Review

CUADb-based oligonucleotide insecticides and RNA biocontrols: molecular bases and potential in plant protection

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Abstract: Recent advances in molecular genetics, nucleic acid synthesis, and bioinformatics have provided novel opportunities for plant protection against insect pests. Currently, both DNA and RNA serve as active insecticidal ingredients, transcending their traditional role as carriers of genetic information. This novel activity is achieved through two fundamentally distinct mechanisms: DNA containment (DNAc), employing oligonucleotide insecticides based on contact unmodified antisense DNA biotechnology (CUADb), also known as 'genetic zipper' technology, and RNA interference (RNAi), employing RNA biocontrols based on double-stranded RNA (dsRNA) technology. The investigation of the molecular mechanism underlying the antisense activity of nucleic acids emerged in the early 1960s. While the antisense function of RNA in gene silencing through interference (RNAi) has been documented in the late1990s as an antiviral immune response in nematodes, the CUADb antisense approach initially emerged as a powerful strategy for pest control against lepidopterans in 2008. CUADb approach relies on disrupting rRNA biogenesis and ribosome production, a process entirely distinct from RNAi. The efficacy of these approaches appears to be species dependent: while CUADb demonstrates optimal activity against Sternorrhyncha (e.g., aphids, mealybugs, psyllids, scale insects), thrips, and mites, RNAi strategy shows a strong insecticidal potential against beetles from the Tenebrionidae and Chrysomelidae families. Here, we will review the differences between the two technologies, their mechanism of action and the current challenges facing their adoption.

Keywords: oligonucleotide insecticides; CUAD biotechnology; DNA containment; RNA biocontrols; double-stranded RNA technology; RNA interference; next-generation insecticides; plant protection

1. Introduction

The relentless innovation in insect pest control drives continuous replacement of older chemistries (carbamates, organophosphates) by contemporary agents (neonicotinoids, diamides) (van den Berg et al. 2021; Sparks et al. 2021; Zhang et al. 2023). Yet the intractable challenge of genetic resistance ensures this cycle persists (Sparks et al. 2021; Mogilicherla et al. 2023), demanding insecticides with extended utility, heightened selectivity, enhanced biodegradability, and reduced carbon footprint. Biomolecules have recently emerged as promising tools that demonstrate high potential in overcoming these challenges (Christiaens et al. 2020; Oberemok et al. 2022; Palli 2023; Gal'chinsky et al. 2024). Both DNA and RNA have shown remarkable insecticidal effects, enabled by advances in molecular genetics, synthesis, and bioinformatics (Oberemok et al. 2018a; Rank and Koch 2021; Oberemok et al. 2023; Koeppe et al. 2023). This led to the development of two distinct technologies: oligonucleotide insecticides based on Contact Unmodified Antisense DNA Biotechnology (CUADb), also known as 'genetic zipper' technology, and RNA biocontrols utilizing double-stranded RNA (dsRNA) technology (Nitnavare et al. 2021; Gal'chinsky et al. 2024). Alongside CRISPR/Cas, all together they represent three core antisense approaches for insect control, each employing

unique molecular mechanisms: RNAi functions through guide RNA-mRNA duplexes cleaved by Argonaute nuclease; CUADb operates via guide DNA-rRNA duplexes processed by DNA-guided rRNase such as RNase H1; and CRISPR/Cas combines guide RNA-genomic DNA complexes with Cas nuclease (Kumar et al. 2025). While all three exhibit taxon-specific efficacy enabling combinatorial strategies, their divergent molecular targets preclude universal application, increasing the chance of efficiently targeting different organisms.

After two decades of development, RNAi still lacks a standardized design algorithm for reliable insecticide development. Conversely, CUADb possesses a validated design framework (Oberemok et al. 2024a) with potential efficiency against up to 50% of all insect pests, demonstrating particular efficacy against Sternorrhyncha (e.g., aphids, mealybugs) and superorder Paraneoptera in general (Oberemok et al. 2025a), whereas RNA biocontrols show strongest activity mainly against Coleoptera. These independent 'fraternal twins', dsRNA technology and 'genetic zipper' technology (CUADb), hold synergistic potential for developing highly selective, next-generation pest control agents. This review addresses the paradigm shift toward nucleic acid-based solutions, with dedicated focus on CUADb as an innovative technology characterized by its novel DNAc mechanism and advanced insecticidal profile.

