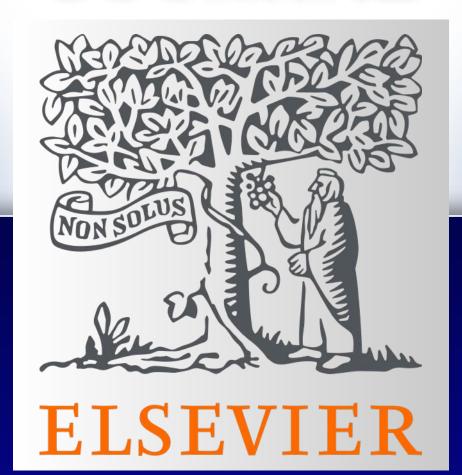
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"Targeted Insect Pest Control Using RNA Interference (RNAi): A short review"

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Abstract:

A novel, naturally occurring gene-silencing process, RNA interference (RNAi) has great promise for creating highly targeted and eco-friendly insect pest management techniques. Exogenous double-stranded RNA (dsRNA) molecules that are complementary to important genes in an insect's genome are introduced as the fundamental component of RNA interference (RNAi)-based pest management. These dsRNAs are converted into smaller interfering RNAs (siRNAs) after being consumed or absorbed. The target gene is therefore "silencing" as a result of these siRNAs directing the RNA-induced silencing complex (RISC) to locate and break the same mRNA. The remarkable species-specificity of RNA interference is a key benefit for pest management. This accuracy significantly lessens the negative effects that broad-spectrum chemical pesticides frequently have on the ecosystem by minimising damage to non-target creatures such beneficial insects, pollinators and natural predators. Moreover, dsRNA molecules are naturally occurring biological substances that have a brief environmental persistence. Despite the significant success of early RNA interference applications, especially against coleopteran pests, difficulties still exist. These include finding universally effective target genes, overcoming physiological hurdles to dsRNA absorption and improving dsRNA delivery techniques (such as through transgenic plants or topical treatments). The goal of ongoing research is to guarantee the ecological safety and public acceptability of this promising technology by improving RNAi efficiency, creating dsRNA manufacturing that is affordable and carrying out comprehensive risk evaluations. RNAi is a ground-breaking technique for integrated pest management (IPM), providing a targeted, environmentally responsible and sustainable method of safeguarding public health and agriculture.

Keywords: RNA Interference (RNAi), Insect Pest Control, Gene Silencing, Double-stranded RNA (dsRNA), Species-specificity, Sustainable Agriculture

Introduction:

Insect pests pose a significant threat to global agriculture, contributing to substantial crop losses and affecting food security. Traditionally, chemical insecticides have been the primary tool for pest management; however, their widespread use has led to concerns about environmental pollution, non-target species effects and the development of pesticide resistance [1-2]. In this context, RNA interference (RNAi) has emerged as a novel and highly specific molecular approach for pest control that leverages the natural gene-silencing pathways found in most eukaryotes.

RNAi is a post-transcriptional gene silencing mechanism initiated by the introduction of doublestranded RNA (dsRNA), which leads to the degradation of complementary messenger RNA (mRNA), effectively silencing the target gene [3]. In insect pest control, RNAi can be used to selectively disrupt genes essential for survival, reproduction, or development, offering a precise method to reduce pest populations with minimal ecological impact [4]. The species-specificity of RNAi makes it particularly appealing compared to broad-spectrum insecticides, as it minimizes risks to pollinators and other beneficial organisms [5]. Recent advancements in dsRNA delivery methods, including plant-mediated RNAi (transgenic crops), topical applications and nanoparticle carriers, have expanded the potential of RNAi-based technologies in field settings [6-7]. Despite its promise, the efficacy of RNAi varies among insect orders, with Coleoptera showing high susceptibility and Lepidoptera often displaying resistance due to differences in gut pH, dsRNA degradation and cellular uptake [8-9]. Addressing these limitations through improved delivery systems and molecular tools remains a critical focus of current research. As the need for sustainable pest control intensifies, RNAi represents a powerful tool that could revolutionize integrated pest management (IPM) strategies. Continued research into its mechanisms, delivery methods and ecological impact will determine its long-term viability and acceptance in agricultural practices.

Mechanisms of RNAi in Insects:

RNA interference (RNAi) is a gene regulatory mechanism conserved across many eukaryotic organisms, including insects. It plays a critical role in antiviral defense, gene regulation and genome stability. In the context of pest management, RNAi can be exploited to silence essential genes in insect pests, leading to growth inhibition, sterility, or mortality. The RNAi mechanism in insects involves several distinct stages: uptake of double-stranded RNA (dsRNA), processing into

small interfering RNAs (siRNAs), incorporation into the RNA-induced silencing complex (RISC) and sequence-specific degradation of target mRNA.

