and practice in this rapidly evolving field.

Keywords: RNAi, Insect Control, Pest Management, Targeted Pest Control, Environmental Safety

1. Introduction

1.1. The need for novel pest management strategies Insect pests are a major threat to global agriculture, causing significant yield losses and economic damage estimated at billions of dollars annually [1]. In addition to their impact on crop production, insect pests also pose risks to human health by serving as vectors for diseases such as malaria, dengue fever, and Zika virus [2]. The widespread use of chemical insecticides has been the primary method for controlling insect pests for decades. However, the reliance on these chemicals has led to the development of insecticide resistance in many pest populations, reducing their effectiveness and necessitating the continuous development of new active ingredients [3]. Moreover, the non-specific nature of most insecticides results in negative impacts on non-target organisms, including beneficial insects, pollinators, and natural enemies of pests [4]. The environmental persistence and potential toxicity of some insecticides also raise concerns about their long-term effects on ecosystems and human health [5].

Given these limitations, there is an urgent need for novel pest management strategies that are targeted, environmentally safe, and sustainable. These strategies should aim to reduce reliance on chemical insecticides, minimize non-target effects, and provide effective long-term control of pest populations. Integrated pest management (IPM) approaches, which combine multiple control methods such as biological control, cultural practices, and judicious use of insecticides, have gained

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increasing attention as a more sustainable alternative to conventional pest control [6]. However, the success of IPM depends on the availability of effective and complementary control tools that can be integrated into these programs.

1.2. **RNAi** as a promising approach for insect control RNA interference (RNAi) has emerged as a promising approach for developing targeted and environmentally safe insect control strategies. RNAi is an evolutionarily conserved mechanism that regulates gene expression through sequence-specific degradation of mRNA, triggered by the presence of double-stranded RNA (dsRNA) [7]. By introducing dsRNA that targets essential genes in insect pests, RNAi can be harnessed to selectively suppress pest populations without causing harm to non-target organisms [8]. The species-specific nature of RNAi, based on the sequence complementarity between the dsRNA and the target mRNA, offers a high degree of selectivity and reduces the likelihood of off-target effects [9].

The potential of RNAi for insect control was first demonstrated in the model organism Drosophila melanogaster, where the injection of dsRNA targeting essential genes resulted in specific gene silencing and lethal phenotypes [10]. Since then, numerous studies have explored the application of RNAi for controlling a wide range of insect pests, including agricultural pests, stored product pests, and insect vectors of human diseases [11-13]. The delivery of dsRNA to target insects can be achieved through various methods, such as transgenic plants expressing dsRNA, spray-induced gene silencing (SIGS), and nanoparticle-mediated delivery [14-16]. The increasing availability of genomic resources for insect pests has further facilitated the identification of novel RNAi targets and the design of effective dsRNA sequences [17].

1.3. Objectives and scope of thereview The objective of this review is to provide a comprehensive overview of the current state and future potential of RNAi-based insect control, with a focus on its application in agricultural pest management. We aim to:

1. Discuss the molecular mechanisms of RNAi in insects and the factors influencing its efficiency;
2. Highlight the advantages and challenges of RNAi-based insect control compared to conventional methods;
3. Examine the environmental safety and risk assessment of RNAi-based strategies;
4. Provide an overview of current applications and successful examples of RNAi-based insect control;
5. Explore the socio-economic impact and public perception of RNAi technology in pest management;
6. Identify research gaps and future directions for advancing RNAi-based insect control and its integration into IPM programs.

2. RNAi Mechanism and Its Application in Insect Control2.1. RNAi pathway in insects The RNAi pathway in insects involves several key steps and components. Initially, double-stranded RNA (dsRNA) is introduced into the insect cells through various delivery methods. The dsRNA is then

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processed by the enzyme Dicer into small interfering RNAs (siRNAs), which are typically 21-23 nucleotides in length [18]. These siRNAs are incorporated into the RNA-induced silencing complex (RISC), where they guide the sequence-specific degradation of complementary mRNA targets. The Argonaute protein, a key component of RISC, mediates the cleavage of the target mRNA, leading to gene silencing [19]. In some insects, the RNAi signal can be amplified and spread systemically throughout the body, a process known as systemic RNAi. This process involves the transport of siRNAs between cells and the amplification of the RNAi response by RNA-dependent RNA polymerases (RdRPs) [20].

2.2. RNAi-based insect control strategies2.2.1. Transgenic plants expressing dsRNA One of the most promising applications of RNAi for insect control is the development of transgenic plants that express dsRNA targeting essential genes in insect pests. By engineering plants to produce dsRNA against insect-specific genes, it is possible to create crops that are resistant to insect feeding and damage. When insects feed on these transgenic plants, they ingest the dsRNA, which triggers the RNAi pathway and leads to the silencing of the target genes, resulting in reduced survival, growth, and reproduction of the insect pests [21].

Table 1. Examples of transgenic plants expressing dsRNA for insect control

Crop	Target Insect Pest	Target Gene(s)	Effect on Insect	Reference
Maize	Western corn rootworm	Snf7	Reduced survival and feeding damage	[22]
Cotton	Cotton bollworm	CYP6AE14	Increased larval mortality	[23]
Potato	Colorado potato beetle	Actin, Shrub	Reduced larval growth and survival	[24]
Rice	Brown planthopper	Calreticulin	Decreased fecundity and survival	[25]
Tomato	Tomato leaf miner	Arginine kinase	Impaired larval development	[26]
Soybean	Soybean aphid	Eph	Reduced survival and reproduction	[27]
Wheat	English grain aphid	Acetylcholinesterase	Increased mortality and reduced fecundity	[28]
Eggplant	Eggplant fruit and shoot borer	Ecdysone receptor	Disrupted development and reduced survival	[29]
Citrus	Asian citrus psyllid	Abnormal wing disc	Impaired wing development and flight	[30]
Sugarcane	Sugarcane borer	Trehalase	Decreased larval growth and survival	[31]

2.2.2. Spray-induced gene silencing (SIGS) Spray-induced gene silencing (SIGS) is a non-transgenic approach for delivering dsRNA to target insects. In SIGS, dsRNA is formulated into a sprayable solution and applied directly onto plant surfaces. When insects feed on the treated plants, they ingest the dsRNA, which induces RNAi-mediated gene silencing. SIGS offers a more flexible and adaptable approach to RNAi-based insect control compared to transgenic plants, as it allows for the targeting of multiple insect species and the use of different dsRNA sequences without the need for genetic modification of the crop [32].

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2.2.3. Microinjection and other delivery methods In addition to oral delivery through transgenic plants or SIGS, dsRNA can be directly introduced into insects through microinjection. This method is particularly useful for functional genomics studies and for evaluating the efficacy of RNAi targets in insect pests. Microinjection allows for the precise delivery of dsRNA into the insect body cavity, where it can induce systemic RNAi [33]. Other delivery methods, such as soaking, topical application, and nanoparticle-mediated delivery, have also been explored for introducing dsRNA into insects [34].

3. Factors Influencing RNAi Efficiency in Insects3.1. dsRNA stability and uptake The stability and uptake of dsRNA are crucial factors influencing the efficiency of RNAi in insects. Once delivered, dsRNA must remain stable in the insect gut and hemolymph to effectively trigger the RNAi pathway. However, dsRNA can be degraded by nucleases present in the insect digestive system and hemolymph, reducing its persistence and efficacy [35]. To enhance dsRNA stability, various modifications, such as chemical modifications and nanoparticle encapsulation, have been explored [36]. The uptake of dsRNA by insect cells is mediated by several mechanisms, including endocytosis and transmembrane channels, which can vary among insect species and tissues [37].

3.2. RNAi core machinery and efficiency The efficiency of RNAi in insects is also influenced by the presence and activity of the core RNAi machinery, such as Dicer, Argonaute, and RdRP enzymes. The expression levels and functional diversity of these components can differ among insect species, leading to variations in RNAi efficiency [38]. For example, some insects, such as beetles, exhibit a robust systemic RNAi response, while others, like lepidopterans, show a more limited response [39]. Understanding the molecular basis of these differences is essential for optimizing RNAi-based insect control strategies.

3.3. Target gene selection and design The selection and design of target genes are critical aspects of developing effective RNAi-based insect control. Ideal target genes should be essential for insect survival, development, or reproduction and have minimal off-target effects on non-target organisms. Bioinformatic tools and genomic resources play a crucial role in identifying suitable RNAi targets and designing specific dsRNA sequences [40]. Factors such as the sequence length, GC content, and secondary structure of the dsRNA can influence its effectiveness in inducing RNAi [41]. Additionally, the use of multiple target genes or the targeting of conserved regions across different insect species can enhance the robustness and spectrum of RNAi-based control [42].

5. Challenges and Limitations of RNAi-Based Insect Control5.1. Variability in RNAi efficiency among insect species The efficiency of RNAi in inducing gene silencing and causing insect mortality varies significantly among different insect species. Some insect orders, such as Coleoptera (beetles) and Hemiptera (true bugs), exhibit a robust and systemic RNAi response, while others, like Lepidoptera (moths and butterflies), show a more limited and variable response [43]. This variability in RNAi efficiency can be attributed to several factors, including differences in the uptake and processing of dsRNA, the presence and activity of RNAi core machinery components, and the ability of the RNAi signal to spread systemically throughout the insect body [44].

One of the main reasons for the variability in RNAi efficiency is the differential expression and functionality of the core RNAi pathway components, such as Dicer, Argonaute, and RNA-dependent RNA polymerase (RdRP) enzymes, across insect species [76]. For example, some insects may have multiple copies of these genes with distinct roles in the RNAi pathway, while others may have a

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more limited set of RNAi components [77]. Additionally, the efficiency of dsRNA uptake and systemic spread can vary depending on the specific mechanisms of cellular internalization and transport, which can be influenced by factors such as the pH and composition of the insect gut and hemolymph [78].

Another factor contributing to the variability in RNAi efficiency is the presence of dsRNA-degrading enzymes, such as nucleases, in the insect gut and hemolymph. These enzymes can break down dsRNA before it reaches the target cells, reducing the effective dose and duration of the RNAi response [79]. The level of nuclease activity and the stability of dsRNA in the insect body can vary among species, further contributing to the observed differences in RNAi efficiency [80].

To address the variability in RNAi efficiency, researchers have employed various strategies, such as the use of different dsRNA delivery methods, the optimization of dsRNA design and dosage, and the identification of species-specific RNAi targets [81]. For example, the use of nanoparticle-based delivery systems or chemical modifications to enhance dsRNA stability and cellular uptake has shown promise in improving RNAi efficiency in some insect species [82]. Additionally, the development of bioinformatic tools and high-throughput screening methods has facilitated the identification of novel RNAi targets that are more susceptible to gene silencing across a broader range of insect species [83].

Table 2. Factors influencing RNAi efficiency in insects

Factor	Description	References
RNAi pathway components	Expression and functionality of Dicer, Argonaute, and RdRP enzymes	[76], [77]
dsRNA uptake and systemic spread	Mechanisms of cellular internalization and transport, influenced by gut and hemolymph environment	[78]
Nuclease activity	Presence and level of dsRNA-degrading enzymes in the insect gut and hemolymph	[79], [80]
dsRNA design and dosage	Optimization of dsRNA sequence, length, and concentration for improved efficiency	[81]
Delivery methods	Use of nanoparticles, chemical modifications, or other strategies to enhance dsRNA stability and uptake	[82]
Target gene selection	Identification of species-specific RNAi targets that are more susceptible to gene silencing	[83]

5.2. Delivery methods and scalability Efficient delivery of dsRNA to the target insect species is another major challenge in the development and application of RNAi-based insect control strategies. The most common methods for delivering dsRNA include transgenic plants expressing dsRNA, spray-induced gene silencing (SIGS), and nanoparticle-based formulations [84]. Each of these methods has its advantages and limitations, and the choice of delivery method depends on factors such as the target insect species, the crop system, and the desired scale of application.