2. CUADb-based oligonucleotide insecticides

In 2008, our research group pioneered the use of short unmodified antisense DNA oligonucleotides as contact insecticides (Oberemok 2008). Initial work on the spongy moth (*Lymantria dispar*) demonstrated that effective gene silencing using oligonucleotide insecticides (oligoRING and oligoRIBO-11) depended critically on target gene and its expression levels (Oberemok et al. 2017, 2019). Subsequent research identified rRNA as a prime target, leading to the development of oligonucleotide insecticides based on 11-mer DNA sequences complementary to pest rRNA in 2019 (Kumar et al. 2025). rRNA is an optimal target due to its abundance (constituting ~80-85% of cellular RNA) and the substantial energy investment (>60% of cellular energy) required for ribosome production and maintenance (Szaflarski et al. 2022; Deng et al. 2022). Insect rRNA comprises 28S rRNA (~3900 nt), 18S rRNA (~1920 nt), 5.8S rRNA (~160 nt), 5S rRNA (~120 nt), mitochondrial rRNAs including 16S rRNA (~1140 nt) and 12S rRNA (~600 nt) (Deng et al. 2022). The first successful rRNA targeting with unmodified antisense DNA oligonucleotides was achieved against 5.8S rRNA of *L. dispar* (Oberemok et al. 2019). CUADb-based oligonucleotide insecticides are designed using the DNAInsector program (dnainsector.com) or manually via GenBank sequences of pest pre-rRNA and mature rRNA. Synthesis employs the phosphoramidite method through liquid-phase or solid-phase synthesis using instruments such as the ASM-800 (BIOSSET, Russia), OligoPilot™ (Cytiva, Sweden), PolyGen 10-Column DNA Synthesizers, and others (Gal'chinsky et al. 2019).

Oligonucleotide insecticides demonstrate high efficacy against sternorrhynchans, thrips, and mites, with successful targeting documented across multiple species: 28S rRNA in *Unaspis euonymi*, *Dynaspidiotus britannicus*, *Icerya purchasi*, *Ceroplastes japonicus*, *Aonidia lauri*, and *Coccus hesperidum* (Gal'chinsky et al. 2020, 2023, 2024; Useinov et al., 2020; Oberemok et al., 2022, 2025a); 18S rRNA in *Pseudococcus viburni* (Novikov et al. 2023); mitochondrial 16S rRNA (Oberemok et al. 2025b); and ITS2 regions in *Macrosiphoniella sanborni*, *Schizolachnus pineti* (Puzanova et al. 2023; Oberemok et al. 2024b), *Trioza alacris* (Oberemok et al. 2024c); and also *Tetranychus urticae* (Gavrilova et al. 2025), demonstrating high insecticidal potential for oligonucleotide acaricides (Kumar et al. 2025). A single contact treatment at 100 ng/µl typically achieves approximately 80% mortality in pests within 3-14 days (Oberemok et al. 2023, 2024a). While highly effective against hemipterans and moderately effective against lepidopterans including *L. dispar*, efficacy is notably lower against coleopterans such as *Leptinotarsa decemlineata* and requires further investigation of their resistance to this approach (Oberemok et al., 2018b). The 11-mer length provides species specificity with a uniqueness frequency exceeding $1/4.19 \cdot 10^{m}$, covering most agricultural applications (Oberemok et al. 2022). rRNA's dual advantages of abundance and inter-species variability make it superior to less concentrated cellular mRNAs as a target. Contact delivery (CUADs) outperforms oral delivery (ODUADs) due to digestive DNases and extra-oral salivary barriers in hemipterans (Cao et al. 2018).

