Review

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Perspectives of RNAi, CUAD and CRISPR/Cas as Innovative Antisense Technologies for Insect Pest Control: From Discovery to Practice

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Abstract: Pest management has entered a new era with the emergence of three innovative antisense technologies: RNAi, CUAD, and CRISPR/Cas. These technologies, which operate through sequence-specific nucleic acid duplex formation and guided nuclease activity, offer unprecedented potential for targeted pest control. While RNA-guided systems such as RNAi and CRISPR/Cas were initially discovered in non-insect models as fundamental biological mechanisms (primarily in antiviral defense), the DNA-guided CUAD system was first identified in insect pests as a practical tool for pest control, while its broader role in ribosomal RNA (rRNA) biogenesis only recently recognized. These surprising discoveries have unveiled an entirely new dimension of gene regulation, with profound implications for sustainable pest management. Despite certain similarities of these technologies, RNAi, CUAD, and CRISPR/Cas differ in their mode of action, specificity, and applicability. No single approach provides a universal solution for all insect pests; instead, each is likely to be most effective against specific pest groups. Moreover, these technologies enable the rapid adaptation of pest management strategies by countering target-site resistance, ensuring long-term efficacy. This review provides a critical synthesis of the unique advantages and limitations of each antisense technology, highlighting their complementary roles in eco-friendly, nucleic acid-guided insect pest control. By bridging fundamental discoveries with applied research, we offer new perspectives on their practical implementation, underscoring the urgent need for their integration into modern pest management strategies.

Keywords: RNAi; CUAD; CRISPR/Cas; antisense technologies; insect pest control

1. Introduction

Nucleic acids, DNA and RNA, orchestrate cellular processes through precise complementary interactions (Minchin and Lodge 2019). The fundamental principles of Watson–Crick base pairing, coupled with the action of specific enzymes, govern essential biological mechanisms such as replication, transcription, translation, and gene expression regulation (Westhof et al. 2014). The specificity and fidelity of these processes arise from the unique combinations of nitrogenous bases, which form the molecular basis of genetic control. Three innovative antisense technologies, RNA interference (RNAi) (Fire et al. 1998), CUAD (contact unmodified antisense DNA) biotechnology (Oberemok 2008; Gal'chinsky et al. 2024; Oberemok et al. 2024a), and CRISPR/Cas (Jinek et al. 2012; Gasiunas et al. 2012; Doudna and Charpentier 2014), have harnessed these nucleic acid interactions to develop targeted genetic interventions. These technologies rely on the formation of sequence-specific duplexes: RNAi (guide RNA–mRNA) (Li et al. 2012), CUAD (guide DNA-rRNA) (Gal'chinsky et al. 2024; Oberemok et al. 2019, 2024b, c, d), and CRISPR/Cas (guide RNA-genomic DNA) (Doudna and Charpentier 2014; Wiles et al. 2015; Li et al. 2023), which then recruit specialized nucleases such as Argonaute (Ago) (Ma et al. 2018), RNase H (Gal'chinsky et al. 2024; Oberemok et al. 2024a), and CRISPR-associated protein (Doudna and Charpentier 2014). In order for a technology to emerge, it is necessary to accumulate a critical mass of data in a certain area, and then, as a rule, an unexpected guess or a successful experiment sheds light on a pattern that can subsequently be applied in practice without fail. In the case of RNA interference, the key finding was the use of an antisense fragment within double-stranded RNA, for CUAD biotechnology it was the use of rRNA as a target for antisense oligonucleotides and sternorrhynchans as model objects, and for CRISPR/Cas it was the understanding that the target molecule for the antisense effect is genomic DNA. These innovations have set new standards in molecular genetics and are now being widely explored for their applications in insect pest control. Each of these technologies emerged from fundamental research, evolving from uncertain beginnings into powerful tools with transformative potential. Since their core mechanism relies on the complementary binding of antisense molecule, either DNA or RNA, to a target nucleic acid, they are collectively referred to as antisense technologies (Figure 1). While RNA- and DNA-guided nucleases have been extensively studied, a targeted DNA-cleaving mechanism utilizing guide DNA to cleave target DNA via a specific nuclease has not yet been developed, representing a potential direction for future research.