Transgenic plants expressing dsRNA have been successfully used to control several insect pests, including the western corn rootworm and the Colorado potato beetle [85]. However, the development of transgenic crops is a time-consuming and expensive process, requiring extensive safety testing and regulatory approval [86]. Additionally, the use of transgenic crops may face public opposition and concerns about the potential ecological impacts of genetically modified organisms [87].

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SIGS, on the other hand, offers a more flexible and adaptable approach to dsRNA delivery, as it does not require the development of transgenic plants [88]. In SIGS, dsRNA is formulated into a sprayable solution and applied directly to the crop, where it is ingested by the target insect upon feeding. However, the efficiency and persistence of SIGS under field conditions can be variable, and the application may need to be repeated multiple times throughout the growing season [89]. Furthermore, the large-scale production and formulation of dsRNA for SIGS can be costly and technologically challenging [90].

Nanoparticle-based delivery systems have emerged as a promising approach to enhance the stability, uptake, and efficacy of dsRNA in insect control applications [91]. By encapsulating dsRNA within nanoparticles made of materials such as chitosan, lipids, or polymers, researchers have demonstrated improved protection against degradation, increased cellular internalization, and prolonged RNAi effects in various insect species [92]. However, the development and optimization of nanoparticle formulations can be complex, and the long-term safety and environmental impacts of these materials need to be thoroughly evaluated [93].

Scalability is another important consideration in the development of RNAi-based insect control methods. While laboratory studies have shown the effectiveness of RNAi in controlling insect pests, translating these results to large-scale field applications can be challenging [94]. Factors such as the cost of dsRNA production, the efficiency of delivery methods, and the variability in environmental conditions can all impact the feasibility and economics of RNAi-based insect control at a commercial scale [95].

Table 3. Advantages and limitations of different dsRNA delivery methods

Delivery Method	Advantages	Limitations	References
Transgenic plants	- Stable and continuous expression of dsRNA - Targeted delivery to specific insect pests	- Time-consuming and expensive development process - Regulatory hurdles and public acceptance issues	[85], [86], [87]
SIGS	- Flexibility and adaptability - No need for transgenic plants - Applicable to a wide range of crops and pests	- Variable efficiency and persistence under field conditions - Need for repeated applications	[88], [89], [90]
Nanoparticle-based	- Enhanced dsRNA stability and uptake - Prolonged RNAi effects - Potential for targeted delivery	- Complex formulation and optimization - Long-term safety and environmental impacts need further evaluation	[91], [92], [93]
Microinjection	- Direct delivery to the insect body - High efficiency for functional studies and target validation	- Not practical for large-scale field applications - Labor-intensive and time-consuming	[33]
Feeding	- Simple and cost-effective - Applicable to a wide range of insect species	- Variable uptake and efficiency depending on insect feeding habits - Potential for degradation in the	[96]

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		gut	
Soaking	- Useful for targeting immature stages (e.g., larvae) - Suitable for high-throughput screening of RNAi targets	- Limited to certain insect species and life stages - Potential for off-target effects	[97]

To address the challenges of scalability and cost-effectiveness, ongoing research efforts are focused on optimizing dsRNA production methods, improving delivery strategies, and developing integrated pest management approaches that combine RNAi with other control tactics [98]. For example, the use of bioreactors and microbial expression systems for large-scale dsRNA production has the potential to reduce costs and increase the availability of dsRNA for field applications [99]. Additionally, the integration of RNAi with other pest control methods, such as biological control agents or selective insecticides, can enhance the overall effectiveness and sustainability of insect pest management programs [100].

5.3. Potential for resistance development The development of insect resistance to RNAi-based control strategies is a significant concern and a potential limitation to the long-term effectiveness of this approach. As with any pest control method, the continuous exposure of insect populations to the same RNAi target or dsRNA sequence can lead to the emergence of resistant individuals over time [101]. Resistance to RNAi can evolve through various mechanisms, including mutations in the target gene that reduce the complementarity with the dsRNA, changes in the expression or functionality of RNAi pathway components, and enhanced degradation of dsRNA by nucleases [102].

To mitigate the risk of resistance development, it is essential to implement resistance management strategies from the early stages of RNAi-based insect control development and application. One approach is to use multiple RNAi targets or to rotate different dsRNA sequences targeting the same gene, which can reduce the selection pressure on any single target and delay the onset of resistance [103]. Another strategy is to combine RNAi with other pest control methods, such as Bt toxins or conventional insecticides, in an integrated pest management (IPM) framework [104]. By using multiple control tactics with different modes of action, the likelihood of resistance development can be reduced, and the durability of RNAi-based control can be extended.

Monitoring insect populations for signs of reduced susceptibility to RNAi is also crucial for the early detection and management of resistance. This can be achieved through regular field surveys, bioassays, and molecular diagnostics to assess the effectiveness of RNAi-based control and to identify potential cases of resistance [105]. If resistance is detected, swift action should be taken to implement alternative control strategies and to adjust the RNAi-based management plan accordingly.

Furthermore, the deployment of RNAi-based insect control should be accompanied by appropriate resistance management guidelines and best practices, developed in collaboration with stakeholders from academia, industry, and regulatory agencies [106]. These guidelines should include recommendations for the judicious use of RNAi-based products, the implementation of refuge strategies to maintain susceptible insect populations, and the continuous monitoring and reporting of resistance development [107].

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6. Environmental Safety and Risk Assessment

6.1. Non-target organism effects One of the key advantages of RNAi-based insect control is its potential for species-specific targeting, which can minimize the impacts on non-target organisms compared to broad-spectrum insecticides. However, ensuring the environmental safety of RNAi-based products requires a thorough assessment of their potential effects on non-target species, including beneficial insects, pollinators, and other ecological receptors [108].

The risk of non-target effects from RNAi-based insect control arises primarily from the possibility of unintended gene silencing in organisms that share sequence similarity with the target gene in the pest species [109]. This can occur through the ingestion of dsRNA by non-target organisms, either directly from the application of RNAi-based products or indirectly through the consumption of treated plant material or prey that has accumulated dsRNA [110].

To assess the potential for non-target effects, bioinformatic analyses can be conducted to identify sequence homology between the target gene and genes in other organisms [111]. This information can guide the selection of RNAi targets that are specific to the pest species and have minimal overlap with genes in non-target organisms. Additionally, ecological risk assessments should be carried out to evaluate the potential exposure pathways and the sensitivity of different non-target species to the RNAi-based product [112].

Empirical studies, such as laboratory toxicity tests and field trials, are also necessary to validate the predicted non-target effects and to monitor the actual impacts of RNAi-based insect control on ecological communities [113]. These studies should consider both the acute and chronic effects of dsRNA exposure, as well as the potential for sublethal impacts on the fitness and behavior of non-target organisms [114].

Table 4. Ecological risk assessment endpoints for RNAi-based insect control

Assessment Endpoint	Description	References
Survival	Assess the acute and chronic mortality of non-target organisms exposed to dsRNA	[115]
Growth and development	Evaluate the potential impacts of dsRNA exposure on the growth, development, and reproduction of non-target organisms	[116]
Behavioral effects	Investigate changes in behavior, such as feeding, locomotion, or mating, in non-target organisms exposed to dsRNA	[117]
Community structure	Assess the potential shifts in the composition and diversity of ecological communities in response to RNAi-based insect control	[118]
Ecosystem services	Evaluate the potential impacts on ecosystem services, such as pollination, nutrient cycling, or biological control, provided by non-target organisms	[119]
Food web	Investigate the potential for trophic transfer of dsRNA and its	[120]

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interactions	effects on food web dynamics and energy flow	
Population dynamics	Assess the long-term impacts of RNAi-based insect control on the population dynamics and genetic diversity of non-target species	[121]
Evolutionary responses	Evaluate the potential for non-target organisms to evolve resistance or adapt to RNAi-based insect control over time	[122]
Sublethal effects	Investigate the potential for sublethal effects, such as reduced fecundity, altered physiology, or compromised immune function, in non-target organisms	[123]

6.2. Persistence and degradation of dsRNA in the environment Understanding the environmental fate and persistence of dsRNA is crucial for assessing the potential ecological impacts of RNAi-based insect control. Once released into the environment, dsRNA can undergo various degradation processes, such as hydrolysis, photolysis, and microbial degradation, which can influence its stability and bioavailability [124].

The rate of dsRNA degradation in the environment depends on several factors, including the specific sequence and structure of the dsRNA, the environmental conditions (e.g., temperature, pH, and moisture), and the presence of degradative enzymes [125]. Studies have shown that the half-life of dsRNA in soil and water can range from a few hours to several days, depending on these factors [126].

To assess the persistence and degradation of dsRNA in the environment, studies should be conducted to monitor the levels of dsRNA over time in different environmental matrices, such as soil, water, and plant tissues [127]. These studies can employ various analytical techniques, such as quantitative PCR (qPCR) or high-performance liquid chromatography (HPLC), to detect and quantify dsRNA in environmental samples [128].

In addition to the direct measurement of dsRNA persistence, it is also important to evaluate the potential for dsRNA to accumulate in food chains and to assess the risks of biomagnification and secondary exposure to non-target organisms [129]. Trophic transfer studies can be conducted to investigate the movement of dsRNA through different levels of the food web and to determine the potential for adverse effects on higher trophic level organisms [130].

The results of environmental fate and persistence studies can inform the development of risk management strategies and guide the selection of appropriate application methods and formulations to minimize the potential for environmental impacts [131]. For example, the use of biodegradable nanoparticles or the targeted delivery of dsRNA to specific plant tissues can help to reduce the persistence and spread of dsRNA in the environment [132].

6.3. Regulatory considerations and guidelines The development and commercialization of RNAi-based insect control products are subject to regulatory oversight to ensure their safety, efficacy, and environmental compatibility. Regulatory agencies, such as the US Environmental Protection Agency (EPA) and the European Food Safety Authority (EFSA), have established guidelines and data requirements for the assessment and approval of RNAi-based products [133].

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One of the key challenges in the regulation of RNAi-based insect control is the need to adapt existing risk assessment frameworks to address the unique properties and modes of action of dsRNA [134].

7. Current Applications and Future Prospects

7.1. Successful examples of RNAi-based insect control RNAi-based insect control has been successfully demonstrated in various crop systems and against a range of insect pests. One prominent example is the development of transgenic corn plants expressing dsRNA targeting the western corn rootworm (WCR), a major pest causing significant yield losses [135]. The introduction of WCR-specific dsRNA in corn plants has shown high efficacy in controlling the pest and reducing root damage [136]. Similar successes have been reported in other crops, such as potato, targeting the Colorado potato beetle [137], and in rice, targeting the brown planthopper [138].

Another notable example is the use of RNAi to control the Asian citrus psyllid (ACP), the vector of the devastating citrus greening disease [139]. By targeting essential genes in the ACP, researchers have demonstrated significant reductions in the insect's survival and transmission of the disease-causing bacterium [140]. These examples highlight the potential of RNAi as a powerful tool for managing insect pests and protecting agricultural crops.

Table 5. Successful examples of RNAi-based insect control in various crop systems

Crop	Target Insect Pest	Target Gene(s)	Delivery Method	References
Corn	Western corn rootworm	Snf7, DvSSJ1	Transgenic plants	[135], [136]
Potato	Colorado potato beetle	Actin, β-tubulin, Shrub	Transgenic plants	[137]
Rice	Brown planthopper	Calreticulin, Cathepsin B	Transgenic plants	[138]
Citrus	Asian citrus psyllid	Abnormal wing disc, Cathepsin B	Topical application	[139], [140]
Cotton	Cotton bollworm	Ecdysone receptor, Juvenile hormone acid methyltransferase	Transgenic plants	[141]
Soybean	Soybean aphid	Salivary protein C002, Acetylcholinesterase	Transgenic plants	[142]
Tomato	Tomato leaf miner	Arginine kinase	Spray application	[143]
Eggplant	Eggplant fruit and shoot borer	Juvenile hormone acid O-methyltransferase	Transgenic plants	[144]
Wheat	English grain aphid	Acetylcholinesterase	Transgenic	[145]

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			plants	
Sugarcane	Sugarcane borer	Aminopeptidase N	Transgenic plants	[146]

7.2. Integration with other pest management strategies The integration of RNAi-based insect control with other pest management strategies is crucial for developing sustainable and effective IPM programs. RNAi can be combined with various control methods, such as biological control, cultural practices, and selective use of insecticides, to achieve optimal pest suppression while minimizing the risk of resistance development and non-target effects [147].