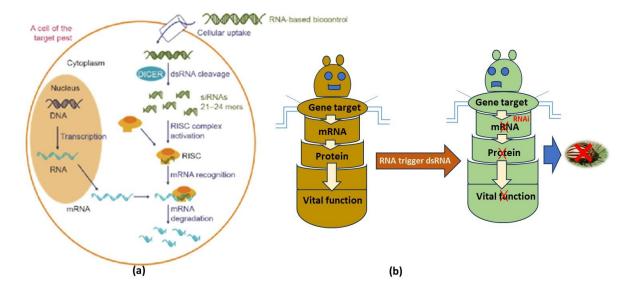


Figure.1: (a) RNA interference's molecular process; (b) the idea behind applying RNA interference to insect control. RISC: RNA-induced silencing complex; siRNA: short interfering RNA; DICER: a ribonuclease name [10].

1. dsRNA Uptake:

The initiation of RNAi begins with the uptake of externally delivered dsRNA, which can occur through feeding, microinjection, or transgenic expression in host plants. In insects, dsRNA uptake occurs primarily in the gut epithelium following ingestion. Two major mechanisms for dsRNA uptake have been described, 1). Clathrin-mediated endocytosis is a primary mode of uptake in many insect species, particularly those with RNAi-responsive systems such as beetles (Coleoptera) [11-12]. 2). SID-like proteins (Systemic RNA Interference Defective proteins), homologs of nematode SID-1, have been implicated in dsRNA uptake and systemic spread in some insects [13]. However, many insects lack functional SID-1 homologs, which may contribute to variability in RNAi efficiency.

2. Processing of dsRNA into siRNAs:

Once inside the cytoplasm, long dsRNA is recognized and cleaved by an RNase III enzyme called Dicer-2 into 21–23 nucleotide long small interfering RNAs (siRNAs) [14]. This is a critical step in initiating gene silencing and the efficiency of this cleavage influences the strength of the RNAi

response. Dicer-2 is responsible for cleaving the dsRNA into siRNAs with characteristic 2-nucleotide 3' overhangs. The resulting siRNAs are double-stranded and then loaded into the RNA-Induced Silencing Complex (RISC).

3. RISC Assembly and Target Cleavage:

The RISC complex, with Argonaute-2 (Ago2) as its catalytic component, unwinds the siRNA duplex and retains the guide strand (complementary to the target mRNA), while discarding the passenger strand. The guide strand directs RISC to complementary mRNA sequences. Ago2 cleaves the target mRNA in the middle of the complementary region, leading to degradation and gene silencing [15].

4. Systemic Spread and Amplification:

In some insects, the RNAi effect remains localized to the cells that initially take up dsRNA. However, in others, the silencing signal can spread systemically to other tissues and organs. The systemic RNAi response is more pronounced in Coleoptera and some Hemiptera but is weak or absent in Lepidoptera and Diptera. Systemic RNAi may be facilitated by extracellular vesicles, endocytic transport, or SID-like proteins [16]. Unlike in plants and nematodes, amplification of the RNAi signal via RNA-dependent RNA polymerases (RdRPs) is absent in insects, which may limit the duration and spread of the effect.

5. Factors Affecting RNAi Efficiency in Insects:

RNAi efficacy in insects is influenced by various physiological and molecular factors, dsRNA Degradation: Some insects possess high levels of dsRNases in the gut and hemolymph, which degrade dsRNA before it can trigger silencing. For example, Lepidoptera (e.g., *Helicoverpa armigera*) exhibit strong dsRNA degradation activity [17]. pH and Digestive Conditions: Alkaline midgut pH in Lepidoptera can denature dsRNA, contributing to RNAi refractoriness [18]. Uptake Efficiency: Insects differ in their endocytosis rates and dsRNA transport capabilities. Gene Target Selection: Some genes are more amenable to silencing than others, depending on their expression levels and cellular localization.

Delivery Strategies:

Successful implementation of RNA interference (RNAi) for insect pest management depends largely on the efficient and species-specific delivery of double-stranded RNA (dsRNA) into the target insect. Various delivery methods have been explored to overcome physiological barriers, improve RNAi efficacy and facilitate field deployment. These strategies can be broadly categorized into plant-mediated, topical, injection-based, nanoparticle-assisted and microbial delivery systems.

1. Transgenic Plant-Mediated Delivery (HIGS/PM-RNAi)

In this strategy, transgenic plants are engineered to express dsRNA targeting essential insect genes. When the insect feeds on the plant, it ingests the dsRNA, leading to gene silencing. Nuclear-transformed plants: The dsRNA is expressed in the nucleus and processed in the cytoplasm. Used effectively in maize, cotton and potato against pests like the corn rootworm and cotton bollworm [19-20]. Plastid-mediated expression: Plastids (e.g., chloroplasts) can accumulate high levels of dsRNA without triggering RNAi machinery, leading to more stable and abundant dsRNA in plant tissues [21]. Continuous delivery during feeding; high target specificity. The limitation are regulatory hurdles for GM crops; potential for resistance development.