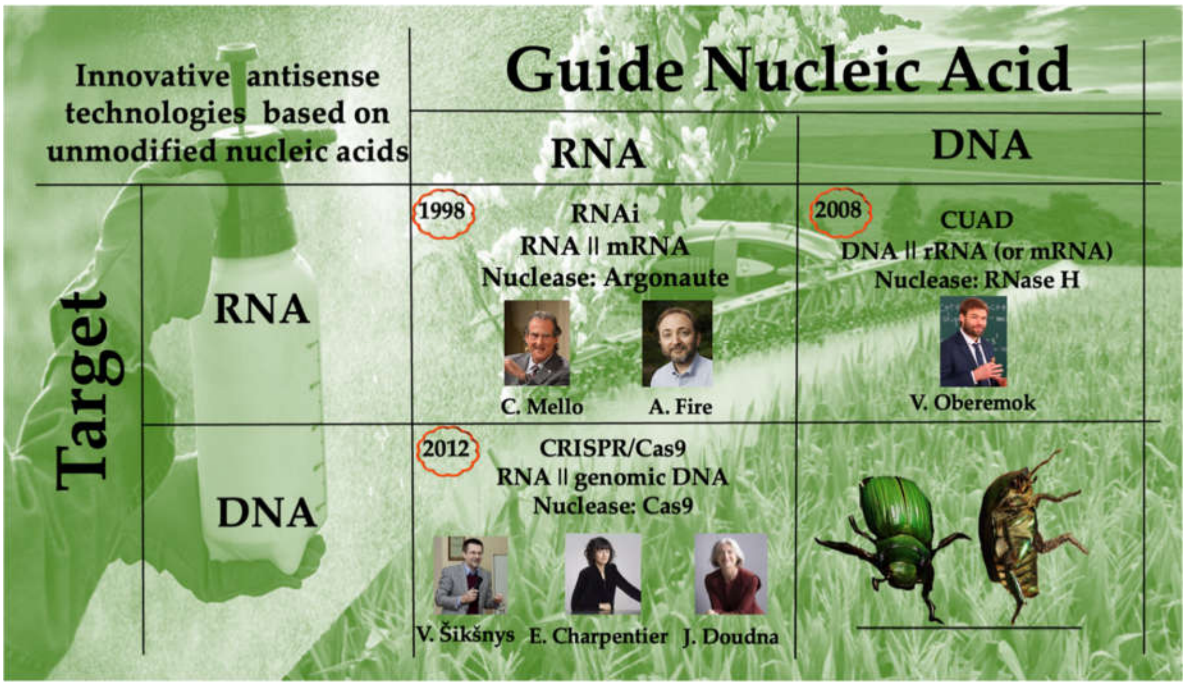

Figure 1. Antisense technologies based on unmodified nucleic acids and used for insect pest control.

Chemical insecticides remain a cornerstone of insect pest management (Araújo et al. 2023). There are several key factors that drive the development of new classes of insecticides and the most important of these is economic cost of insect pest damage to agriculture and insecticide resistance which has dramatically and relentlessly increased since the mid-20th century (Siddiqui et al. 2023; Araújo et al. 2023; Gul et al. 2023). The general mechanism underlying insecticide resistance is natural selection, which leads to an increase in frequency of resistance alleles formed as a result of random mutations in insect pest population (Hawkins et al. 2019). Antisense technologies (RNAi, CUAD, and CRISPR/Cas) are able to counteract insecticide resistance targeting conserved genes and conserved parts of the genes and easily re-create efficient pest control agents in the case of target-site resistance. While CUAD (Oberemok et al. 2024a) and RNAi (Fire et al. 1998) show great potential to be used as the next-generation chemical insecticides (Oberemok et al. 2024b), CRISPR/Cas is used to genetically attenuate insect pest populations through genetic engineering (Jinek et al. 2012). Nevertheless, these innovative antisense technologies and their combinations offer an endless repertoire in controlling insect pests, and the main question will be in selecting the optimal tactics of insect pest management in each individual case.