For example, RNAi can be used in conjunction with natural enemies, such as predators and parasitoids, to enhance the overall efficacy of biological control. By selectively targeting specific insect pests, RNAi can help to conserve the populations of beneficial insects and promote their role in regulating pest populations [148]. Additionally, RNAi can be integrated with cultural practices, such as crop rotation and intercropping, to disrupt pest life cycles and reduce their population densities [149].

Furthermore, RNAi can be used as a component of insecticide resistance management programs. By alternating or combining RNAi-based control with other modes of action, such as Bt toxins or selective insecticides, the selection pressure on any single control method can be reduced, thereby delaying the onset of resistance [150]. This approach can help to prolong the effectiveness of RNAi-based control and ensure its long-term sustainability in IPM systems.

7.3. Emerging technologies and improvements Advances in biotechnology and molecular biology continue to drive the development and improvement of RNAi-based insect control strategies. One promising area of research is the use of nanotechnology for the targeted delivery of dsRNA [151]. Nanoparticles, such as liposomes, polymers, and inorganic materials, can be engineered to encapsulate and protect dsRNA from degradation, enhance its cellular uptake, and improve its stability in the environment [152]. These nanoformulations can also be functionalized with specific ligands or receptors to achieve targeted delivery to specific insect tissues or stages [153].

Another emerging technology is the use of CRISPR-based gene editing for the development of RNAi-resistant crops [154]. By precisely modifying the target gene sequences in the crop genome, researchers can create plants that are less susceptible to the effects of RNAi, thereby reducing the risk of off-target effects and improving the specificity of RNAi-based control [155]. CRISPR technology can also be used to identify and validate novel RNAi targets in insect pests, accelerating the development of new RNAi-based control strategies [156].

Furthermore, advances in high-throughput screening and bioinformatics are enabling the discovery and optimization of more effective RNAi targets and dsRNA sequences [157]. By leveraging genomic and transcriptomic data, researchers can design dsRNAs that are highly specific to the target insect species and have minimal off-target effects on non-target organisms [158]. These computational tools can also help to predict the potential for resistance development and guide the selection of RNAi targets that are less likely to evolve resistance rapidly [159].

Table 6. Emerging technologies and improvements in RNAi-based insect control

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Technology	Description	Advantages	References
Nanotechnology	Use of nanoparticles for targeted delivery of dsRNA	Enhances dsRNA stability, cellular uptake, and specificity; reduces environmental persistence	[151], [152], [153]
CRISPR-based gene editing	Precise modification of target gene sequences in crops to create RNAi-resistant plants; identification and validation of novel RNAi targets in insects	Improves specificity and reduces off-target effects; accelerates discovery of new RNAi targets	[154], [155], [156]
High-throughput screening	Use of genomic and transcriptomic data to design highly specific and effective dsRNA sequences	Minimizes off-target effects and potential for resistance development; enables optimization of RNAi target selection	[157], [158]
Bioinformatics	Computational tools for predicting resistance development and guiding the selection of durable RNAi targets	Helps to prolong the effectiveness of RNAi-based control and ensure its long-term sustainability	[159]
Microbiome engineering	Manipulation of insect gut microbiome to enhance RNAi efficiency and overcome barriers to dsRNA uptake and processing	Improves RNAi efficacy in recalcitrant insect species; provides new opportunities for RNAi-based control	[160]
Topical RNAi formulations	Development of sprayable dsRNA formulations for foliar application and direct uptake by insect pests	Offers a non-transgenic alternative to RNAi-based control; improves flexibility and adoptability	[161]
Inducible RNAi systems	Use of inducible promoters or chemical switches to control the expression of dsRNA in transgenic plants	Allows for targeted and timely induction of RNAi response; reduces potential for off-target effects	[162]
Combinatorial RNAi	Simultaneous targeting of multiple genes or pathways in insect pests using a combination of dsRNAs	Enhances RNAi efficacy and reduces the risk of resistance development; provides a more robust control strategy	[163]
RNAi synergists	Use of chemical compounds or natural products that enhance the RNAi response in insects	Improves RNAi efficiency and reduces the effective dose of dsRNA required for insect	[164]

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		control	
RNAi-based pest monitoring	Use of RNAi-based sensors or biomarkers for the early detection and monitoring of insect pest populations	Enables timely and targeted application of control measures; facilitates the development of precision IPM strategies	[165]

8. Socio-Economic Impact and Public Perception8.1. Potential benefits for farmers and consumers The development and adoption of RNAi-based insect control strategies can offer numerous potential benefits for farmers and consumers alike. For farmers, RNAi technology provides a novel and effective tool for managing insect pests that are difficult to control with conventional methods [166]. By reducing crop losses and improving yields, RNAi-based control can help to increase farm profitability and ensure a more stable income for agricultural producers [167]. Additionally, the species-specific nature of RNAi can help to reduce the reliance on broad-spectrum insecticides, thereby promoting the conservation of beneficial insects and reducing the environmental impacts of pest management [168].

For consumers, the adoption of RNAi-based insect control can lead to increased availability of high-quality, affordable food products [169]. By minimizing crop damage and reducing the need for insecticide applications, RNAi technology can help to ensure a more stable and sustainable food supply [170]. Furthermore, the reduced use of insecticides can contribute to a lower risk of pesticide residues in food products, addressing consumer concerns about food safety and quality [171].

8.2. Public acceptance and communication strategies Public acceptance is a critical factor in the successful adoption and commercialization of RNAi-based insect control technologies. Despite the potential benefits, the public may have concerns or misconceptions about the safety and environmental impacts of RNAi-based products [172]. To address these concerns and build public trust, effective communication and engagement strategies are essential [173].

One key aspect of public communication is the clear and transparent sharing of information about the science behind RNAi, its mode of action, and its potential applications in insect control [174]. This can be achieved through various channels, such as public outreach events, educational materials, and media campaigns [175]. It is important to engage with diverse stakeholders, including farmers, consumers, environmental groups, and policymakers, to understand their perspectives and address their specific concerns [176].

Another important strategy is the involvement of the public in the research and development process of RNAi-based insect control. By fostering a participatory approach and seeking input from different stakeholders, researchers and industry can ensure that the technology is developed in a socially responsible and acceptable manner [177]. This can include the establishment of multi-stakeholder advisory groups, the conduct of public consultations, and the incorporation of feedback into the design and implementation of RNAi-based control programs [178].

Table 7. Strategies for enhancing public acceptance of RNAi-based insect control

Strategy	Description	Examples	References

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Public outreach and education	Engaging with the public through various channels to provide clear and accessible information about RNAi and its applications in insect control	Public lectures, webinars, educational materials, social media campaigns	[174], [175]
Stakeholder engagement	Involving diverse stakeholders in the research and development process to understand their perspectives and address their concerns	Multi-stakeholder advisory groups, public consultations, incorporation of feedback into technology design	[176], [177], [178]
Transparency and communication	Sharing information about the science, safety, and environmental impacts of RNAi-based insect control in a transparent and proactive manner	Regular updates on research progress, safety assessments, and regulatory decisions; open access to data and materials	[179]
Responsible innovation	Ensuring that the development and deployment of RNAi-based insect control are guided by principles of social responsibility, sustainability, and ethical considerations	Integration of social and ethical considerations into research and development; adherence to responsible conduct guidelines	[180]
Benefit-sharing mechanisms	Developing equitable mechanisms for sharing the benefits of RNAi-based insect control with farmers, consumers, and local communities	Farmer participatory research, technology transfer programs, benefit-sharing agreements	[181]
Risk communication	Communicating the potential risks and uncertainties associated with RNAi-based insect control in a clear, balanced, and context-specific manner	Risk assessment reports, risk communication materials, dialogue with stakeholders	[182]
Trust-building measures	Establishing trust and credibility through transparency, accountability, and responsiveness to public concerns and values	Third-party certifications, independent audits, grievance redressal mechanisms	[183]
Inclusive innovation	Ensuring that the benefits of RNAi-based insect control are accessible and affordable to small-scale farmers and marginalized communities	Participatory technology development, capacity-building programs, subsidies and incentives	[184]

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Science-policy interface	Strengthening the interface between science and policy to ensure that RNAi-based insect control is developed and regulated in a evidence-based and socially accountable manner	Science-policy dialogues, policy briefs, engagement with regulatory agencies	[185]

8.3. Intellectual property and commercialization The commercialization of RNAi-based insect control technologies involves complex intellectual property (IP) and licensing considerations. Many of the key technologies and gene sequences underlying RNAi are protected by patents, which can impact the development and accessibility of RNAi-based products [186]. Navigating the IP landscape and establishing appropriate licensing agreements are crucial for the successful commercialization of RNAi-based insect control solutions [187].

One challenge is the potential for IP fragmentation, where different aspects of RNAi technology are owned by multiple entities, making it difficult to acquire the necessary licenses for commercialization [188]. This can lead to high transaction costs and delays in bringing RNAi-based products to market [189]. To address this issue, collaborative IP management strategies, such as patent pools and cross-licensing agreements, can be explored to facilitate access to key technologies and promote innovation [190].

Another consideration is the need to balance IP protection with the accessibility and affordability of RNAi-based insect control for small-scale farmers and developing countries [191]. This may require the development of alternative IP models, such as open-source licensing or humanitarian use exemptions, to ensure that the benefits of RNAi technology are widely distributed [192]. Additionally, the establishment of public-private partnerships and technology transfer programs can help to build local capacity and promote the adoption of RNAi-based solutions in resource-limited settings [193].

Table 8. Intellectual property and commercialization considerations for RNAi-based insect control

Consideration	Description	Challenges	Strategies	References
Patent landscape	Understanding the existing patents and IP rights related to RNAi technologies and their applications in insect control	IP fragmentation, overlapping patent claims, freedom-to-operate issues	Patent landscaping, freedom-to-operate analysis, due diligence	[186], [187]
Licensing agreements	Establishing appropriate licensing agreements for accessing and using RNAi technologies for insect control	High transaction costs, negotiation complexities, potential for disputes	Collaborative licensing models, standardized agreements, alternative dispute resolution	[188], [189]

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			mechanisms	
IP management strategies	Developing strategies for managing IP assets and promoting innovation in RNAi-based insect control	Balancing IP protection with accessibility and affordability, encouraging knowledge sharing and technology transfer	Patent pools, cross-licensing agreements, open-source models	[184,188]

9. Research Gaps and Future Directions9.1. Enhancing RNAi efficiency and delivery Despite the significant progress made in RNAi-based insect control, there are still several research gaps and challenges that need to be addressed to enhance the efficiency and delivery of RNAi in the field. One key area of research is the development of more effective dsRNA delivery methods that can overcome the barriers to RNAi uptake and persistence in insects [194]. This may involve the design of novel nanocarriers or formulations that can protect dsRNA from degradation, enhance its cellular uptake, and prolong its residence time in the insect body [195].

Another important research direction is the exploration of strategies to enhance the RNAi response in recalcitrant insect species, such as some lepidopteran pests, which have shown limited sensitivity to RNAi [196]. This may involve the identification of new RNAi targets, the use of more potent dsRNA sequences, or the co-delivery of RNAi enhancers or synergists [197]. Additionally, the development of high-throughput screening methods and bioinformatics tools can help to accelerate the discovery and optimization of effective RNAi targets and sequences [198].

9.2. Long-term effects and ecological studies To fully assess the environmental safety and sustainability of RNAi-based insect control, long-term studies and ecological investigations are needed. While most studies to date have focused on the short-term effects of RNAi on target and non-target organisms, the potential long-term impacts on insect populations, ecological communities, and ecosystem functions remain largely unexplored [199].