2. Topical Application (Spray-Induced Gene Silencing - SIGS)

SIGS involves direct application of dsRNA to the surface of plants or insects through spraying, trunk injection, or root soaking. dsRNA can be sprayed onto crops or applied through irrigation systems. First successful demonstration in Colorado potato beetle showed significant gene knockdown and mortality [22]. Non-GMO; flexible and fast to deploy. The limitations are dsRNA instability due to UV light, nucleases and environmental conditions.

3. Microinjection

Microinjection delivers dsRNA directly into the insect's body cavity or tissues. Often used in laboratory experiments for mechanistic studies or proof-of-concept research [8]. Microinjection into eggs, embryos, or adults allows precise control over dose and timing.

Advantages are High precision and reproducibility. Limitations are Labor-intensive, impractical for field applications and limited to research use.

4. Nanoparticle-Based Delivery or Carriers:

Nanocarriers enhance the stability, uptake and delivery efficiency of dsRNA in hostile environments (e.g., alkaline gut conditions). Chitosan nanoparticles, liposomes, carbon quantum dots and layered double hydroxides (BioClay) are explored for encapsulating dsRNA [23-24]. Nanoparticles protect dsRNA from enzymatic degradation and improve cellular uptake via endocytosis. Advantages are Protection from degradation, increased bioavailability, potential for targeted delivery. Limitations are Cost, regulatory complexity and need for environmental risk assessment.

5. Microbial and Symbiont-Based Delivery or Vector-Mediated Delivery

Genetically modified microorganisms (bacteria, viruses, or endosymbionts) are engineered to produce and deliver dsRNA inside the insect. Bacillus thuringiensis (Bt) and yeast-based systems have been used to express dsRNA targeting pest genes [25]. Endosymbionts are particularly useful in hemipterans (e.g., aphids) where direct delivery is challenging. Advantages are Long-term, systemic delivery; potential for persistence in insect populations. Limitations are Ecological risks, genetic stability of engineered microbes, regulatory hurdles.

6. Feeding-Based Delivery (Artificial Diet)

This approach is widely used in lab assays, where insects are fed artificial diets containing dsRNA. Effective for gene function studies and screening target genes [26]. Works well in RNAi-sensitive species like coleopterans. Advantages are Easy to implement in lab; dosage can be precisely controlled. Limitations are Not practical for field use; not suitable for all feeding behaviors.

Table1: RNAi Delivery Strategies

Strategy	Application	Key Pros	Key Cons
Plant-mediated	Field crops	Continuous delivery,	GMO regulations,
(HIGS)		high yield	resistance risk
Spray-based (SIGS)	Field crops	Non-GMO, flexible	dsRNA degradation,
			short-term effect
Microinjection	Lab assays	Precise, reliable	Labor-intensive, not
			scalable

Nanoparticles	Field and lab	Protection, enhanced	Expensive, still under
		uptake	development
Microbial/Symbiont	Experimental/lab	Potential for systemic	Ecological concerns,
		delivery	stability issues
Feeding (diet-based)	Laboratory	Controlled exposure	Not field-relevant
	studies		

Challenges and Limitations:

A number of biological, technological and regulatory obstacles prevent RNA interference (RNAi) from being widely used in agriculture, despite its potential as a species-specific and eco-friendly substitute for traditional pesticides. To increase RNAi's effectiveness, delivery and scalability, it is imperative to comprehend these constraints.

1. Variability in RNAi Efficiency Across Insect Orders

RNAi response is highly variable across insect taxa, with Coleoptera (e.g., *Leptinotarsa decemlineata*) being highly responsive, while Lepidoptera (e.g., *Helicoverpa armigera*) and Diptera (e.g., *Drosophila melanogaster*) often show poor response. This variability is attributed to differences in gut environment, dsRNA uptake mechanisms and RNAi machinery components [18-17]. Some insects lack SID-like transmembrane proteins that mediate systemic RNAi spread [13].

2. dsRNA Degradation

Insects, particularly Lepidoptera, possess nucleases in the gut lumen and hemolymph that rapidly degrade dsRNA before it can be taken up or processed. This is a major bottleneck in RNAi efficiency and is one reason why topical or ingested RNAi fails in many species [27]. Strategies such as dsRNA encapsulation with nanoparticles or co-delivery with nuclease inhibitors are being investigated to mitigate this problem [25].

3. Limited Systemic Spread

Unlike plants and nematodes, most insects do not amplify or efficiently spread the RNAi signal systemically due to the absence of RNA-dependent RNA polymerase (RdRP) genes. As a result,

silencing often remains localized to the tissues where dsRNA is delivered [8]. This limits the effectiveness of RNAi for targeting genes expressed in internal organs or across multiple tissues.