The prerequisite for the development of antisense technologies was the discovery of the DNA double helix, as well as pre-birth period of antisense technologies marked by the pioneer research works of Nina Grineva and co-workers on site-specific modification of valine tRNA (Belikova et al. 1967) and Paul Zamecnik and Mary Stephenson with modified DNA on the Rous sarcoma virus (Zamecnik and Stephenson 1978). However, unmodified nucleic acids are fundamental to controlling cellular processes, making it crucial for scientists to develop methods to regulate gene expression using duplexes of unmodified nucleic acids and transform them into cost-effective antisense technologies that operate efficiently. While the RNAi was discovered by one research group of scientists in the USA in 1998 (Fire et al. 1998) and CUAD technology was discovered in 2008 and later developed in Crimea by another research group of scientists (Oberemok 2008; Oberemok et al. 2024a), several other research groups, mainly from Lithuania, Sweden and USA contributed to the creation of CRISPR/Cas9 approach in 2012 (Jinek et al. 2012; Lander 2016; Shmakova et al. 2022) and eventually formed three main antisense technologies for pest control at the turn of the 21st century. Whilst CUAD technology has a comparatively easy algorithm for creation of pesticides, RNAi and CRISPR/Cas do not have trouble-free algorithms for creation of selective and efficient end-products for pest control and are still being elaborated upon. The main idea of this review is to briefly describe the emergence of antisense technologies in historical retrospective, to demonstrate the potential of RNAi, CUAD, and CRISPR/Cas mainly in insect pest control. Overall, we provide an overview of the current potentials and limitations of antisense technologies in insect pest control and try to determine their further development.

2. RNAi

2.1. History of Discovery

The discovery of RNA interference was inspired by the pioneering studies of Paul Zamecnik and Mary Stephenson (1978), who showed that a short antisense sequence of modified nucleic acid could inhibit the replication of the Rous sarcoma virus (Zamecnik and Stephenson 1978). In 1998, Craig Mello and Andrew Fire studied the effect of antisense and sense RNA fragments on the development of the nematode *Caenorhabditis elegans*. They sought to explain the effectiveness of the sense RNA fragment synthesized by bacteriophage RNA polymerase and used a double-stranded RNA fragment as a control. Bacteriophage polymerases, although highly specific, produce some random or ectopic transcripts, likewise, DNA transgene arrays generate a fraction of aberrant RNA products. Craig Mello and Andrew Fire hypothesized that interfering RNA populations might include some molecules with double-stranded character. To their surprise, it was the double-stranded RNA fragment that triggered a potent reduction or elimination of the endogenous *mex-3* mRNA transcript, which is abundant in the gonad and early embryos of the nematode. The first publication on RNAi

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appeared in Nature in 1998 (Fire et al. 1998). Double-stranded RNA fragments initiate RNA interference (RNAi), leading to the silencing of target genes (Tomoyasu et al. 2008; Svoboda 2020). In the later stages of RNAi, short antisense RNA fragments (21–23 nucleotides in length) are generated and, with the involvement of Argonaute nuclease, cleave the target mRNA (Zhao et al. 2021) (Figure 2).