Future research should aim to conduct multi-year, field-scale studies to monitor the effects of RNAi-based control on insect population dynamics, resistance development, and ecological interactions [200]. This may involve the use of advanced monitoring techniques, such as population genetics tools, to track the spatial and temporal changes in insect populations and their associated ecological networks [201].

Furthermore, the potential effects of RNAi-based control on soil health, microbial communities, and biogeochemical processes should be investigated [202]. This may require the development of sensitive and specific methods for detecting and quantifying dsRNA in environmental matrices, as well as the assessment of its fate and persistence under different environmental conditions [203].

Table 9. Research gaps and future directions in RNAi-based insect control

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Research Gap	Future Directions	Potential Outcomes	References
dsRNA delivery methods	Development of novel nanocarriers and formulations for enhanced dsRNA stability, uptake, and persistence	Improved RNAi efficiency and field performance, reduced environmental persistence	[194], [195]
RNAi efficiency in recalcitrant species	Identification of new RNAi targets, use of potent dsRNA sequences, co-delivery of RNAi enhancers or synergists	Expanded range of insect pests controllable by RNAi, improved RNAi efficacy	[196], [197]
High-throughput screening and bioinformatics	Development of advanced screening methods and computational tools for RNAi target and sequence optimization	Accelerated discovery and design of effective RNAi strategies, reduced off-target effects	[198]
Long-term ecological effects	Multi-year, field-scale studies on insect population dynamics, resistance development, and ecological interactions	Comprehensive assessment of the environmental safety and sustainability of RNAi-based control	[199], [200], [201]
Soil health and microbial communities	Investigation of the effects of RNAi-based control on soil health, microbial diversity, and biogeochemical processes	Improved understanding of the ecological impacts of RNAi, development of mitigation strategies	[202]
dsRNA environmental fate and persistence	Development of sensitive and specific methods for detecting and quantifying dsRNA in environmental matrices, assessment of dsRNA persistence under different conditions	Informed risk assessment and management of RNAi-based control, optimization of dsRNA formulations and application strategies	[203]
Resistance management	Integration of RNAi with other control strategies, use of refuge areas, monitoring of resistance development, and implementation of proactive management plans	Prolonged effectiveness of RNAi-based control, reduced risk of resistance evolution	[204]
Socio-economic and policy aspects	Assessment of the economic feasibility, social acceptance, and regulatory frameworks for RNAi-based insect control	Informed decision-making and policy development, enhanced public trust and adoption of RNAi technology	[205]
Capacity building and technology	Strengthening of research and extension services, development	Improved access to and adoption of RNAi	[206]

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transfer	of partnerships and collaborations, and enhancement of local capacity for RNAi-based insect control	technology, particularly in developing countries	

9.3. Addressing resistance development The potential for insects to develop resistance to RNAi-based control is a major concern and an important research gap that needs to be addressed. As with any insect control strategy, the continuous exposure of insect populations to the same RNAi target or sequence can lead to the evolution of resistance over time [204]. Therefore, proactive resistance management strategies must be developed and implemented to ensure the long-term sustainability of RNAi-based insect control.

Future research should focus on understanding the molecular mechanisms of RNAi resistance in insects, including the identification of potential resistance genes and the characterization of resistance development processes [207]. This knowledge can inform the design of more durable RNAi targets and sequences, as well as the development of resistance monitoring and management plans [208].

Moreover, the integration of RNAi with other control strategies, such as biological control, cultural practices, and selective insecticide use, can help to reduce the selection pressure on insect populations and delay the onset of resistance [209]. The use of refuge areas, where a portion of the crop is left untreated to maintain susceptible insect populations, can also be explored as a strategy to manage RNAi resistance [210].

Experimental Results

1. Transgenic corn expressing dsRNA targeting the Snf7 gene of the western corn rootworm (Diabroticavirgiferavirgifera) showed significant reduction in root damage and adult emergence compared to non-transgenic controls [211].
2. Silencing of the P450 monooxygenase gene CYP6AE14 in the cotton bollworm (Helicoverpaarmigera) by plant-mediated RNAi resulted in increased larval mortality and reduced tolerance to gossypol, a natural defense compound in cotton [212].
3. Knockdown of the ecdysone receptor gene in the Colorado potato beetle (Leptinotarsa decemlineata) by feeding on dsRNA-expressing potato plants led to significant developmental abnormalities and reduced survival [213].
4. RNAi-mediated silencing of the wing development gene Distal-less (Dll) in the Asian citrus psyllid (Diaphorinacitri) caused wing malformations and reduced flight ability, potentially limiting the insect's dispersal and transmission of citrus greening disease [214].
5. Oral delivery of dsRNA targeting the V-ATPase gene in the western flower thrips (Frankliniella occidentalis) resulted in significant mortality and reduced fecundity, demonstrating the potential of RNAi for controlling this polyphagous pest [215].

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6. Transgenic rice expressing dsRNA against the midgut genes hexose transporter (HT1) and carboxypeptidase (CAR1) of the brown planthopper (Nilaparvatalugens) showed enhanced resistance to the insect, with reduced survival and population growth [216].

7. Silencing of the olfactory co-receptor gene Orco in the diamondback moth (Plutellaxylostella) by plant-mediated RNAi disrupted the insect's olfactory responses and reduced its ability to locate host plants [217].

8. RNAi-mediated knockdown of the gap gene hunchback in the tobacco hornworm (Manduca sexta) resulted in severe embryonic defects and developmental arrest, highlighting the potential of targeting developmentally critical genes for insect control [218].

9. Feeding of dsRNA targeting the acetylcholinesterase gene in the green peach aphid (Myzuspersicae) led to significant mortality and reduced fecundity, demonstrating the feasibility of RNAi-based control in hemipteran pests [219].

10. Transgenic soybean plants expressing dsRNA against the Rack1 gene of the soybean aphid (Aphis glycines) exhibited enhanced resistance to the pest, with reduced aphid population growth and plant damage [220].

11. Spray application of dsRNA targeting the juvenile hormone acid O-methyltransferase (JHAMT) gene in the red flour beetle (Triboliumcastaneum) caused significant developmental abnormalities and reduced fertility, showcasing the potential of spray-based RNAi delivery [221].

12. Injection of dsRNA targeting the vitellogenin gene in the honey bee (Apis mellifera) led to reduced egg production and impaired reproductive performance, demonstrating the need for careful selection of RNAi targets to avoid off-target effects on beneficial insects [222].

13. Plant-mediated RNAi silencing of the chitin synthase gene in the soybean looper (Chrysodeixisincludens) resulted in significant larval mortality and reduced feeding damage, indicating the potential for controlling lepidopteran pests in soybean [223].

14. Oral delivery of dsRNA targeting the gamma-aminobutyric acid (GABA) receptor gene in the tomato leafminer (Tuta absoluta) caused significant mortality and reduced larval feeding, showcasing the potential of RNAi for controlling this invasive pest [224].

15. RNAi-mediated knockdown of the ecdysone receptor gene in the Asian long-horned beetle (Anoplophoraglabripennis) led to significant developmental defects and reduced survival, highlighting the potential for controlling wood-boring pests [225].

16. Transgenic wheat expressing dsRNA against the salivary sheath protein gene in the English grain aphid (Sitobionavenae) showed enhanced resistance to the pest, with reduced aphid population growth and plant damage [226].

17. Silencing of the molting hormone receptor gene Methoprene-tolerant (Met) in the red palm weevil (Rhynchophorusferrugineus) by injection of dsRNA caused significant developmental abnormalities and reduced survival, indicating the potential for controlling this invasive pest [227].

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18. Plant-mediated RNAi targeting the acetylcholinesterase gene in the striped flea beetle (Phyllotretastriolata) resulted in significant mortality and reduced feeding damage, demonstrating the feasibility of RNAi-based control in cruciferous crops [228].

19. Oral delivery of dsRNA targeting the voltage-gated sodium channel gene in the fall armyworm (Spodoptera frugiperda) caused significant mortality and reduced larval growth, showcasing the potential of RNAi for controlling this polyphagous pest [229].

20. RNAi-mediated silencing of the ecdysone receptor gene in the Africanized honey bee (Apis mellifera scutellata) led to significant developmental defects and reduced survival, highlighting the need for species-specific RNAi targets in social insects [230].

21. Transgenic sugarcane expressing dsRNA against the aminopeptidase N gene of the sugarcane borer (Diatraeasaccharalis) showed enhanced resistance to the pest, with reduced larval survival and plant damage [231].

22. Spray application of dsRNA targeting the juvenile hormone esterase gene in the cabbage looper (Trichoplusiani) resulted in significant developmental abnormalities and reduced pupal weight, indicating the potential of spray-based RNAi delivery in lepidopteran pests [232].

23. Oral delivery of dsRNA targeting the cytochrome P450 gene CYP321A1 in the cotton aphid (Aphis gossypii) caused significant mortality and reduced fecundity, demonstrating the feasibility of RNAi-based control in this polyphagous pest [233].

24. Plant-mediated RNAi silencing of the voltage-gated sodium channel gene in the beet armyworm (Spodoptera exigua) resulted in significant larval mortality and reduced feeding damage, showcasing the potential of RNAi for controlling this pest in various crops [234].

25. Injection of dsRNA targeting the vitellogenin gene in the bumble bee (Bombus terrestris) led to reduced egg production and impaired colony development, highlighting the need for careful assessment of RNAi effects on non-target pollinators [235].

26. RNAi-mediated knockdown of the olfactory receptor co-receptor gene Orco in the oriental fruit fly (Bactrocera dorsalis) disrupted the insect's olfactory responses and reduced its ability to locate host fruits, indicating the potential for RNAi-based control of this invasive pest [236].

27. Transgenic eggplant expressing dsRNA against the acetylcholinesterase gene of the eggplant fruit and shoot borer (Leucinodesorbonalis) showed enhanced resistance to the pest, with reduced larval survival and plant damage [237].

28. Oral delivery of dsRNA targeting the juvenile hormone acid methyltransferase gene in the pea aphid (Acyrthosiphonpisum) caused significant developmental abnormalities and reduced fecundity, demonstrating the potential of RNAi for controlling this important vector of plant viruses [238].

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29. Plant-mediated RNAi silencing of the chitin synthase gene in the spotted wing drosophila (Drosophila suzukii) resulted in significant adult mortality and reduced oviposition, indicating the potential for controlling this invasive pest in fruit crops [239].

30. RNAi-mediated knockdown of the ecdysone receptor gene in the Russian wheat aphid (Diuraphisnoxia) led to significant developmental defects and reduced survival, showcasing the potential of RNAi for controlling this important pest in cereal crops [240].

10. Conclusion

10.1. Summary of key findings This review has highlighted the significant potential of RNAi-based insect control as a promising approach for sustainable pest management. The specific and potent gene silencing effects of RNAi, combined with its adaptability to different insect species and delivery methods, make it a valuable tool for controlling a wide range of insect pests in agriculture and other sectors.

The research community has made substantial progress in elucidating the molecular mechanisms of RNAi in insects, identifying effective target genes and delivery strategies, and demonstrating the feasibility of RNAi-based control in various crop systems. The development of transgenic plants, sprayable dsRNA formulations, and other innovative delivery methods has opened up new possibilities for the field application of RNAi.

However, several challenges and research gaps still need to be addressed to fully realize the potential of RNAi-based insect control. These include the variability in RNAi efficiency among insect species, the need for improved dsRNA delivery and stability, the potential for resistance development, and the long-term ecological impacts of RNAi use.

10.2. Prospects for RNAi-based insect control in integrated pest management Despite the challenges, the prospects for RNAi-based insect control in integrated pest management (IPM) are promising. The compatibility of RNAi with other control strategies, such as biological control and cultural practices, makes it a valuable component of IPM programs. By selectively targeting specific insect pests, RNAi can help to reduce the reliance on broad-spectrum insecticides and promote the conservation of beneficial insects and natural enemies.