4. Target Gene Selection and Off-Target Effects

Designing effective dsRNA requires careful selection of essential and insect-specific genes to avoid: Off-target silencing, where unintended genes with partial sequence homology are affected [28]. Non-target effects on beneficial insects (e.g., pollinators or predators), particularly if conserved gene sequences are targeted [29]. Solution: Bioinformatics tools are used to screen for off-target matches, but this is not foolproof.

5. Resistance Development

Just like with chemical pesticides, there is potential for evolution of resistance to RNAi in insect populations. Mechanisms may include mutations in target genes, upregulation of nucleases, downregulation of dsRNA uptake genes, or changes in RNAi pathway components [30]. Repeated exposure to the same dsRNA increases selection pressure, making resistance a realistic concern. Solution is Use of multiple gene targets, rotational strategies, or cocktail dsRNAs can delay resistance.

6. Environmental Stability of dsRNA

Unprotected dsRNA degrades quickly in the field due to UV exposure, rain, temperature and microbial activity. This makes foliar sprays (SIGS) short-lived and reduces field-level RNAi success [31]. Solution is Incorporation into nanocarriers or formulations can improve dsRNA stability and uptake.

7. Delivery Challenges

Efficient delivery remains a central technical barrier, especially for Non-chewing insects (e.g., sapsucking Hemiptera), which have limited ingestion of dsRNA. Spray-based applications, which may not ensure sufficient dsRNA uptake. Plant-mediated RNAi (HIGS) is effective but requires genetic transformation, which is time-consuming and faces regulatory resistance in some regions.

8. Scalability and Cost of dsRNA Production

Producing large quantities of high-quality dsRNA for field application remains expensive. In vitro transcription systems are not scalable. Microbial fermentation (e.g., in *E. coli*) is a more affordable method but may involve regulatory complexities [32]. Efforts are ongoing to optimize cost-effective production platforms, such as yeast-based and chloroplast-expressed dsRNAs.

9. Regulatory and Public Acceptance

Regulatory pathways for RNAi-based biopesticides are still being defined in many countries. Concerns include off-target effects, ecotoxicity, persistence in the environment and impact on biodiversity. Public perception of RNAi as a genetic technology may lead to the same resistance seen with GMOs, despite many RNAi products being non-transgenic.

Case Studies and Field Applications:

1. Colorado Potato Beetle (Leptinotarsa decemlineata)

The Colorado potato beetle (CPB) (**Figure.2**), is one of the most notorious pests of solanaceous crops (e.g., potatoes, tomatoes). It has developed resistance to nearly all major classes of chemical insecticides, making it an ideal candidate for RNAi-based control. RNAi Strategy: Target genes: *Actin*, *v-ATPase A and E*, *Snf7*, *chitin synthase* and others critical for cellular function and molting [19-34]. Delivery methods: Topical sprays, plant-mediated RNAi and ingestion through artificial diet have all been tested. Mode of action: dsRNA ingestion leads to gene knockdown, resulting in developmental arrest, impaired feeding and mortality. Field Application & Results, Monsanto and Devgen demonstrated that transgenic potato plants expressing dsRNA targeting CPB genes could provide effective protection [19]. Sprayable RNAi formulations are being developed as non-GMO alternatives. RNAi-based product (e.g., LedpronaTM, developed by GreenLight Biosciences) was approved by the EPA in 2023 for field use in the U.S. Impact is CPB remains the most successful field case for RNAi pest control to date due to its high RNAi sensitivity and consistent gene silencing through ingestion.



Figure.2: Adult Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Credit: Whitney Cranshaw, Colorado State University, www.insectimages.org

2. Varroa Mite (Varroa destructor)

The Varroa mite (**figure.3**) is a parasitic arachnid that infests honeybee (*Apis mellifera*) colonies, contributing significantly to colony collapse disorder (CCD). RNAi Strategy, Target genes: *VdSnf7*, *VdUbiquitin*, *VdChitinase* and other essential genes [35]. Delivery methods: dsRNA is fed to bees, which then transfer it to mites through trophallaxis or contact. Mode of action: After ingestion by the mite via bee hemolymph, dsRNA silences target genes leading to mite death or reproductive failure. Field Application & Results: **Garbian et al. (2012)** demonstrated that bees fed sugar syrup containing mite-specific dsRNA significantly reduced mite populations without harming the bees. Companies like SiRNAgen are working on commercial RNAi-based Varroa control products. Challenges are Ensuring consistent delivery to mites via the host is complex and Environmental persistence and protection from degradation are critical for success.