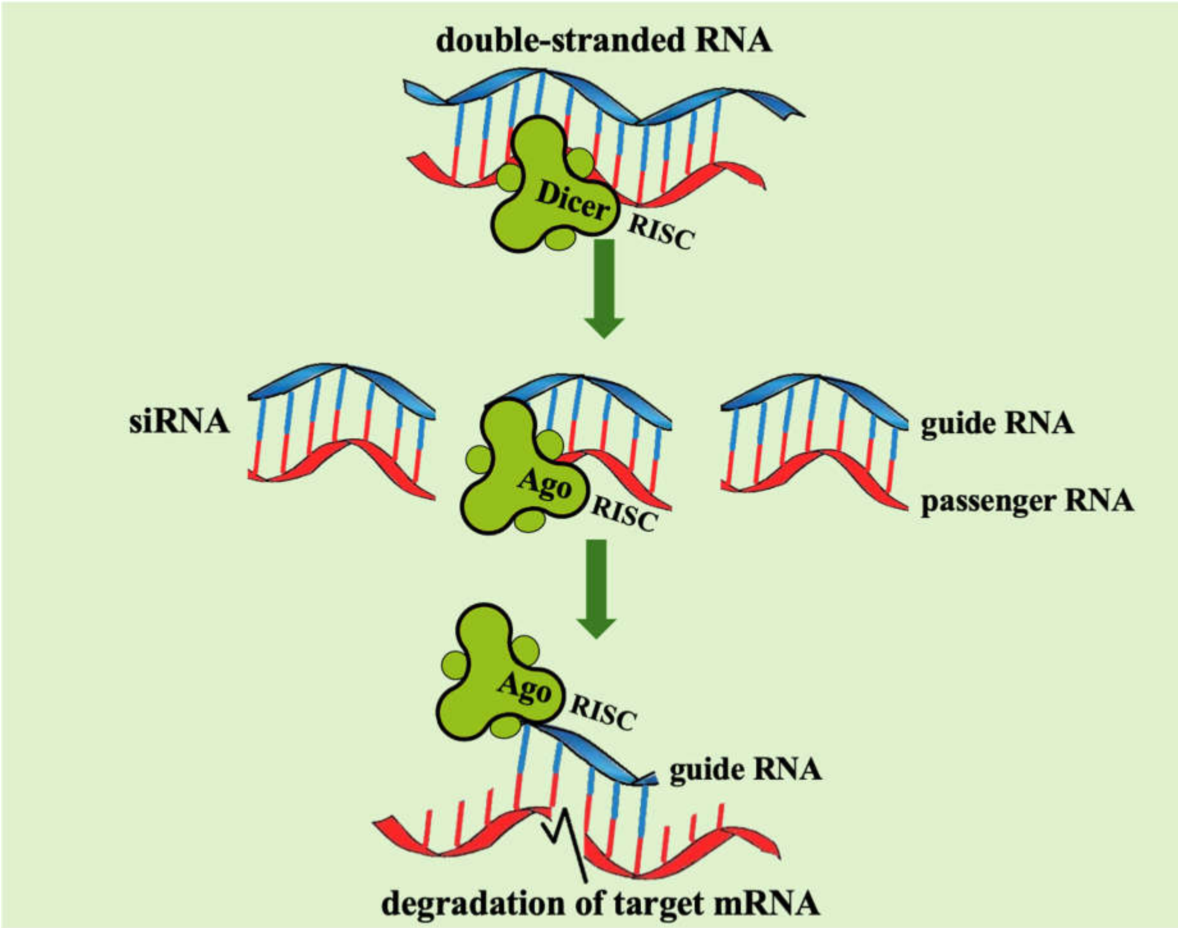

Figure 2. Main route of RNAi used for creation of dsRNA insecticides.

Thus, the basic principle of efficient RNAi involves the application of long (>200 bp) dsRNA fragments to initiate robust gene silencing through short antisense RNA fragments (Tomoyasu et al. 2008; Svoboda 2020). Although the effects of dsRNA-mediated interference are highly potent and specific, there are several limitations that should be taken into account when designing RNAi-based experiments. First, if a sequence is shared among multiple closely related genes, RNAi may unintentionally silence several members of the gene family or homologous genes in related species (Chen et al. 2021a). Second, genes with low expression levels may exhibit resistance to RNAi, at least partially. For example, if the target protein is very stable, its depletion occurs much more slowly despite transcript degradation. Moreover, the function of a target protein can be compensated for by related proteins, which may even be upregulated by the cell to counteract the loss. Additionally, the transcription of the target gene itself could be upregulated by a regulatory gene networks in response to RNAi mediated knock-down (Cedden and Bucher 2024).