To fully harness the potential of RNAi in IPM, ongoing research and development efforts should focus on enhancing the efficiency and specificity of RNAi-based control, while minimizing the risks of resistance development and off-target effects. This will require a multidisciplinary approach, integrating advances in molecular biology, biotechnology, ecology, and socio-economic sciences.

Furthermore, effective communication and engagement with stakeholders, including farmers, consumers, policymakers, and the general public, will be crucial for the successful adoption and implementation of RNAi-based insect control. Building trust, transparency, and participation in the development and regulation of RNAi technologies will be essential for ensuring their social acceptance and responsible use.

References

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1. Baum, J. A., Bogaert, T., Clinton, W., Heck, G. R., Feldmann, P., Ilagan, O., Johnson, S., Plaetinck, G., Munyikwa, T., Pleau, M., Vaughn, T., & Roberts, J. (2007). Control of coleopteran insect pests through RNA interference. Nature Biotechnology, 25(11), 1322-1326. https://doi.org/10.1038/nbt1359

2. Mao, Y. B., Cai, W. J., Wang, J. W., Hong, G. J., Tao, X. Y., Wang, L. J., Huang, Y. P., & Chen, X. Y. (2007). Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nature Biotechnology, 25(11), 1307-1313. https://doi.org/10.1038/nbt1352

3. Zhu, J. Q., Liu, S., Ma, Y., Zhang, J. Q., Qi, H. S., Wei, Z. J., Yao, Q., Zhang, W. Q., & Li, S. (2012). Improvement of pest resistance in transgenic tobacco plants expressing dsRNA of an insect-associated gene EcR. PLoS One, 7(6), e38572. https://doi.org/10.1371/journal.pone.0038572

4. El-Shesheny, I., Hajeri, S., El-Hawary, I., Gowda, S., &Killiny, N. (2013). Silencing abnormal wing disc gene of the Asian citrus psyllid, Diaphorinacitri disrupts adult wing development and increases nymph mortality. PLoS One, 8(5), e65392. https://doi.org/10.1371/journal.pone.0065392

5. Badillo-Vargas, I. E., Rotenberg, D., Schneweis, B. A., & Whitfield, A. E. (2015). RNA interference tools for the western flower thrips, Frankliniella occidentalis. Journal of Insect Physiology, 76, 36-46. https://doi.org/10.1016/j.jinsphys.2015.03.009

6. Zha, W., Peng, X., Chen, R., Du, B., Zhu, L., & He, G. (2011). Knockdown of midgut genes by dsRNA-transgenic plant-mediated RNA interference in the hemipteran insect Nilaparvatalugens. PLoS One, 6(5), e20504. https://doi.org/10.1371/journal.pone.0020504

7. Gong, L., Yang, X., Zhang, B., Zhong, G., & Hu, M. (2011). Silencing of Rieske iron-sulfur protein using chemically synthesised siRNA as a potential biopesticide against Plutellaxylostella. Pest Management Science, 67(5), 514-520. https://doi.org/10.1002/ps.2086

8. Asokan, R., Chandra, G. S., Manamohan, M., Kumar, N. K., & Sita, T. (2014). Response of various target genes to diet-delivered dsRNA mediated RNA interference in the cotton bollworm, Helicoverpaarmigera. Journal of Pest Science, 87(1), 163-172. https://doi.org/10.1007/s10340-013-0541-7

9. Pitino, M., Coleman, A. D., Maffei, M. E., Ridout, C. J., &Hogenhout, S. A. (2011). Silencing of aphid genes by dsRNA feeding from plants. PLoS One, 6(10), e25709. https://doi.org/10.1371/journal.pone.0025709

10. Bansal, R., Mamidala, P., Mian, M. R., Mittapalli, O., & Michel, A. P. (2012). Validation of reference genes for gene expression studies in Aphis glycines (Hemiptera: Aphididae). Journal of Economic Entomology, 105(4), 1432-1438. https://doi.org/10.1603/EC12095

UNDER PEER REVIEW

11. Xiao, D., Gao, X., Xu, J., Liang, X., Li, Q., Yao, J., & Zhu, K. Y. (2015). Clathrin-dependent endocytosis plays a predominant role in cellular uptake of double-stranded RNA in the red flour beetle. Insect Biochemistry and Molecular Biology, 60, 68-77. https://doi.org/10.1016/j.ibmb.2015.03.009

12. Amdam, G. V., Simões, Z. L., Guidugli, K. R., Norberg, K., & Omholt, S. W. (2003). Disruption of vitellogenin gene function in adult honeybees by intra-abdominal injection of double-stranded RNA. BMC Biotechnology, 3(1), 1. https://doi.org/10.1186/1472-6750-3-1

13. Fishilevich, E., Vélez, A. M., Storer, N. P., Li, H., Bowling, A. J., Rangasamy, M., Worden, S. E., Narva, K. E., & Siegfried, B. D. (2016). RNAi as a management tool for the western corn rootworm, Diabroticavirgiferavirgifera. Pest Management Science, 72(9), 1652-1663. https://doi.org/10.1002/ps.4324

14. Camargo, C., Manhaes, V., Leal, S., Amaral, I. M. R., Melo, A. C. V., Almeida, F., Dias, R. C. S., & Fernandes, G. W. (2018). Effects of dsRNA on Tuta absoluta larvae: oral delivery by droplet feeding, molecular analysis and in silico modeling of TaDVP1 gene. Pesticide Biochemistry and Physiology, 152, 58-67. https://doi.org/10.1016/j.pestbp.2018.09.011

15. Sun, D., Chen, S., Qin, J., Zhang, F., & Zhang, H. (2019). Development of an artificial diet for the Asian longhorned beetle, Anoplophoraglabripennis, and its effect on laboratory rearing and RNA interference. Insects, 10(10), 350. https://doi.org/10.3390/insects10100350

16. Abdellatef, E., Will, T., Koch, A., Imani, J., Vilcinskas, A., & Kogel, K. H. (2015). Silencing the expression of the salivary sheath protein causes transgenerational feeding suppression in the aphid Sitobionavenae. Plant Biotechnology Journal, 13(6), 849-857. https://doi.org/10.1111/pbi.12322

17. Al-Ayedh, H., Rizwan-ul-Haq, M., Hussain, A., &Aljabr, A. M. (2016). Insecticidal potency of RNAi-based catalase knockdown in Rhynchophorusferrugineus (Oliver)(Coleoptera: Curculionidae). Pest Management Science, 72(11), 2118-2127. https://doi.org/10.1002/ps.4242

18. Sun, N., Meng, F., Song, P., & Qiu, L. (2019). Silencing of olfactory coreceptor gene Orco impairs mating and oviposition behaviors of Phyllotretastriolata (Coleoptera: Chrysomelidae). Pesticide Biochemistry and Physiology, 158, 118-125. https://doi.org/10.1016/j.pestbp.2019.04.015

19. Garcia, R. A., Macedo, L. L., Cabral do Nascimento, D., Gillet, F. X., Moreira-Pinto, C. E., Faheem, M., Basso, A. M. M., Silva, M. C. M., & Grossi-de-Sa, M. F. (2017). Nucleases as a barrier to gene silencing in the cotton boll weevil, Anthonomus grandis. PLoS One, 12(12), e0189600. https://doi.org/10.1371/journal.pone.0189600

20. Leelesh, R. S., & Rieske, L. K. (2020). Hydrolytic enzymes in honey bee (Apis mellifera) larvae act as a double-edged sword for RNAi-mediated control of Varroa mites. Journal of Pest Science, 93, 811-822. https://doi.org/10.1007/s10340-020-01199-6

UNDER PEER REVIEW

21. Li, H., Guan, R., Guo, H., & Miao, X. (2015). New insights into an RNAi approach for plant defence against piercing-sucking and stem-borer insect pests. Plant, Cell & Environment, 38(11), 2277-2285. https://doi.org/10.1111/pce.12546

22. Mogilicherla, K., Howell, J. L., & Palli, S. R. (2018). Improving RNAi in the brown marmorated stink bug: Identification of target genes and reference genes for RT-qPCR. Scientific Reports, 8(1), 1-9. https://doi.org/10.1038/s41598-018-27605-9

23. Christiaens, O., Tardajos, M. G., Martinez Reyna, Z. L., Dash, M., Dubruel, P., &Smagghe, G. (2018). Increased RNAi efficacy in Spodoptera exigua via the formulation of dsRNA with guanylated polymers. Frontiers in Physiology, 9, 316. https://doi.org/10.3389/fphys.2018.00316

24. Whitten, M., & Dyson, P. (2017). Gene silencing in non-model insects: Overcoming hurdles using symbiotic bacteria for trauma-free sustainable delivery of RNA interference: Sustained RNA interference in insects mediated by symbiotic bacteria: applications as a genetic tool and as a biocide. BioEssays, 39(3), 1600247. https://doi.org/10.1002/bies.201600247

25. Rangasamy, M., & Siegfried, B. D. (2012). Validation of RNA interference in western corn rootworm Diabroticavirgiferavirgifera LeConte (Coleoptera: Chrysomelidae) adults. Pest Management Science, 68(4), 587-591. https://doi.org/10.1002/ps.2301

26. Jarosch, A., & Moritz, R. F. (2011). Systemic RNA-interference in the honeybee Apis mellifera: tissue dependent uptake of fluorescent siRNA after intra-abdominal application observed by laser-scanning microscopy. Journal of Insect Physiology, 57(7), 851-857. https://doi.org/10.1016/j.jinsphys.2011.03.013

27. Guan, R. B., Li, H. C., Fan, Y. J., Hu, S. R., Christiaens, O., Smagghe, G., & Miao, X. X. (2018). A nuclease specific to lepidopteran insects suppresses RNAi. Journal of Biological Chemistry, 293(16), 6011-6021. https://doi.org/10.1074/jbc.RA117.001553

28. Zhang, J., Khan, S. A., Heckel, D. G., & Bock, R. (2017). Next-generation insect-resistant plants: RNAi-mediated crop protection. Trends in Biotechnology, 35(9), 871-882. https://doi.org/10.1016/j.tibtech.2017.04.009

29. Knorr, E., Fishilevich, E., Tenbusch, L., Frey, M. L., Rangasamy, M., Billion, A., Worden, S. E., Gandra, P., Arora, K., Lo, W., Schulenberg, G., Valverde-Garcia, P., Vilcinskas, A., & Narva, K. E. (2018). Gene silencing in Triboliumcastaneum as a tool for the targeted identification of candidate RNAi targets in crop pests. Scientific Reports, 8(1), 1-15. https://doi.org/10.1038/s41598-018-20416-y

30. Guan, R. B., Li, H. C., Fan, Y. J., Hu, S. R., Christiaens, O., Smagghe, G., & Miao, X. X. (2018). A nuclease specific to lepidopteran insects suppresses RNAi. Journal of Biological Chemistry, 293(16), 6011-6021. https://doi.org/10.1074/jbc.RA117.001553

31. Li, H., Khajuria, C., Rangasamy, M., Gandra, P., Fitter, M., Geng, C., Woosely, A., Hasler, J., Schulenberg, G., Worden, S., McEwan, R., Evans, C., Siegfried, B., & Narva, K. E. (2015). Long

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dsRNA but not siRNA initiates RNAi in western corn rootworm larvae and adults. Journal of Applied Entomology, 139(6), 432-445. https://doi.org/10.1111/jen.12224

32. Taning, C. N. T., Christiaens, O., Berkvens, N., Casteels, H., Maes, M., &Smagghe, G. (2016). Oral RNAi to control Drosophila suzukii: laboratory testing against larval and adult stages. Journal of Pest Science, 89(3), 803-814. https://doi.org/10.1007/s10340-016-0736-9

33. Ramaseshadri, P., Segers, G., Flannagan, R., Wiggins, E., Clinton, W., Ilagan, O., McNulty, B., Clark, T., & Bolognesi, R. (2013). Physiological and cellular responses caused by RNAi-mediated suppression of Snf7 orthologue in western corn rootworm (Diabroticavirgiferavirgifera) larvae. PLoS One, 8(1), e54270. https://doi.org/10.1371/journal.pone.0054270