The 2008 discovery of contact oligonucleotide insecticides was unexpected, contradicting established views that unmodified oligodeoxyribonucleotides are rapidly degraded by DNases (Dias and Stein 2002) and that rRNA resists RNase H-dependent antisense DNA degradation (Will and Lührmann 2001; Bachellerie et

al. 2002). Previous research focused exclusively on gene downregulation, overlooking insecticidal potential (Dias and Stein 2002; Oberemok 2008). Oligonucleotide insecticides (briefly olinscides or DNA insecticides) refuted these assumptions through a two-step DNA containment (DNAc) mechanism (Figure 1, left part): initial target rRNA 'arrest' halts ribosome function and triggers rDNA transcription hypercompensation, followed by degradation of target rRNA by DNA-guided rRNases such as RNase H1 (Kumar et al. 2025; Gal'chinsky et al. 2024). This 'genetic zipper' mechanism (Oberemok et al. 2024c) induces metabolic shifts toward lipid-based energy synthesis, enhancing biogenesis of ribosomes and ATP production. Ultimately, widespread kinase downregulation – including mTOR, which regulates ribosome biogenesis through mTORC1 – causes 'kinase disaster' due to ATP insufficiency, while significant RNase H1 upregulation occurs throughout DNAc (Oberemok et al. 2025a).

In 2011, three years after this discovery, Wang et al. (2011) adapted the contact concept for dsRNA insecticides. Crucially, DNAc downregulates key RNAi enzymes (DICER1, Argonaute 2, DROSHA), underscoring its mechanistic distinction from RNAi (Oberemok et al., 2025a). Thus, ssDNA in CUADb and dsRNA in RNAi represent complementary but fundamentally distinct antisense technologies with synergistic potential in pest management (Kumar et al. 2025) (Table 1).

Table 1. Comparative overview of CUAD biotechnology and dsRNA technology, highlighting differences in effectors, mechanisms, synthesis methods, and pest targets.

Key features	**CUAD biotechnology ('genetic zipper' technology)**	**dsRNA technology**
Effector	ssDNA	dsRNA
Mechanism	DNA containment	RNA interference
Mode of action	ssDNA:rRNA	ssRNA:mRNA
Nuclease involved	DNA-guided rRNase (such as RNase H1)	Argonaute
Synthesis	Liquid-phase solid-phase oligonucleotide synthesis based on phosphoramidite chemistry	Large-scale cell-free biomanufacturing (cell-free dsRNA production)
Target pests with best outcome	Sternorrhynchans	Beetles

A working hypothesis proposes that DNA and RNA viruses may exploit the rRNA gene expression hypercompensation observed during the initial step of the DNA containment mechanism to increase cellular ribosome numbers essential for viral replication (Oberemok et al. 2025a). Bioinformatics studies indicate viral hijacking of host genes is common (Cerio et al. 2020). Given rRNA's dominance (~80% of cellular RNA) and the significant energy investment (>60%) in ribosome production (Deng et al. 2022; Szaflarski et al. 2022), DNA viruses likely co-opted rRNA-like sequences during co-evolution (Evseev et al. 2021) but their function is still unknown.