Figure.3: One of the main parasites of honey bees (Apis mellifera) and a major cause of colony losses in North America is the Varroa destructor mite. According to a recent study, honey bee

colonies that consume pollen tainted with neonicotinoid pesticide exhibit increased Varroa mite parasitism. According to the study's researchers, this is the first experimental field proof of how neonic exposure might boost honey bee populations of Varroa (Photo by Gilles San Martin via Naturalist, CC BY-SA 4.0).

3. Western Flower Thrips (Frankliniella occidentalis)

Western flower thrips (WFT) (**figure.2**), is a polyphagous pest that causes direct feeding damage and transmits viruses such as Tomato spotted wilt virus (TSWV). Control is challenging due to small size, cryptic behavior and insecticide resistance. RNAi Strategy are Target genes, *Vacuolar ATPase*, *Actin*, *Chitin synthase*, α-tubulin and *Cytochrome P450s* [36]. Delivery methods: Oral feeding via artificial diet, microinjection and plant-mediated RNAi. Mode of action: Gene silencing results in disrupted development, reduced reproduction and increased mortality. Field Application & Results are Most studies are currently at the lab and greenhouse level. Feeding assays confirmed significant knockdown and phenotypic effects. RNAi via topical application or HIGS remains challenging due to poor dsRNA uptake and high nuclease activity in WFT [37]. Challenges are Low RNAi efficiency and instability of dsRNA in field conditions and Limited systemic RNAi spread in thrips limits broader application potential.



Figure.4: Frankliniella occidentalis [38].

Table.2: RNAi applications in three key insect pests.

Pest Species	RNAi	Target Genes	Delivery Methods	Field Readiness
	Sensitivity			

Colorado Potato	High	v-ATPase,	Plant-mediated,	Approved product
Beetle		Snf7, etc.	Spray	(U.S.)
Varroa Mite	Moderate-	VdSnf7,	Sugar syrup (bee	In development
	High	Ubiquitin	feeding)	
Western Flower	Low-	Chitin	Feeding,	Experimental stage
Thrips	Moderate	synthase, Actin	microinjection	

Integration into Integrated Pest Management (IPM):

A comprehensive strategy for crop protection, integrated pest management (IPM) uses biological, cultural, physical and chemical methods to control pest populations in an environmentally and economically responsible manner. Because of its target specificity, environmental safety and potential to decrease the usage of chemical pesticides, RNA interference (RNAi) is a good fit for this framework.

1. RNAi as a Precision Tool in IPM

RNAi's mechanism allows for species-specific gene silencing, minimizing off-target effects on non-target and beneficial organisms such as pollinators, parasitoids and predators [5]. This aligns with IPM's core goal of minimizing ecosystem disruption. Targeted Control: dsRNA can silence genes essential for survival or reproduction of pest species without affecting natural enemies. Compatibility with Monitoring: RNAi products can be deployed based on pest population thresholds, as with traditional IPM decision tools.

2. RNAi as a Resistance Management Strategy

Pesticide resistance is a major concern in IPM. RNAi offers a novel mode of action distinct from conventional insecticides, which can help manage resistance in pest populations when used in rotation or combination with other control methods [34]. Gene stacking or multi-target RNAi can delay resistance evolution. Combination strategies: RNAi may be used alongside microbial pesticides or selective insecticides to manage resistance.

3. Environmental and Human Safety Benefits

RNAi-based products (especially topical dsRNA sprays) generally do not persist in the environment and dsRNA is rapidly degraded in soil and water, minimizing contamination risks [31]. Non-toxic to humans and vertebrates due to sequence specificity and degradation by mammalian nucleases. Suitable for organic or low-input farming systems if proven effective.

4. Integration with Biological Control

RNAi can complement biological control by sparing natural enemies that are often harmed by broad-spectrum pesticides. For example, RNAi targeting *Leptinotarsa decemlineata* has no effect on predatory ladybird beetles or pollinators like bees [39]. Selective RNAi ensures functional biodiversity is maintained, enhancing ecosystem resilience.

5. Delivery Compatibility with IPM Practices

RNAi can be delivered through multiple channels that fit into IPM systems Spray-Induced Gene Silencing (SIGS): Compatible with foliar application schedules. Host-Induced Gene Silencing (HIGS): Long-term protection through transgenic plants. Bait stations, microbial carriers, or root drenches: Precision targeting in confined environments like greenhouses.

6. Challenges for IPM Integration

Notwithstanding its potential, a number of obstacles need to be overcome before RNAi can be completely included into IPM systems. The cost and scalability of producing dsRNA. varying effectiveness in different insect taxa. Clarity in regulations for environmental usage. training and awareness of stakeholders among extension agents and farmers. To guarantee proper deployment and stewardship of RNAi technologies, scientists, regulatory agencies, industry and farmers must work together for effective integration.