34. Zhang, J., Khan, S. A., Hasse, C., Ruf, S., Heckel, D. G., & Bock, R. (2015). Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. Science, 347(6225), 991-994. https://doi.org/10.1126/science.1261680

35. Bally, J., McIntyre, G. J., Doran, R. L., Lee, K., Perez, A., Jung, H., Naim, F., Larrinua, I. M., Narva, K. E., & Waterhouse, P. M. (2016). In-plant protection against Helicoverpaarmigera by production of long hpRNA in chloroplasts. Frontiers in Plant Science, 7, 1453. https://doi.org/10.3389/fpls.2016.01453

36. Jin, S., Singh, N. D., Li, L., Zhang, X., & Daniell, H. (2015). Engineered chloroplast dsRNA silences cytochrome p450 monooxygenase, V-ATPase and chitin synthase genes in the insect gut and disrupts Helicoverpaarmigera larval development and pupation. Plant Biotechnology Journal, 13(3), 435-446. https://doi.org/10.1111/pbi.12355

37. Mao, Y. B., Tao, X. Y., Xue, X. Y., Wang, L. J., & Chen, X. Y. (2011). Cotton plants expressing CYP6AE14 double-stranded RNA show enhanced resistance to bollworms. Transgenic Research, 20(3), 665-673. https://doi.org/10.1007/s11248-010-9450-1

38. Mamta, B., & Rajam, M. V. (2017). RNAi technology: a new platform for crop pest control. Physiology and Molecular Biology of Plants, 23(3), 487-501. https://doi.org/10.1007/s12298-017-0443-x

39. Baum, J. A., & Roberts, J. K. (2014). Progress towards RNAi-mediated insect pest management. In Advances in Insect Physiology (Vol. 47, pp. 249-295). Academic Press. https://doi.org/10.1016/B978-0-12-800197-4.00005-1

40. Scott, J. G., Michel, K., Bartholomay, L. C., Siegfried, B. D., Hunter, W. B., Smagghe, G., Zhu, K. Y., & Douglas, A. E. (2013). Towards the elements of successful insect RNAi. Journal of Insect Physiology, 59(12), 1212-1221. https://doi.org/10.1016/j.jinsphys.2013.08.014

41. Joga, M. R., Zotti, M. J., Smagghe, G., & Christiaens, O. (2016). RNAi efficiency, systemic properties, and novel delivery methods for pest insect control: what we know so far. Frontiers in Physiology, 7, 553. https://doi.org/10.3389/fphys.2016.00553

UNDER PEER REVIEW

42. Palli, S. R. (2014). RNA interference in Colorado potato beetle: steps toward development of dsRNA as a commercial insecticide. Current Opinion in Insect Science, 6, 1-8. https://doi.org/10.1016/j.cois.2014.09.011

43. Kolliopoulou, A., Taning, C. N., Smagghe, G., & Swevers, L. (2017). Viral delivery of dsRNA for control of insect agricultural pests and vectors of human disease: prospects and challenges. Frontiers in Physiology, 8, 399. https://doi.org/10.3389/fphys.2017.00399

44. Terenius, O., Papanicolaou, A., Garbutt, J. S., Eleftherianos, I., Huvenne, H., Kanginakudru, S., ... &Smagghe, G. (2011). RNA interference in Lepidoptera: an overview of successful and unsuccessful studies and implications for experimental design. Journal of Insect Physiology, 57(2), 231-245. https://doi.org/10.1016/j.jinsphys.2010.11.006

45. Khajuria, C., Vélez, A. M., Rangasamy, M., Wang, H., Fishilevich, E., Frey, M. L., ... & Siegfried, B. D. (2015). Parental RNA interference of genes involved in embryonic development of the western corn rootworm, Diabroticavirgiferavirgifera LeConte. Insect Biochemistry and Molecular Biology, 63, 54-62. https://doi.org/10.1016/j.ibmb.2015.05.011

46. Fishilevich, E., Bowling, A. J., Frey, M. L., Wang, P. H., Lo, W., Rangasamy, M., ... & Narva, K. E. (2019). RNAi targeting of rootworm Troponin I transcripts confers root protection in maize. Insect Biochemistry and Molecular Biology, 104, 20-29. https://doi.org/10.1016/j.ibmb.2018.09.006

47. Niu, X., Kassa, A., Hu, X., Robeson, J., McMahon, M., Richtman, N. M., ... & Wu, G. (2017). Control of western corn rootworm (Diabroticavirgiferavirgifera) reproduction through plant-mediated RNA interference. Scientific Reports, 7(1), 1-13. https://doi.org/10.1038/s41598-017-12638-3

48. Hu, X., Richtman, N. M., Zhao, J. Z., Duncan, K. E., Niu, X., Procyk, L. A., ... & Wu, G. (2016). Discovery of midgut genes for the RNA interference control of corn rootworm. Scientific Reports, 6(1), 1-12. https://doi.org/10.1038/srep30542

49. Vélez, A. M., Fishilevich, E., Rangasamy, M., Khajuria, C., McCaskill, D. G., Pereira, A. E., ... & Siegfried, B. D. (2020). Control of western corn rootworm via RNAi traits in maize: lethal and sublethal effects of Sec23 dsRNA. Pest Management Science, 76(4), 1500-1512. https://doi.org/10.1002/ps.5666

50. Pereira, A. E., Vélez, A. M., Meinke, L. J., & Siegfried, B. D. (2017). Sublethal effects of vATPase-A and Snf7 dsRNAs on biology of southern corn rootworm, Diabroticaundecimpunctatahowardi Barber. Journal of Economic Entomology, 110(6), 2545-2553. https://doi.org/10.1093/jee/tox263

51. Knorr, E., Fishilevich, E., Tenbusch, L., Frey, M. L., Rangasamy, M., Billion, A., ... & Narva, K. E. (2018). Gene silencing in Triboliumcastaneum as a tool for the targeted identification of candidate RNAi targets in crop pests. Scientific Reports, 8(1), 1-15. https://doi.org/10.1038/s41598-018-20416-y

UNDER PEER REVIEW

52. Rodrigues, T. B., Rieske, L. K., Duan, J. J., Mogilicherla, K., & Palli, S. R. (2017). Development of RNAi method for screening candidate genes to control emerald ash borer, Agrilus planipennis. Scientific Reports, 7(1), 1-8. https://doi.org/10.1038/s41598-017-07605-9

53. Wu, K., Taylor, C. E., Fishilevich, E., Narva, K. E., & Siegfried, B. D. (2018). Rapid and persistent RNAi response in western corn rootworm adults. Pesticide Biochemistry and Physiology, 150, 66-70. https://doi.org/10.1016/j.pestbp.2018.07.002

54. Vélez, A. M., Fishilevich, E., Matz, N., Storer, N. P., Narva, K. E., & Siegfried, B. D. (2017). Parameters for successful parental RNAi as an insect pest management tool in western corn rootworm, Diabroticavirgiferavirgifera. Genes, 8(1), 7. https://doi.org/10.3390/genes8010007

55. Pinheiro, D. H., Vélez, A. M., Fishilevich, E., Wang, H., Carneiro, N. P., Valencia-Jiménez, A., ... & Monteiro-Vitorello, C. B. (2018). Clathrin-dependent endocytosis is associated with RNAi response in the western corn rootworm, Diabroticavirgiferavirgifera LeConte. PLoS One, 13(8), e0201849. https://doi.org/10.1371/journal.pone.0201849

56. Yoon, J. S., Shukla, J. N., Gong, Z. J., Mogilicherla, K., & Palli, S. R. (2016). RNA interference in the Colorado potato beetle, Leptinotarsa decemlineata: identification of key contributors. Insect Biochemistry and Molecular Biology, 78, 78-88. https://doi.org/10.1016/j.ibmb.2016.09.002

57. Fishilevich, E., Vélez, A. M., Khajuria, C., Frey, M. L., Hamm, R. L., Wang, H., ... & Siegfried, B. D. (2016). Use of chromatin remodeling ATPases as RNAi targets for parental control of western corn rootworm (Diabroticavirgiferavirgifera) and Neotropical brown stink bug (Euschistusheros). Insect Biochemistry and Molecular Biology, 71, 58-71. https://doi.org/10.1016/j.ibmb.2016.02.004

58. Shukla, J. N., Kalsi, M., Sethi, A., Narva, K. E., Fishilevich, E., Singh, S., ... & Palli, S. R. (2016). Reduced stability and intracellular transport of dsRNA contribute to poor RNAi response in lepidopteran insects. RNA Biology, 13(7), 656-669. https://doi.org/10.1080/15476286.2016.1191728

59. Parsons, K. H., Mondal, M. H., McCormick, C. L., & Flynt, A. S. (2018). Guanidinium-functionalized interpolyelectrolyte complexes enabling RNAi in resistant insect pests. Biomacromolecules, 19(4), 1111-1117. https://doi.org/10.1021/acs.biomac.7b01717

60. Gillet, F. X., Garcia, R. A., Macedo, L. L., Albuquerque, E. V., Silva, M., & Grossi-de-Sa, M. F. (2017). Investigating engineered ribonucleoprotein particles to improve oral RNAi delivery in crop insect pests. Frontiers in Physiology, 8, 256. https://doi.org/10.3389/fphys.2017.00256

61. Christiaens, O., Prentice, K., Pertry, I., Ghislain, M., Bailey, A., Niblett, C., ... &Smagghe, G. (2016). RNA interference: a promising biopesticide strategy against the African Sweetpotato Weevil Cylas brunneus. Scientific Reports, 6(1), 1-11. https://doi.org/10.1038/srep38836

UNDER PEER REVIEW

62. Cagliari, D., Dias, N. P., Galdeano, D. M., dos Santos, E. Á., Smagghe, G., & Zotti, M. J. (2019). Management of pest insects and plant diseases by non-transformative RNAi. Frontiers in Plant Science, 10, 1319. https://doi.org/10.3389/fpls.2019.01319

63. Head, G. P., Carroll, M. W., Evans, S. P., Rule, D. M., Willse, A. R., Clark, T. L., ... & Meinke, L. J. (2017). Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management. Pest Management Science, 73(9), 1883-1899. https://doi.org/10.1002/ps.4554

64. Khajuria, C., Ivashuta, S., Wiggins, E., Flagel, L., Moar, W., Pleau, M., ... & Clark, T. (2018). Development and characterization of the first dsRNA-resistant insect population from western corn rootworm, Diabroticavirgiferavirgifera LeConte. PLoS One, 13(5), e0197059. https://doi.org/10.1371/journal.pone.0197059

65. Agrawal, A., Rajamani, V., Reddy, V. S., Mukherjee, S. K., & Bhatnagar, R. K. (2015). Transgenic plants over-expressing insect-specific microRNA acquire insecticidal activity against Helicoverpaarmigera: an alternative to Bt-toxin technology. Transgenic Research, 24(5), 791-801. https://doi.org/10.1007/s11248-015-9880-x

66. Kola, V. S., Renuka, P., Madhav, M. S., &Mangrauthia, S. K. (2015). Key enzymes and proteins of crop insects as candidate for RNAi based gene silencing. Frontiers in Physiology, 6, 119. https://doi.org/10.3389/fphys.2015.00119

67. Yu, X., Killiny, N., & Cicero, J. M. (2017). Double stranded RNA delivery to citrus blight-associated Liberibacter asiaticus-infected citrus plants using trunk injections. Crop Protection, 100, 148-153. https://doi.org/10.1016/j.cropro.2017.06.020

68. Luo, Y., Chen, Q., Luan, J., Chung, S. H., Van Eck, J., Turgeon, R., & Douglas, A. E. (2017). Towards an understanding of the molecular basis of effective RNAi against a global insect pest, the whitefly Bemisiatabaci. Insect Biochemistry and Molecular Biology, 88, 21-29. https://doi.org/10.1016/j.ibmb.2017.07.005

69. Yan, S., Ren, B. Y., & Shen, J. (2021). Nanoparticle-mediated double-stranded RNA delivery system: a promising approach for sustainable pest management. Insect Science, 28(1), 21-34. https://doi.org/10.1111/1744-7917.12822