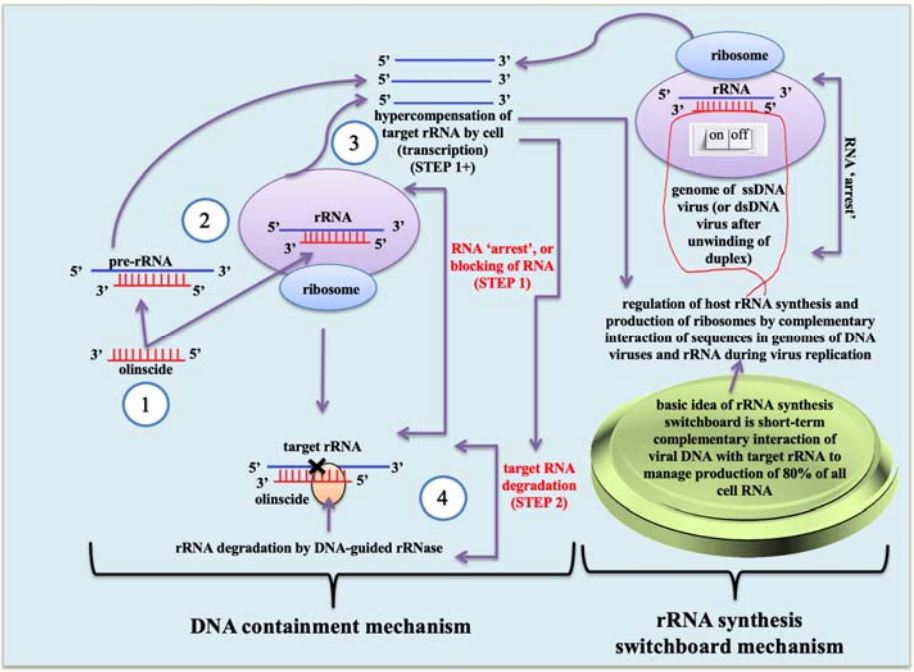

Figure 1. Mechanisms underlying oligonucleotide insecticides and rRNA regulation. Left: DNA containment mechanism (DNAc) for insect pest control. (1) Antisense DNA oligonucleotides complementary to pest mature rRNA or pre-rRNA are applied as insecticides. (2) Duplex formation occurs between the oligonucleotide and target rRNA/pre-rRNA. (3) In the first step of DNAc, antisense DNA 'arrests' rRNA or pre-rRNA, leading to hypercompensation and containment of ribosome biogenesis ('arrested ribosomes'). (4) In the second step, DNA-guided rRNase cleaves the target rRNA/pre-rRNA, reducing its concentration. Right: rRNA synthesis switchboard mechanism, where DNA viruses regulate host rRNA synthesis and ribosome production by introducing complementary sequences of viral DNA, influencing up to 80% of rRNA output.

Most DNA viruses replicate within the nucleus and interact with the nucleolus – a site critical for ribosome assembly and virus life cycle (Salvetti et al. 2024). Nucleoli facilitate rRNA synthesis, processing, and pre-ribosomal subunit assembly (Rawlinson et al. 2018). Our studies suggest that specific DNA virus genome fragments could regulate host rRNA synthesis via an ON/OFF switch mechanism, using complementary interactions between viral DNA and host rRNA to initiate ribosome biogenesis with minimal energy expenditure (Oberemok et al. 2025a). This adaptation is likely to be very important since helps quickly and efficiently bypass host limitations. For example, viruses may need to replicate while the host cell is in resting phase (e.g., G2/M resting phase), where polymerases may not be readily available. Also some large DNA viruses replicate in the cytoplasm, a region of the cell that lacks the necessary host polymerases for DNA replication, which is typically carried out in the nucleus. Thus, fast and elegant rRNA synthesis switchboard mechanism for turning on the ribosome biogenesis is required for DNA viruses in both nucleus and cytoplasm (Figure 1, right part). Additionally, DNAc may function within the innate immune system against ssDNA viruses (their major vectors are hemipterans) and DNA viruses infecting hemipterans (Barnes and Turner 2016; Wang and Blanck 2021; Guo et al. 2022). DNAc can help degrading virus mRNAs using DNA fragments of partially cleaved virus genome and enhancing ribosome biogenesis to counteract viral pathogen (Gal'chinsky et al. 2024). Of note, in our investigations all DNA oligos with either high or moderate complementarity to target rRNA initiated rRNA hypercompensation, but subsequent substantial rRNA degradation and insect mortality occurred only when the oligo sequence perfectly matched the rRNA. Also long DNA oligos with either high or moderate complementarity triggered stronger rRNA hypercompensation than short ones (Oberemok et al., 2025a). Thus, viruses obviously will tend to undergo microevolution toward having long but somewhat similar complementary sequences to host rRNAs rather than highly similar complementary sequences. This strategy will

likely trigger robust rRNA biogenesis without risk of its further substantial degradation and death of the host. Interestingly, we found that many RNA viruses found in GenBank, including those of hemipterans, also contain long (>100 nt) rRNA-like sequences of insect pests located either near or in the locus of RNA-dependent RNA polymerase gene performing virus replication function. Obtained data indicates potential role of these sequences in regulation of rRNA synthesis and ribosome biogenesis required for fast synthesis of RNA-dependent RNA polymerase and subsequent replication of RNA viruses and managed through a process similar to rRNA synthesis switchboard mechanism (Figure 1, right part) via viral RNA-host rRNA interactions.