7. Case Study Integration Example: Colorado Potato Beetle

In a field trial using RNAi spray or transgenic plants targeting *Snf7* gene in CPB, populations declined significantly, reducing the need for neonicotinoids. Result are Fewer chemical applications, preservation of beneficial arthropods and delayed resistance development [19-34]. Demonstrates RNAi's potential to replace or reduce insecticide use in high-resistance pests.

Future Directions and Perspectives:

Although RNA interference (RNAi) has shown great promise for managing insect pests sustainably, present barriers must be removed before it can be widely used and have an impact. Enhancing delivery methods, increasing effectiveness, guaranteeing long-term field success and broadening the use of RNA interference through innovative mechanisms are the main goals of future research. The four main paths to progress are shown below.

1. Enhanced Carriers for dsRNA Delivery

Goal are Improve the stability, target specificity and cellular uptake of dsRNA in harsh environmental and insect gut conditions. Nanoparticle-based delivery systems (e.g., liposomes, chitosan, clay nanosheets, carbon dots) are being developed to protect dsRNA from degradation and improve uptake [40-5]. Smart carriers that respond to pH or enzymatic conditions can enable controlled release of dsRNA in specific insect environments. Microbial delivery systems, such as genetically engineered *E. coli* or yeast, show promise for scalable, cost-effective production and oral delivery [32]. Perspective are Future RNAi formulations will likely include multi-functional nanocarriers tailored to specific insect physiology, crop systems and field conditions.

2. Combination Strategies

Goal are Combine RNAi with other pest control methods to enhance efficacy and delay resistance. RNAi + biopesticides: dsRNA can be combined with Bacillus thuringiensis (Bt) toxins or entomopathogenic fungi to produce synergistic effects [41]. RNAi + chemical insecticides: Sublethal doses of insecticides can improve dsRNA uptake, particularly in RNAi-recalcitrant insects like Lepidoptera. Stacked RNAi constructs: Targeting multiple essential genes in a single dsRNA construct can reduce the likelihood of resistance and improve robustness. Perspective are Integrated use of RNAi in combination treatments will help it transition from a stand-alone technology to a component of modern IPM systems.

3. Field Validation and Large-Scale Trials

Goal are Conduct rigorous field studies to evaluate RNAi performance under real-world environmental conditions. Most RNAi research remains confined to lab or greenhouse settings. Field trials are needed to test factors such as, dsRNA persistence, Rainfastness, Delivery method efficiency, Nontarget organism safety. Products like LedpronaTM for Colorado potato beetle mark progress in regulatory approval and commercial-scale testing [42]. Perspective are Success of field

validation will determine the commercial viability and regulatory acceptance of RNAi technologies.

4. Transgenerational RNAi

Goal are Explore and utilize transgenerational RNAi (tgRNAi), where gene silencing effects persist into the next generation of insects. Documented in some species like *Tribolium castaneum*, tgRNAi could offer longer-lasting pest suppression from a single application [43]. Mechanisms include transmission of small RNAs or chromatin modifications that persist through reproduction. TgRNAi may reduce the frequency and cost of applications in the field. Perspective are with better understanding and control, transgenerational RNAi could transform pest control into a semi-permanent, heritable solution.

Conclusion:

In conclusion, the utilization of RNA interference (RNAi) presents a promising approach to manage insect pest populations by offering a targeted, environmentally friendly and cost-effective solution for sustainable agriculture and integrated pest management strategies, which can be further optimized and integrated with existing pest management practices to enhance their efficacy and minimize the development of resistance in pest populations, leading to long-term agricultural sustainability and reduced environmental impact. To fully realize the potential of RNAi technology, it is essential to continue research and development in this area, focusing on improving the delivery mechanisms, stability and specificity of RNAi-based pesticides, as well as addressing regulatory and public acceptance concerns through transparent and effective communication, education and outreach programs, ensuring the safe and responsible deployment of RNAi technology for the betterment of agricultural practices worldwide, thereby contributing to global food security, human health and environmental protection.

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References:

[1] D. Pimentel, "Environmental and economic costs of the application of pesticides primarily in the United States," *Environ. Dev. Sustain.*, vol. 7, no. 2, pp. 229–252, 2005.