70. McLoughlin, A. G., Walker, P. L., Wytinck, N., Sullivan, D. S., Whyard, S., & Belmonte, M. F. (2018). Developing new RNA interference technologies to control fungal pathogens. Canadian Journal of Plant Pathology, 40(3), 325-335. https://doi.org/10.1080/07060661.2018.1495268

71. Fletcher, S. J., Reeves, P. T., Hoang, B. T., & Mitter, N. (2020). A perspective on RNAi-based biopesticides. Frontiers in Plant Science, 11, 51. https://doi.org/10.3389/fpls.2020.00051

72. Zhu, K. Y., & Palli, S. R. (2020). Mechanisms, applications, and challenges of insect RNA interference. Annual Review of Entomology, 65, 293-311. https://doi.org/10.1146/annurev-ento-011019-025224

UNDER PEER REVIEW

73. Niu, J., Yang, W. J., Tian, Y., Fan, J. Y., Ye, C., Shang, F., ... & An, X. (2019). Topical dsRNA delivery induces gene silencing and mortality in the pea aphid. Pest Management Science, 75(11), 2873-2881. https://doi.org/10.1002/ps.5457

74. Vogel, E., Santos, D., Mingels, L., Verdonckt, T. W., & Broeck, J. V. (2019). RNA interference in insects: protecting beneficials and controlling pests. Frontiers in Physiology, 9, 1912. https://doi.org/10.3389/fphys.2018.01912

75. Zotti, M., Dos Santos, E. A., Cagliari, D., Christiaens, O., Taning, C. N. T., &Smagghe, G. (2018). RNA interference technology in crop protection against arthropod pests, pathogens and nematodes. Pest Management Science, 74(6), 1239-1250. https://doi.org/10.1002/ps.4813

76. Taning, C. N. T., Arpaia, S., Christiaens, O., Dietz-Pfeilstetter, A., Jones, H., Mezzetti, B., ... &Smagghe, G. (2020). RNA-based biocontrol compounds: current status and perspectives to reach the market. Pest Management Science, 76(3), 841-845. https://doi.org/10.1002/ps.5686

77. Pugsley, C. E., Isaac, R. E., Warren, N. J., & Cayre, O. J. (2020). Recent Advances in Engineered Nanoparticles for RNAi-Mediated Crop Protection Against Insect Pests. Frontiers in Agronomy, 2, 4. https://doi.org/10.3389/fagro.2020.00004

78. Dubrovina, A. S., & Kiselev, K. V. (2019). Exogenous RNAs for gene regulation and plant resistance. International Journal of Molecular Sciences, 20(9), 2282. https://doi.org/10.3390/ijms20092282

79. Worrall, E. A., Bravo-Cazar, A., Nilon, A. T., Fletcher, S. J., Robinson, K. E., Carr, J. P., & Mitter, N. (2019). Exogenous application of RNAi-inducing double-stranded RNA inhibits aphid-mediated transmission of a plant virus. Frontiers in Plant Science, 10, 265. https://doi.org/10.3389/fpls.2019.00265

80. Castellanos, N. L., Smagghe, G., Sharma, R., Oliveira, E. E., & Christiaens, O. (2019). Liposome encapsulation and EDTA formulation of dsRNA targeting essential genes increase oral RNAi-caused mortality in the Neotropical stink bug Euschistusheros. Pest Management Science, 75(2), 537-548. https://doi.org/10.1002/ps.5167

81. Guan, R. B., Li, H. C., Fan, Y. J., Hu, S. R., Christiaens, O., Smagghe, G., & Miao, X. X. (2018). A nuclease specific to lepidopteran insects suppresses RNAi. Journal of Biological Chemistry, 293(16), 6011-6021. https://doi.org/10.1074/jbc.RA117.001553

82. Garbutt, J. S., Belles, X., Richards, E. H., & Reynolds, S. E. (2013). Persistence of double-stranded RNA in insect hemolymph as a potential determiner of RNA interference success: evidence from Manduca sexta and Blattella germanica. Journal of Insect Physiology, 59(2), 171-178. https://doi.org/10.1016/j.jinsphys.2012.05.013

83. Song, H., Zhang, J., Li, D., Cooper, A. M., Silver, K., Li, T., ... & Zhang, J. (2017). A double-stranded RNA degrading enzyme reduces the efficiency of oral RNA interference in migratory locust. Insect Biochemistry and Molecular Biology, 86, 68-80. https://doi.org/10.1016/j.ibmb.2017.05.008

UNDER PEER REVIEW

84. Prentice, K., Smagghe, G., Gheysen, G., & Christiaens, O. (2019). Nuclease activity decreases the RNAi response in the sweetpotato weevil Cylas puncticollis. Insect Biochemistry and Molecular Biology, 110, 80-89. https://doi.org/10.1016/j.ibmb.2019.04.001

85. Prentice, K., Christiaens, O., Pertry, I., Bailey, A., Niblett, C., Ghislain, M., ... &Smagghe, G. (2017). RNAi-based gene silencing through dsRNA injection or ingestion against the African sweet potato weevil Cylas puncticollis (Coleoptera: Brentidae). Pest Management Science, 73(1), 44-52. https://doi.org/10.1002/ps.4337

86. Spit, J., Philips, A., Wynant, N., Santos, D., Plaetinck, G., & Broeck, J. V. (2017). Knockdown of nuclease activity in the gut enhances RNAi efficiency in the Colorado potato beetle, Leptinotarsa decemlineata, but not in the desert locust, Schistocerca gregaria. Insect Biochemistry and Molecular Biology, 81, 103-116. https://doi.org/10.1016/j.ibmb.2017.01.004

87. Tayler, A., Heschuk, D., Giesbrecht, D., Park, J. Y., & Whyard, S. (2019). Efficiency of RNA interference is improved by knockdown of dsRNA nucleases in tephritid fruit flies. Open Biology, 9(12), 190198. https://doi.org/10.1098/rsob.190198

88. Christiaens, O., &Smagghe, G. (2014). The challenge of RNAi-mediated control of hemipterans. Current Opinion in Insect Science, 6, 15-21. https://doi.org/10.1016/j.cois.2014.09.012

89. Ivashuta, S., Zhang, Y., Wiggins, B. E., Ramaseshadri, P., Segers, G. C., Johnson, S., ... & Bolognesi, R. (2015). Environmental RNAi in herbivorous insects. RNA, 21(5), 840-850. https://doi.org/10.1261/rna.048116.114

90. Cooper, A. M., Silver, K., Zhang, J., Park, Y., & Zhu, K. Y. (2019). Molecular mechanisms influencing efficiency of RNA interference in insects. Pest Management Science, 75(1), 18-28. https://doi.org/10.1002/ps.5126

91. Gould, F., Brown, Z. S., & Kuzma, J. (2018). Wicked evolution: Can we address the sociobiological dilemma of pesticide resistance?. Science, 360(6390), 728-732. https://doi.org/10.1126/science.aar3780

92. Fishilevich, E., Vélez, A. M., Storer, N. P., Li, H., Bowling, A. J., Rangasamy, M., ... & Siegfried, B. D. (2016). RNAi as a management tool for the western corn rootworm, Diabroticavirgiferavirgifera. Pest Management Science, 72(9), 1652-1663. https://doi.org/10.1002/ps.4324

93. Vélez, A. M., &Fishilevich, E. (2018). The mysteries of insect RNAi: A focus on dsRNA uptake and transport. Pesticide Biochemistry and Physiology, 151, 25-31. https://doi.org/10.1016/j.pestbp.2018.08.005

94. Wang, K., Peng, Y., Pu, J., Fu, W., Wang, J., & Han, Z. (2016). Variation in RNAi efficacy among insect species is attributable to dsRNA degradation in vivo. Insect Biochemistry and Molecular Biology, 77, 1-9. https://doi.org/10.1016/j.ibmb.2016.07.007

UNDER PEER REVIEW

95. Parsons, K. H., Holley, A. C., Munn, G. A., Flynt, A. S., & McCormick, C. L. (2020). Block ionomer complexes consisting of siRNA and aRAFT-synthesized hydrophilic-block-cationic copolymers II: the influence of cationic block charge density on gene suppression. Polymer Chemistry, 11(17), 2959-2969. https://doi.org/10.1039/D0PY00052C

96. Kunte, N., McGraw, E., Bell, S., Held, D., & Avila, L. A. (2020). Prospects, challenges and current status of RNAi through insect feeding. Pest Management Science, 76(1), 26-41. https://doi.org/10.1002/ps.5588

97. Bachman, P. M., Bolognesi, R., Moar, W. J., Mueller, G. M., Paradise, M. S., Ramaseshadri, P., ... & Anderson, J. A. (2013). Characterization of the spectrum of insecticidal activity of a double-stranded RNA with targeted activity against Western Corn Rootworm (Diabroticavirgiferavirgifera LeConte). Transgenic Research, 22(6), 1207-1222. https://doi.org/10.1007/s11248-013-9716-5

98. Pan, H., Xu, L., Noland, J. E., Li, H., Siegfried, B. D., & Zhou, X. (2016). Assessment of potential risks of dietary RNAi to a soil micro-arthropod, Sinellacurviseta Brook (Collembola: Entomobryidae). Frontiers in Plant Science, 7, 1028. https://doi.org/10.3389/fpls.2016.01028

99. Paces, J., Nic, M., Novotny, T., & Svoboda, P. (2017). Literature review of baseline information to support the risk assessment of RNAi-based GM plants. EFSA Supporting Publications, 14(6), 1246E. https://doi.org/10.2903/sp.efsa.2017.EN-1246

100. Haller, S., Widmer, F., Siegfried, B. D., Zhuo, X., & Romeis, J. (2019). Responses of two ladybird beetle species (Coleoptera: Coccinellidae) to dietary RNAi. Pest Management Science, 75(11), 2652-2662. https://doi.org/10.1002/ps.5370

101. Vélez, A. M., Jurzenski, J., Matz, N., Zhou, X., Wang, H., Ellis, M., & Siegfried, B. D. (2016). Developing an in vivo toxicity assay for RNAi risk assessment in honey bees, Apis mellifera L. Chemosphere, 144, 1083-1090. https://doi.org/10.1016/j.chemosphere.2015.09.068

102. Tan, J., Levine, S. L., Bachman, P. M., Jensen, P. D., Mueller, G. M., Uffman, J. P., ... & Beevers, M. H. (2016). No impact of DvSnf7 RNA on honey bee (Apis mellifera L.) adults and larvae in dietary feeding tests. Environmental Toxicology and Chemistry, 35(2), 287-294. https://doi.org/10.1002/etc.3075

103. Taning, C. N. T., Christiaens, O., Li, X., Swevers, L., Casteels, H., Maes, M., &Smagghe, G. (2018). Engineered flock house virus for targeted gene suppression through RNAi in fruit flies (Drosophila melanogaster) in vitro and in vivo. Frontiers in Physiology, 9, 805. https://doi.org/10.3389/fphys.2018.00805

104. Jiang, L., Ding, L., He, B., Shen, J., Xu, Z., Yin, M., & Zhang, X. (2014). Systemic gene silencing in plants triggered by fluorescent nanoparticle-delivered double-stranded RNA. Nanoscale, 6(17), 9965-9969. https://doi.org/10.1039/C4NR03481C

UNDER PEER REVIEW

105. Koch, A., Biedenkopf, D., Furch, A., Weber, L., Rossbach, O., Abdellatef, E., ... & Kogel, K. H. (2016). An RNAi-based control of Fusarium graminearum infections through spraying of long dsRNAs involves a plant passage and is controlled by the fungal silencing machinery. PLoS Pathogens, 12(10), e1005901. https://doi.org/10.1371/journal.ppat.1005901

106. Wang, M., Weiberg, A., Lin, F. M., Thomma, B. P., Huang, H. D., & Jin, H. (2016). Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection. Nature Plants, 2(10), 1-10. https://doi.org/10.1038/nplants.2016.151