From practical point of view, targeting conserved regions of insect pest rRNA genes for oligonucleotide insecticide design delays target-site resistance development, as mutations occur less frequently in these areas (Sharma et al. 2014; Oberemok et al. 2019). When resistance develops, new effective CUADb insecticides could be designed by shifting the target site adjacent to the resistant region within the rRNA genes (Gal'chinsky et al. 2024). Notably, current CUADb-based pesticides demonstrate efficacy against approximately 30% of the most resistant arthropods (Whalon and Mota-Sanchez 2015), showing substantial activity against Sternorrhyncha and spider mites (Kumar et al. 2025).

Oligonucleotide insecticides undergo rapid and clean biodegradation in the environment (soils, water, plants) via abiotic factors (temperature, pH, salinity, UV radiation) and biotic factors (microbes, extracellular enzymes) (Barnes and Turner 2016; Oberemok et al. 2024d). Deoxyribonucleases in tissue homogenates of *Lymantria dispar*, *Leptinotarsa decemlineata*, *Icerya purchasi*, *Dynaspidiotus britannicus*, *Aonidia lauri*, and their host plants (*Quercus pubescens*, *Solanum tuberosum*, *Pittosporum tobira*, *Laurus nobilis*) degrade olinscides within 24 hours at 27°C (Oberemok et al. 2018b, 2019; Gal'chinsky et al., 2023, 2024), while *Macrosiphoniella sanborni* nucleases achieve degradation within 1 hour (Puzanova et al., 2023). Minimal negative impacts are observed on non-target organisms including *Triticum aestivum* (Oberemok et al. 2013; Nyadar et al. 2018), *Quercus robur*, *Malus domestica* (Zaitsev et al. 2015), *Manduca sexta*, *Agrotis ipsilon* (Oberemok et al. 2015), and *Galleria mellonella* (Oberemok et al. 2019). Unprecedented selectivity is evidenced by dramatic efficacy loss following single nucleotide substitutions (Puzanova et al. 2023; Gal'chinsky et al. 2024), although tolerance for non-canonical base pairs (A:C, C:A, G:U) must be considered during design (Gal'chinsky et al. 2024; Oberemok et al. 2024b). Efficacy extends to mixed pest populations (Gal'chinsky et al. 2024), and modern liquid-phase synthesis minimizes greenhouse gas emissions (nitrogen oxide, methane, carbon dioxide) compared to solid-phase methods (Gal'chinsky et al. 2023), addressing climate concerns.

In conclusion, overcoming widespread pesticide resistance requires the development of novel and highly specific pesticide classes to reduce environmental impact. Oligonucleotide insecticides represent a remarkable advancement using unmodified antisense oligonucleotides. Mirroring the trajectory of therapeutic oligonucleotides – which overcame early challenges through sustained research and investment (TriLink BioTechnologies 2025) – CUADb-based pesticides offer selective action, rapid biodegradation, and increasingly cost-effective production, presenting a highly promising approach (Kumar et al. 2022). Based on our estimations, the 'genetic zipper' technology (CUADb) can potentially control up to 50% of all insect pests using a simple and flexible algorithm (Oberemok et al. 2024c). Formulation additives (spreaders, adhesives, penetrators, UV protectants) may enhance efficacy for other insect orders, pending environmental safety assessment. Future research will undoubtedly uncover deeper mechanistic details of DNAc and its potential. CUADb paves the way for sustainable, xenobiotic-free agriculture, positioning oligonucleotide pesticides as prospective cornerstone agents in pest control (Oberemok et al. 2025b).