2.2. How RNAi Works in Insect Pests

RNAi is a highly specific, naturally occurring gene silencing mechanism found in a wide range of organisms, including plants, animals, and insects, playing critical roles in post-transcriptional gene regulation, antiviral defense, and genome maintenance (Malakondaiah et al. 2024). This mechanism has been extensively studied for agricultural applications, particularly in pest management, by silencing essential insect genes to control pest populations. The siRNA pathway consists of two branches: the exogenous siRNA (exo-siRNA) pathway, which provides defense against viral infections, and the endogenous siRNA (endo-siRNA) pathway, responsible for transposon suppression within the genome (Zhu and Palli 2020). The exo-siRNA pathway is often harnessed in experimental settings by introducing dsRNA to silence specific genes (Cooper et al. 2019, 2021). The process begins when dsRNA enters the cell, either naturally or via external introduction. The enzyme Dicer, a member of the RNase III family, recognizes and cleaves the dsRNA into short fragments known as siRNAs, typically 21–25 nucleotides in length (Vogel et al. 2019). These siRNAs are then incorporated into the RNA-induced silencing complex (RISC), where Ago proteins, along with dsRNA-binding proteins such as R2D2 or loquacious, play a crucial role (Ortolá and Daròs 2024).

The RISC complex selects one strand of the siRNA, known as the guide strand, while the other strand, termed the passenger strand, is degraded. The guide strand directs RISC to recognize and bind to complementary mRNA sequences within the cell (Matranga et al. 2005; Matranga and Pyle 2010). Upon binding, the Ago protein, particularly through its PIWI domain, cleaves the target mRNA, leading to its degradation and preventing protein translation. Dicer enzymes in animals exhibit relatively low diversity (Sioud 2021). These enzymes belong to the RNase III family and play a key role in processing long dsRNAs, cleaving them approximately each 21 nucleotides (Treiber et al. 2019). Dicer-like enzymes contain two RNase III domains, which enable the cleavage of both RNA strands while leaving characteristic two-nucleotide 3′ overhangs due to their specific positioning on the dsRNA (Treiber et al. 2019; Koo and Palli 2024).

Argonaute proteins share a highly conserved structure and are composed of four key domains. The N domain facilitates the unwinding of siRNA duplexes. The PAZ domain anchors the 3′ end of the guide strand, while the MID domain binds to the 5′ end. The PIWI domain, in some Argonaute proteins, contains a slicer active site that cleaves target RNA complementary to small RNA (Sheu-Gruttadauria and MacRae 2017; Yang et al. 2020). Research has primarily focused on the siRNA machinery. However, the broader RNAi pathway is more complex, consisting of three sub-pathways: siRNA, miRNA, and piwiRNA. The application of artificially synthesized dsRNA not only includes the siRNA pathway core genes but also affects those of the miRNA pathway, indicating an interactive network of RNAi core genes (Silver et al. 2021).

RNAi is highly efficient and systemic in coleopterans (Zhu and Palli 2020). However, systemic RNAi, the uptake of dsRNA from the environment and the subsequent distribution of the RNAi signal between cells and tissues, is low in Diptera, Lepidoptera (Terenius et al. 2011; Lucena-Leandro et al. 2022; Christiaens et al. 2018), and sap-feeding Hemiptera (Jain et al. 2021; Kaplanoglu et al. 2022), which limits its application in plant protection. Despite these challenges, RNAi has opened new avenues for insect pest control using double-stranded RNA-based approaches. To date, RNAi has demonstrated the most effective results in controlling pests from various insect orders, particularly Coleoptera, by targeting key genes involved in development, detoxification, and reproduction (Table 1).

Table 1. List of pest species successfully targeted by RNAi.