107. Mitter, N., Worrall, E. A., Robinson, K. E., Li, P., Jain, R. G., Taochy, C., ... & Xu, Z. P. (2017). Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. Nature Plants, 3(2), 1-10. https://doi.org/10.1038/nplants.2016.207

108. Numata, K., Ohtani, M., Yoshizumi, T., Demura, T., & Kodama, Y. (2014). Local gene silencing in plants via synthetic dsRNA and carrier peptide. Plant Biotechnology Journal, 12(8), 1027-1034. https://doi.org/10.1111/pbi.12208

109. Li, H., Guan, R., Guo, H., & Miao, X. (2015). New insights into an RNAi approach for plant defence against piercing-sucking and stem-borer insect pests. Plant, Cell & Environment, 38(11), 2277-2285. https://doi.org/10.1111/pce.12546

110. Zhang, T., Jin, Y., Zhao, J. H., Gao, F., Zhou, B. J., Fang, Y. Y., & Guo, H. S. (2016). Host-induced gene silencing of the target gene in fungal cells confers effective resistance to the cotton wilt disease pathogen Verticillium dahliae. Molecular Plant, 9(6), 939-942. https://doi.org/10.1016/j.molp.2016.02.008

112. Dalakouras, A., Wassenegger, M., Dadami, E., Ganopoulos, I., Pappas, M. L., & Papadopoulou, K. (2020). Genetically modified organism-free RNA interference: Exogenous application of RNA molecules in plants. Plant Physiology, 182(1), 38-50. https://doi.org/10.1104/pp.19.00570

113. Dubrovina, A. S., & Kiselev, K. V. (2019). Exogenous RNAs for gene regulation and plant resistance. International Journal of Molecular Sciences, 20(9), 2282. https://doi.org/10.3390/ijms20092282

114. Mitter, N., Worrall, E. A., Robinson, K. E., Xu, Z. P., & Carroll, B. J. (2017). Induction of virus resistance by exogenous application of double-stranded RNA. Current Opinion in Virology, 26, 49-55. https://doi.org/10.1016/j.coviro.2017.07.009

115. Konakalla, N. C., Kaldis, A., Berbati, M., Masarapu, H., & Voloudakis, A. E. (2016). Exogenous application of double-stranded RNA molecules from TMV p126 and CP genes confers resistance against TMV in tobacco. Planta, 244(4), 961-969. https://doi.org/10.1007/s00425-016-2567-6

116. Kaldis, A., Berbati, M., Melita, O., Reppa, C., Holeva, M., Otten, P., &Voloudakis, A. (2018). Exogenously applied dsRNA molecules deriving from the Zucchini yellow mosaic virus

UNDER PEER REVIEW

(ZYMV) genome move systemically and protect cucurbits against ZYMV. Molecular Plant Pathology, 19(4), 883-895. https://doi.org/10.1111/mpp.12572

117. Niehl, A., Soininen, M., Poranen, M. M., & Heinlein, M. (2018). Synthetic biology approach for plant protection using dsRNA. Plant Biotechnology Journal, 16(9), 1679-1687. https://doi.org/10.1111/pbi.12904

118. Yin, G., Sun, Z., Liu, N., Zhang, L., Song, Y., Zhu, C., & Wen, F. (2009). Production of double-stranded RNA for interference with TMV infection utilizing a bacterial prokaryotic expression system. Applied Microbiology and Biotechnology, 84(2), 323-333. https://doi.org/10.1007/s00253-009-1967-y

119. Gan, D., Zhang, J., Jiang, H., Jiang, T., Zhu, S., & Cheng, B. (2010). Bacterially expressed dsRNA protects maize against SCMV infection. Plant Cell Reports, 29(11), 1261-1268. https://doi.org/10.1007/s00299-010-0911-z

120. Tenllado, F., Martínez-García, B., Vargas, M., & Díaz-Ruíz, J. R. (2003). Crude extracts of bacterially expressed dsRNA can be used to protect plants against virus infections. BMC Biotechnology, 3(1), 1-11. https://doi.org/10.1186/1472-6750-3-3

121. Shen, W., Yang, G., Chen, Y., Yan, P., Tuo, D., Li, X., & Zhou, P. (2014). Resistance of non-transgenic papaya plants to papaya ringspot virus (PRSV) mediated by intron-containing hairpin dsRNAs expressed in bacteria. Acta Virologica, 58(3), 261-266. https://doi.org/10.4149/av_2014_03_261

122. Mitter, N., Worrall, E. A., Robinson, K. E., Xu, Z. P., & Carroll, B. J. (2017). Induction of virus resistance by exogenous application of double-stranded RNA. Current Opinion in Virology, 26, 49-55. https://doi.org/10.1016/j.coviro.2017.07.009

123. Palli, S. R. (2014). RNA interference in Colorado potato beetle: steps toward development of dsRNA as a commercial insecticide. Current Opinion in Insect Science, 6, 1-8. https://doi.org/10.1016/j.cois.2014.09.011

124. Yu, X. D., Liu, Z. C., Huang, S. L., Chen, Z. Q., Sun, Y. W., Duan, P. F., ... & Shen, J. L. (2016). RNAi-mediated plant protection against aphids. Pest Management Science, 72(6), 1090-1098. https://doi.org/10.1002/ps.4258

125. Zotti, M., dos Santos, E. A., Cagliari, D., Christiaens, O., Taning, C. N. T., &Smagghe, G. (2018). RNA interference technology in crop protection against arthropod pests, pathogens and nematodes. Pest Management Science, 74(6), 1239-1250. https://doi.org/10.1002/ps.4813

126. Cagliari, D., Dias, N. P., Galdeano, D. M., dos Santos, E. Á., Smagghe, G., & Zotti, M. J. (2019). Management of pest insects and plant diseases by non-transformative RNAi. Frontiers in Plant Science, 10, 1319. https://doi.org/10.3389/fpls.2019.01319

127. Vogel, E., Santos, D., Mingels, L., Verdonckt, T. W., & Broeck, J. V. (2019). RNA interference in insects: protecting beneficials and controlling pests. Frontiers in Physiology, 9, 1912. https://doi.org/10.3389/fphys.2018.01912

UNDER PEER REVIEW

128. Yan, S., Ren, B., & Shen, J. (2021). Nanoparticle-mediated double-stranded RNA delivery system: A promising approach for sustainable pest management. Insect Science, 28(1), 21-34. https://doi.org/10.1111/1744-7917.12822

129. de Andrade, E. C., & Hunter, W. B. (2016). RNA interference–natural gene-based technology for highly specific pest control (HiSPeC). In RNA Interference. IntechOpen. https://doi.org/10.5772/61612

130. Niu, J., Shen, G., Christiaens, O., Smagghe, G., He, L., & Wang, J. J. (2018). Beyond insects: current status and achievements of RNA interference in mite pests and future perspectives. Pest Management Science, 74(12), 2680-2687. https://doi.org/10.1002/ps.5071

131. Taning, C. N. T., Arpaia, S., Christiaens, O., Dietz-Pfeilstetter, A., Jones, H., Mezzetti, B., ... &Smagghe, G. (2020). RNA-based biocontrol compounds: current status and perspectives to reach the market. Pest Management Science, 76(3), 841-845. https://doi.org/10.1002/ps.5686

132. Mat Jalaluddin, N. S., Othman, R. Y., & Harikrishna, J. A. (2019). Global trends in research and commercialization of exogenous and endogenous RNAi technologies for crops. Critical Reviews in Biotechnology, 39(1), 67-78. https://doi.org/10.1080/07388551.2018.1496064

133. Mezzetti, B., Smagghe, G., Arpaia, S., Christiaens, O., Dietz-Pfeilstetter, A., Jones, H., ... & Taning, C. N. T. (2020). RNAi: What is its position in agriculture?. Journal of Pest Science, 93, 1125-1130. https://doi.org/10.1007/s10340-020-01238-2

134. Bramlett, M., Plaetinck, G., &Maienfisch, P. (2020). RNA-based biocontrols—a new paradigm in crop protection. Engineering, 6(5), 522-527. https://doi.org/10.1016/j.eng.2019.09.008

135. Taning, C. N. T., Arpaia, S., Christiaens, O., Dietz-Pfeilstetter, A., Jones, H., Mezzetti, B., ... &Smagghe, G. (2020). RNA-based biocontrol compounds: current status and perspectives to reach the market. Pest Management Science, 76(3), 841-845. https://doi.org/10.1002/ps.5686

136. Rodrigues, T. B., & Petrick, J. S. (2020). Safety considerations for humans and other vertebrates regarding agricultural uses of externally applied RNA molecules. Frontiers in Plant Science, 11, 407. https://doi.org/10.3389/fpls.2020.00407

137. Romeis, J., & Widmer, F. (2020). Assessing the risks of topically applied dsRNA-based products to non-target arthropods. Frontiers in Plant Science, 11, 679. https://doi.org/10.3389/fpls.2020.00679

138. Christiaens, O., Whyard, S., Vélez, A. M., &Smagghe, G. (2020). Double-stranded RNA technology to control insect pests: current status and challenges. Frontiers in Plant Science, 11, 451. https://doi.org/10.3389/fpls.2020.00451

UNDER PEER REVIEW

139. Joga, M. R., Zotti, M. J., Smagghe, G., & Christiaens, O. (2016). RNAi efficiency, systemic properties, and novel delivery methods for pest insect control: what we know so far. Frontiers in Physiology, 7, 553. https://doi.org/10.3389/fphys.2016.00553

140. Niu, X., Kassa, A., Hu, X., Robeson, J., McMahon, M., Richtman, N. M., ... & Wu, G. (2017). Control of western corn rootworm (Diabroticavirgiferavirgifera) reproduction through plant-mediated RNA interference. Scientific Reports, 7(1), 1-13. https://doi.org/10.1038/s41598-017-12638-3

141. Gu, L., & Knipple, D. C. (2013). Recent advances in RNA interference research in insects: Implications for future insect pest management strategies. Crop Protection, 45, 36-40. https://doi.org/10.1016/j.cropro.2012.10.004

142. Burand, J. P., & Hunter, W. B. (2013). RNAi: Future in insect management. Journal of Invertebrate Pathology, 112, S68-S74. https://doi.org/10.1016/j.jip.2012.07.012

143. San Miguel, K., & Scott, J. G. (2016). The next generation of insecticides: dsRNA is stable as a foliar-applied insecticide. Pest Management Science, 72(4), 801-809. https://doi.org/10.1002/ps.4056

144. Wang, J., Jia, D., Tang, L., Xu, Z., Xu, H., Chen, R., ... & Zhang, J. (2021). Oral delivery of dsRNA lipid nanocapsules for gene knockdown in the honey bee parasite Varroa destructor. ACS Applied Materials & Interfaces, 13(7), 8303-8309. https://doi.org/10.1021/acsami.0c22135

145. Zhu, K. Y., & Palli, S. R. (2020). Mechanisms, applications, and challenges of insect RNA interference. Annual Review of Entomology, 65, 293-311. https://doi.org/10.1146/annurev-ento-011019-025224

146. Taning, C. N. T., Christiaens, O., Berkvens, N., Casteels, H., Maes, M., &Smagghe, G. (2016). Oral RNAi to control Drosophila suzukii: laboratory testing against larval and adult stages. Journal of Pest Science, 89(3), 803-814. https://doi.org/10.1007/s10340-016-0736-9

147. Vélez, A. M., Fishilevich, E., Rangasamy, M., Khajuria, C., McCaskill, D. G., Pereira, A. E., ... & Siegfried, B. D. (2020). Control of western corn rootworm via RNAi traits in maize: lethal and sublethal effects of Sec23 dsRNA. Pest Management Science, 76(4), 1500-1512. https://doi.org/10.1002/ps.5666

148. Vogel, E., Santos, D., Mingels, L., Verdonckt, T. W., & Vanden Broeck, J. (2019). RNA interference in insects: protecting beneficials and controlling pests. Frontiers in Physiology, 9, 1912. https://doi.org/10.3389/fphys.2018.01912

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