3. RNA biocontrols

The foundation of RNA interference (RNAi) was established by Andrew Fire and Craig Mello's seminal discovery that antisense strand within double-stranded RNA (dsRNA) mediates sequence-specific gene silencing – a breakthrough recognized with the 2006 Nobel Prize in Physiology or Medicine (Fire et al. 1998). This pivotal work catalyzed extensive biotechnological applications in agricultural and forestry research for plant protection. RNAi operates through post-transcriptional and translational suppression of gene expression, utilizing dsRNA molecules typically exceeding 200 base pairs in length that are complementary to target

genes (Tomoyasu 2008; Svoboda 2020). The mechanism initiates when the RNase III enzyme (Dicer) cleaves long dsRNA into 20–30 nucleotide small interfering RNAs (siRNAs) (Carmell et al. 2002; Obbard et al. 2009; Koeppe et al. 2023). These siRNAs subsequently associate with Argonaute-family proteins to form RNA-induced silencing complexes (RISC) for cytoplasmic mRNA degradation, or RNA-induced transcriptional silencing complexes (RITS) for nuclear gene suppression (Wu et al. 2020; Müller et al. 2020). A parallel pathway involves PIWI-interacting RNAs (piRNAs; 23–36 nucleotides), germline-specific molecules that complex with PIWI-subfamily proteins to repress transposable elements through distinct silencing mechanisms (Bodemann et al. 2012; Chattopadhyay et al. 2022). Collectively, these RNAi pathways play crucial regulatory roles during insect ontogenesis (Figure 2), ultimately disrupting biosynthesis of specific proteins through targeted mRNA degradation or translational blockade.

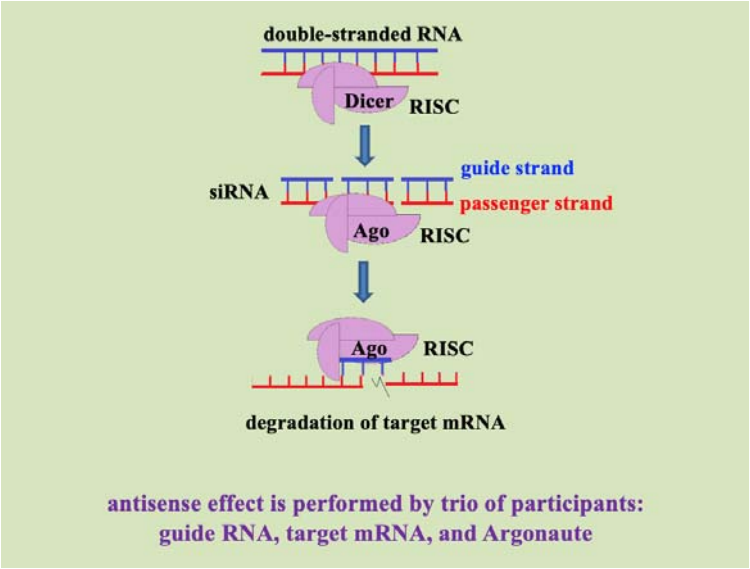

Figure 2. Main pathway of RNA interference (RNAi) utilized for the development of RNA-based biocontrols. Double-stranded RNA (dsRNA) is processed by Dicer into siRNAs, which are incorporated into the RNA-induced silencing complex (RISC). The guide strand directs Argonaute (Ago) to complementary target mRNA, resulting in mRNA cleavage and degradation. This mechanism, distinct from DNA containment (DNAc) and contact unmodified antisense DNA biotechnology (CUADb), forms the basis for RNAi-mediated pest control strategies.

RNAi efficacy exhibits profound taxonomic variation among economically significant insect pests. Coleopterans demonstrate consistently high systemic efficiency (Bodemann et al. 2012; Powell et al. 2017; Rodrigues et al. 2017), while Orthoptera (e.g., locusts) and Blattodea (cockroaches) generally exhibit robust responses (Irles et al. 2013; Santos et al. 2014; Li et al. 2021; Hoang et al. 2022). Efficiency is variable in Hymenoptera and Hemiptera due to limited systemic spreading in piercing-sucking insects, attributed primarily to deficient RNA-directed RNA polymerase (RdRP) activity (Christiaens et al. 2014; Jain et al. 2020, 2021). Lepidopterans and dipterans typically display low susceptibility to RNAi (Miller et al. 2008; Terenius et al. 2011). Consequently, commercial RNAi biopesticide development remains largely confined to coleopteran targets (Bramlett et al. 2020; Rodrigues et al. 2021; Koo et al. 2024), with target mRNAs spanning genes governing development, detoxification, and reproduction (Liu et al. 2020) (Table 1).