Sl. No.	Names of model insects	Targeted gene(s)	Affected processes	References
1.	Beet armyworm, *Spodoptera exigua*	Chitin synthase gene A (SeCHSA)	Chitin synthesis	Tian et al. 2009
2.	Brown planthopper, *Nilaparvata lugens*	NlHT1, Nlcar, Nltry	Digestive system	Zha et al. 2011

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3.	African sweet potato weevil, *Cylas puncticollis*	Snf7	Digestive system	Prentice et al. 2017
4.	Tomato pinworm, *Tuta absoluta*	Vacuolar ATPase-A and Arginine kinase	High mortality	Camargo et al. 2016
5.	Oriental fruit fly, *Bactrocera dorsalis*	α-Spectrin	Oviposition and ovary size	Sun et al. 2023
6.	Cotton mealybug, *Phenacoccus solenopsis*	Krüppel homologue-1, ADP-ATP/Translocase, IDGF-1	Not specified	Arya et al. 2021
7.	Diamond back moth, *Plutella xylostella*	PxCht	Chitin synthesis	Chen et al. 2021b
8.	Fall armyworm, *Spodoptera frugiperda*	Met, EcR, USP genes	Reproductive system, fertility	Li et al. 2024a
9.	White-backed planthopper, *Sogatella furcifera*	hsc70-3, PP-α	Insect metamorphosis	Ma et al. 2024
10.	Soybean aphid, *Aphis glycines*	Cytochrome P450 monooxygenases (CYP450s)	Insect resistance	Li et al. 2024b
11.	Asian citrus psyllid, *Diaphorina citri*	CHC, vATPase-A, Snf7	Transmembrane system	Saberi et al. 2024
12.	*Trichogramma dendrolimi*	Vitellogenin receptor (VgR)	Female reproductive system	Wang et al. 2024
13.	Domestic silk moth, *Bombyx mori*	BmToll9-2 gene	Chitin synthesis	Liu et al. 2025
14.	Silverleaf whitefly, *Bemisia tabaci*	Cysteine protease	Digestive system	Darweesh et al. 2025
15.	Cowpea weevil, *Callosobruchus maculatus*	Olfactory receptor coreceptor (Cmac\Orco)	Insect sensory system	Shimomura et al. 2025
16.	White-backed planthopper, *S. furcifera*	β-N-acetylhexosaminidase genes	Insect metamorphosis	Guo et al. 2025
17.	Fall armyworm, *S. frugiperda*	COPIα, COPIβ, GSTU1	Insect reproduction	Bera et al. 2025
18.	Desert locust, *Schistocerca gregaria*	Cytochrome P450	Ecdysteroid pathway	Schellens et al. 2022
19.	Red flour beetle, *Tribolium castaneum*	CPAPs	Cuticular proteins	Mun et al. 2015
20.	Chinese white pine beetle,	Aquaporin	Osmoregulation	Fu et al. 2019

	Dendroctonus armandi			
21.	Cotton mealybug, *P. solenopsis*	Bursicon, V-ATPase	Cuticle hardening and V-ATPases act as proton pumps	Khan et al. 2018
22	Kissing bug, *Rhodnius prolixus*	Nitrophorin 2 (NP2)	Anticoagulant and apyrase activities in saliva	Araujo et al. 2006
23	Brown plant hopper, *N. lugens*	NlTPS	Enzymatic activity	Chen et al. 2010
24	Citrus aphid, *Toxoptera citricida*	TCiCHS	Chitin synthesis	Shang et al. 2016
25	Potato psyllid, *Bactericera cockerelli*	SUC1, ST4	Osmoregulatory	Lu et al. 2024

2.3. Perspectives and Limitations of RNAi For Insect Pest Control

Although RNA interference has greatly advanced insect biology research over the past 25 years, progress toward its application in pest and disease vector control has been limited. Currently, there is only one commercial product used as conventional chemical insecticides (three more products are present as transgenic crops), Calantha™, and a few others in the development pipeline. Calantha™, the first sprayable dsRNA-based biopesticide targeting the Colorado potato beetle, was commercially released by GreenLight Biosciences in 2023 (Pallis et al. 2023; GreenLight Biosciences 2025). Several challenges have inhibited commercialization of RNAi-based products, including variable RNAi efficacy across insect species, competition from transgenic Bt crops, and inability to effectively control some major sucking pests. Another concern is the potential evolution of RNAi resistance. Nonetheless, RNAi, when combined with precision agriculture and integrated pest management (IPM) strategies, could significantly enhance sustainability. Opportunities for synergy exist with advancing the technologies such as CUAD biotechnology, CRISPR/Cas technology, nanoformulations for improved dsRNA delivery, and microbial-based RNAi production. Delivering dsRNA effectively into insects remains a major hurdle. However, recent advancements in delivery methods and modern technology have significantly improved the efficiency of RNAi applications.