- [2] T. C. Sparks and R. Nauen, "IRAC: Mode of action classification and insecticide resistance management," *Pestic. Biochem. Physiol.*, vol. 121, pp. 122–128, 2015.
- [3] A. Fire, S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello, "Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*," *Nature*, vol. 391, no. 6669, pp. 806–811, 1998.
- [4] K. Y. Zhu and S. R. Palli, "Mechanisms, applications and challenges of insect RNA interference," *Annu. Rev. Entomol.*, vol. 65, pp. 293–311, 2020.
- [5] O. Christiaens, S. Whyard, A. M. Velez, and G. Smagghe, "Double-stranded RNA technology to control insect pests: Current status and challenges," *Front. Plant Sci.*, vol. 11, p. 451, 2020. doi: 10.3389/fpls.2020.00451.
- [6] L. Gu and D. C. Knipple, "Recent advances in RNA interference research in insects: Implications for future insect pest management strategies," *Crop Prot.*, vol. 45, pp. 36–40, 2013.
- [7] M. R. Joga, M. J. Zotti, G. Smagghe, and O. Christiaens, "RNAi efficiency, systemic properties and novel delivery methods for pest insect control: What we know so far," *Front. Physiol.*, vol. 7, p. 553, 2016.
- [8] S. Whyard, A. D. Singh, and S. Wong, "Ingested double-stranded RNAs can act as species-specific insecticides," *Insect Biochem. Mol. Biol.*, vol. 39, no. 11, pp. 824–832, 2009.
- [9] L. Xu, H. Wang, K. He, and S. Bai, "Advances in understanding the mechanisms of RNAi in insects and potential use for pest management," *Pest Manag. Sci.*, vol. 77, no. 7, pp. 3141–3151, 2021.
- [10] M. Bramlett, G. Plaetinck, and P. Maienfisch, "RNA-based biocontrols: A new paradigm in crop protection," *Engineering*, vol. 5, no. 5, pp. 867–872, 2019.
- [11] M. C. Saleh, M. Tassetto, R. P. van Rij, B. Goic, V. Gausson, and B. Berry, "Antiviral immunity in *Drosophila* requires systemic RNA interference spread," *Nature*, vol. 439, no. 7072, pp. 746–750, 2006.
- [12] N. Yu, O. Christiaens, J. Liu *et al.*, "Delivery of dsRNA for RNAi in insects: An overview and future directions," *Insect Sci.*, vol. 20, no. 1, pp. 4–14, 2013.
- [13] Y. Tomoyasu, S. C. Miller, S. Tomita, M. Schoppmeier, D. Grossmann, and G. Bucher, "Exploring systemic RNA interference in insects: A genome-wide survey for RNAi genes in *Tribolium*," *Genome Biol.*, vol. 9, no. 1, p. R10, 2008.
- [14] E. Bernstein, A. A. Caudy, S. M. Hammond, and G. J. Hannon, "Role for a bidentate ribonuclease in the initiation step of RNA interference," *Nature*, vol. 409, no. 6818, pp. 363–366, 2001.

- [15] S. M. Hammond, E. Bernstein, D. Beach, and G. J. Hannon, "An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells," *Nature*, vol. 404, no. 6775, pp. 293–296, 2000.
- [16] O. Christiaens, L. Swevers, and G. Smagghe, "DsRNA degradation in the hemolymph limits the efficiency of RNAi in the pine sawfly *Diprion pini*," *Biomed. Res. Int.*, vol. 2014, pp. 1–10, 2014.
- [17] J. N. Shukla, M. Kalsi, A. Sethi, K. E. Narva, and S. R. Palli, "Reduced stability and intracellular transport of dsRNA contribute to poor RNAi response in lepidopteran insects," *RNA Biol.*, vol. 13, no. 7, pp. 656–669, 2016.
- [18] O. Terenius, A. Papanicolaou, J. S. Garbutt *et al.*, "RNA interference in Lepidoptera: An overview of successful and unsuccessful studies and implications for experimental design," *J. Insect Physiol.*, vol. 57, no. 2, pp. 231–245, 2011.
- [19] J. A. Baum, T. Bogaert, W. Clinton *et al.*, "Control of coleopteran insect pests through RNA interference," *Nat. Biotechnol.*, vol. 25, no. 11, pp. 1322–1326, 2007.
- [20] Y. B. Mao, W. J. Cai, J. W. Wang *et al.*, "Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance to gossypol," *Nat. Biotechnol.*, vol. 25, no. 11, pp. 1307–1313, 2011.
- [21] J. Zhang, S. A. Khan, C. Hasse, S. Ruf, D. G. Heckel, and R. Bock, "Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids," *Science*, vol. 347, no. 6225, pp. 991–994, 2015.
- [22] K. San Miguel and J. G. Scott, "The next generation of insecticides: dsRNA is stable as a foliar-applied insecticide," *Pest Manag. Sci.*, vol. 72, no. 4, pp. 801–809, 2016.
- [23] N. Mitter, E. A. Worrall, K. E. Robinson *et al.*, "Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses," *Nat. Plants*, vol. 3, p. 16207, 2017.
- [24] O. Christiaens, S. Whyard, A. M. Vélez, and G. Smagghe, "Double-stranded RNA technology to control insect pests: Current status and challenges," *Front. Plant Sci.*, vol. 9, p. 1172, 2018.
- [25] C. N. T. Taning, S. Arpaia, O. Christiaens *et al.*, "RNA-based biocontrols for insect pest management," *Insect Biochem. Mol. Biol.*, vol. 71, pp. 1–11, 2016.
- [26] K. Y. Zhu *et al.*, "Ingested RNA interference for managing insect pests: Current status and perspectives," *Insect Sci.*, vol. 18, no. 3, pp. 229–244, 2011.
- [27] J. S. Garbutt, X. Bellés, E. H. Richards, and S. E. Reynolds, "Persistence of double-stranded RNA in insect hemolymph as a potential determining factor in RNA interference success," *FEBS J.*, vol. 280, no. 11, pp. 2869–2878, 2013.