So far, two primary delivery strategies have been developed for RNAi: genetically modified (GM) plants expressing dsRNA, and topical application of formulated sprayable products (Ivashuta et al. 2015). GM approaches undergo protracted regulatory evaluation under biotechnology frameworks, whereas sprayable formulations – classified as biochemical pesticides – offer potential time and cost advantages, with registration timelines dependent on final product composition (Dietz-Pfeilstetter et al. 2021; Galli et al. 2024). Currently, few RNAi products are commercially deployed. The landmark SmartStax® PRO corn (US EPA-registered 2017) combines two Bt insecticidal proteins (Cry3Bb1 and Cry34/35Ab1) with DvSnf7 dsRNA targeting vesicle transport in corn rootworm (Head et al. 2017; EPA 2024). This triple-mode-of-action

pyramid achieves 99% suppression of western (*Diabrotica virgifera virgifera*) and northern (*D. barberi*) corn rootworm larvae, though dsRNA-induced mortality manifests slower than Bt toxicity (≥5 days post-ingestion) (Head et al. 2017). The targeted Snf7 protein functions within the ESCRT-III pathway governing transmembrane protein sorting and degradation (Vaccari et al. 2009; Bolognesi et al. 2012), with documented absence of cross-resistance between DvSnf7 RNAi and Bt toxins (Moar et al. 2017; Khajuria et al. 2018; Reinders et al. 2023). Experimental GM crops demonstrate efficacy against diverse pests including fall armyworm (*Spodoptera frugiperda*; Xiong et al. 2013), aphids (Mao et al. 2014), Colorado potato beetle (*Leptinotarsa decemlineata*; Guo et al. 2018), and spider mites (*Tetranychus urticae*; Shen et al. 2017).

The first exogenous sprayable dsRNA biopesticide, Calantha™ (ledprona; IRAC Group 35), was commercialized by GreenLight Biosciences in 2023 against Colorado potato beetle (Pallis et al. 2023; GreenLight Biosciences 2025; Narva et al. 2025). Applied at 9.4 g/ha, it induces near-complete feeding cessation within 2-3 days and achieves 90-95% mortality in 1st-2nd instar larvae within 11-26 days. Operational constraints include strict larval-stage specificity, necessity for sequential applications, and recommended rotation with insecticides possessing alternate modes of action. Proof-of-concept successes encompass numerous taxa: brown planthopper (*Nilaparvata lugens*; Zha et al. 2011), African sweet potato weevil (*Cylas puncticollis*; Prentice et al. 2017), tomato pinworm (*Tuta absoluta*; Camargo et al. 2016), oriental fruit fly (*Bactrocera dorsalis*; Sun et al. 2023), cotton mealybug (*Phenacoccus solenopsis*; Arya et al. 2021), diamondback moth (*Plutella xylostella*; Chen et al. 2021), fall armyworm (*Spodoptera frugiperda*; Li et al. 2024; Bera et al. 2025), white-backed planthopper (*Sogatella furcifera*; Mao et al. 2014; Guo et al. 2025), desert locust (*Schistocerca gregaria*; Schellens et al. 2022), and potato psyllid (*Bactericera cockerelli*; Lu et al. 2024) – though none have advanced beyond experimental stages.

Despite promising efficacy dsRNA technology is in its infancy and require broader adoption. There are main challenges restricting this adoption. Target gene selection lacks consensus due to compensatory cellular responses including protein stability, functional redundancy among paralogs, and transcriptional feedback loops (Ulrich et al., 2015; Buer et al., 2024; Cedden et al., 2024). Efficacy varies substantially with developmental stage, tissue type, and delivery method. The Spray-Induced Gene Silencing (SIGS) paradigm suffers mechanistic ambiguity – whether dsRNA uptake occurs primarily via integument penetration or oral ingestion and remains unresolved, confounding optimization efforts (Dalakouras et al. 2021). Off-target effects may arise from as few as 15 contiguous base matches (Powell et al. 2017; Chen et al. 2021) or sequence-independent immune activation (Hirai et al. 2004; Brutscher et al. 2017), compounded by formulants (nanocarriers, adjuvants) potentially enhancing non-target exposure (Castellanos et al. 2019; Reinders et al. 2023). Rapid environmental degradation occurs via microbial nucleases in soil/phyllosphere, UV radiation, and precipitation runoff (Dubelman et al. 2014; Parker et al. 2019), with insect-derived nucleases in hemolymph (e.g., *Spodoptera litura*, *Manduca sexta*) and saliva (*Acyrthosiphon pisum*) degrading dsRNA within 0.3–3 hours and interferes with robust insecticidal effect (Christiaens et al. 2014; Garbutt et al. 2013; Peng et al. 2018). While dsRNA production costs have decreased through cell-free platforms like GreenLight's enzymatic NMP→NTP→dsRNA pipeline (Cunningham et al. 2020; Taning et al. 2020), it is not publicly available and affordable price appears dubious. RNA biocontrols remain challenged by slower lethality and shorter residual activity than conventional insecticides. Sprayable dsRNA offers theoretical advantages over GM crops but requires deeper mechanistic understanding of RNAi pathways and environmental safety validation.

4. Conclusion

Although both CUAD biotechnology and double-stranded RNA technology utilize nucleic acids as active ingredients, they represent fundamentally distinct insecticidal approaches with divergent mechanisms and applications (Oberemok et al. 2018a). Oligonucleotide insecticides employ short, unmodified antisense DNA fragments delivered mainly via contact, operating through the DNA containment (DNAc) mechanism to degrade target ribosomal RNAs in pests. The deoxyribose backbone of DNA fragments confers greater resistance to environmental hydrolysis than RNA-based counterparts (Thorp, 2000), while their compact size (~11 nucleotides) enables exceptional targeting precision. Ribosomal RNA – constituting approximately 80% of cellular RNA – serves as the optimal target due to its abundance and metabolic significance (Oberemok et

al. 2019, 2023, 2025a; Gal'chinsky et al., 2024), with current efficacy being most pronounced against sap-feeding sternorrhynchans (Gal'chinsky et al. 2024; Oberemok et al. 2024a). In contrast, RNA biocontrols utilize longer double-stranded RNA molecules delivered through either contact or oral routes, functioning via the RNA interference (RNAi) pathway to degrade messenger RNAs. The ribose backbone of these molecules increases susceptibility to environmental hydrolysis (Williams et al. 2016), while their extended length (typically >200 bp) and subsequent enzymatic dicing into smaller fragments complicate selectivity control, necessitating careful risk assessment for non-target organisms (Roberts et al. 2015; Christiaens et al. 2022; Rank and Koch 2021; Liu et al. 2020). Presently, dsRNA insecticides demand optimized design algorithms and expanded target pest spectra, whereas CUADb-based oligonucleotide insecticides primarily require broader validation across additional pest groups (Oberemok et al. 2024d).

Both classes benefit from cost-effective synthesis: liquid-phase phosphoramidite methods for DNA oligonucleotides (Gal'chinsky et al. 2023; Oberemok et al. 2024b) and cell-free enzymatic production for dsRNA (Taning et al. 2020). Notably, while RNAi mechanisms were characterized years before practical dsRNA insecticides emerged, the DNAc mechanism was discovered following persistent applied research with antisense oligodeoxyribonucleotides in pest control. These complementary technologies will likely demonstrate optimal efficacy against specific pest taxa, offering extended utility through careful design and potential for synergistic integration in multi-target formulations. Their unprecedented safety profiles for non-target organisms and ecosystems – combining high selectivity, rapid environmental biodegradation, and innovative mechanisms – position nucleic acid-based insecticides as indispensable biomolecules for sustainable agriculture's future.

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