- [28] J. Liu, G. Smagghe, L. Swevers, and H. Huvenne, "RNAi in insects: A challenge for transgenic pest control," *Trends Biotechnol.*, vol. 30, no.
- [29] O. Christiaens, S. Whyard, A. M. Vélez, and G. Smagghe, "Double-stranded RNA technology to control insect pests: Current status and challenges," *Front. Plant Sci.*, vol. 11, p. 451, 2020. doi: 10.3389/fpls.2020.00451.
- [30] J. A. Peterson, J. Sweet, and K. Y. Zhu, "RNA interference resistance mechanisms in insects: A moving target," *Insects*, vol. 13, no. 2, p. 124, 2022.
- [31] D. Cagliari, N. P. Dias, D. M. Galdeano, E. Á. dos Santos, and G. Smagghe, "Management of pest insects and plant diseases by non-transformative RNAi," *Front. Plant Sci.*, vol. 10, p. 1319, 2019. doi: 10.3389/fpls.2019.01319.
- [32] M. Zotti, E. A. dos Santos, D. Cagliari, O. Christiaens, C. N. T. Taning, and G. Smagghe, "RNA interference technology in crop protection against arthropod pests, pathogens and nematodes," *Pest Manag. Sci.*, vol. 74, no. 6, pp. 1239–1250, 2018. doi: 10.1002/ps.4813.
- [33] J. A. Baum, T. Bogaert, W. Clinton *et al.*, "Control of coleopteran insect pests through RNA interference," *Nat. Biotechnol.*, vol. 25, no. 11, pp. 1322–1326, 2007.
- [34] S. R. Palli, "RNA interference in Colorado potato beetle: Steps toward development of dsRNA as a commercial insecticide," *Curr. Opin. Insect Sci.*, vol. 6, pp. 1–8, 2014. doi: 10.1016/j.cois.2014.09.011.
- [35] Y. Garbian, E. Maori, H. Kalev, S. Shafir, and I. Sela, "Bidirectional transfer of RNAi between honey bee and *Varroa destructor*: Varroa gene silencing reduces mite populations," *PLoS Pathog.*, vol. 8, no. 12, p. e1003035, 2012. doi: 10.1371/journal.ppat.1003035.
- [36] I. E. Badillo-Vargas, D. Rotenberg, and A. E. Whitfield, "RNA interference tools for the study of vector-virus interactions," *J. Virol. Methods*, vol. 231, pp. 8–17, 2016. doi: 10.1016/j.jviromet.2016.02.007.
- [37] X. Shangguan, J. Zhang, B. Liu *et al.*, "A mucin-like protein is essential for osmoregulation in planthoppers," *Nat. Commun.*, vol. 9, p. 4391, 2018. doi: 10.1038/s41467-018-06605-w.
- [38] R. H. Pergande, "The Corn Root-Worm and Its Work," U.S. Department of Agriculture Division of Entomology Bulletin, no. 4, pp. 1–10, 1895.
- [39] P. M. Bachman, R. Bolognesi, W. J. Moar *et al.*, "Characterization of the spectrum of insecticidal activity of a double-stranded RNA with targeted activity against Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte)," *Transgenic Res.*, vol. 22, no. 6, pp. 1207–1222, 2013. doi: 10.1007/s11248-013-9716-5.

- [40] N. Mitter, E. A. Worrall, K. E. Robinson *et al.*, "Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses," *Nat. Plants*, vol. 3, p. 16207, 2017. doi: 10.1038/nplants.2016.207.
- [41] M. Chitkara, O. Christiaens, and G. Smagghe, "RNAi-based insecticidal strategies: New avenues for synergistic combinations," *Pest Manag. Sci.*, vol. 78, no. 4, pp. 1237–1246, 2022. doi: 10.1002/ps.6740.
- [42] EPA (Environmental Protection Agency), "Ledprona Registration of an RNAi-based biopesticide for Colorado potato beetle," 2023. [Online]. Available: https://www.epa.gov.
- [43] G. Bucher, J. Scholten, and M. Klingler, "Parental RNAi in *Tribolium* (Coleoptera)," *Curr. Biol.*, vol. 12, no. 3, pp. R85–R86, 2002. doi: 10.1016/S0960-9822(02)00666-8.