Microinjection. Direct injection of dsRNA into insect embryos or larvae. Although highly precise, this method is labor-intensive and impractical for large-scale applications (Socha et al. 2022).

Topical application. Application of dsRNA onto the insect's body or feeding sources. Innovations in formulation have enhanced dsRNA stability and uptake, making this method more effective (Yang et al. 2022).

Plant-based expression systems. Genetically modified plants engineered to produce dsRNA. Feeding of insects on these plants ingest dsRNA, leading to gene silencing. This approach offers a sustainable and eco-friendly pest control solution (Nitnavare et al. 2021).

Some advances are also required to improve development of dsRNA-based insecticides.

High-throughput screening. Utilization of genomic and transcriptomic analyses to pinpoint candidate genes for targeted RNAi silencing.

Functional genomics. Research that uncovers the role of specific genes in insect biology, enabling the design of more precise and effective RNAi strategies.

Combination strategies. Integrating RNAi with other pest control methods (for example, with CUAD biotechnology or CRISPR/Cas technology) to minimize the chances of resistance emergence.

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Target-site resistance. Investigating the most variable genes that enable pests to develop resistance and finding ways to counteract them. Double-stranded RNA biocontrols are perceived as 'difficult' insecticides, since they do not have clear and easy algorithm of creation, there is no strategy for RNAi how to avoid target-site resistance in insects, success of their application in the field is unpredictable. As with all pesticides, appropriate insect resistance management (IRM) programmes are required to mitigate the selection for resistance in target insect populations and extend product durability in the field (Narva et al., 2025).

Target specificity. Ensuring that RNAi molecules selectively silence pest genes without affecting non-target organisms are critical. Advances in computational tools and high-throughput sequencing are helping to identify and minimize off-target effects.

Environmental stability. RNA molecules are prone to degradation in the environment. To enhance their effectiveness, researchers are developing more stable RNA formulations and innovative delivery methods.

Regulatory and public acceptance. The adoption of RNAi-based pest control faces regulatory challenges and public concerns. Transparent research, clear communication of benefits, and comprehensive safety assessments are essential for gaining approval and widespread acceptance.

Production of dsRNA. Affordable production of dsRNA is not publicly available, while publicly available in vitro production is still very expensive (>50 USD/mg) (Verdonckt and Vanden Broeck 2022).

Overcoming these constraints will require the collective efforts of researchers, policy-makers, and industry stakeholders at the global level to accelerate innovation and maximize the impact of RNAi in pest management. The technology adapted is expected to evolve over the next 25 years to address current challenges and pave the way for the widespread adoption of RNAi in agriculture and other sectors (Palli 2023).

3. CUAD Biotechnology

3.1. History of Discovery

DNA insecticidal activity was discovered out of curiosity in the spongy moth *Lymantria dispar*. In 2007, Oberemok Vol began research in the field of transovarial transmission of *L. dispar* multiple nucleopolyhedrovirus (LdMNPV) as a part of his doctoral studies. Two specific primers were selected within the anti-apoptotic gene (IAP-3) of LdMNPV: a forward primer from the sense strand (5′-GCCGGCGGAACTGGCCCA-3; oligoBIR fragment) and a reverse primer from the antisense strand (5′-CGACGTGGTGGCACGGCG-3′; oligoRING fragment) (Oberemok et al., 2017). These primers initiate the formation of amplicon a 317 bp long amplicon during PCR in the presence of the LdMNPV DNA. On purified virus preparations, the primers formed the expected 317 bp long amplicon (Oberemok 2011). However, when searching for the virus in the tissues of the virus-free *L. dispar*, primers initiated the formation of several amplicons of different lengths, indicating that they were not specific enough to detect LdMNPV in host tissues. Consequently, obtained data indicated that the *L. dispar* genome contained regions homologous to fragments of the IAP-3 gene of the LdMNPV, a phenomenon previously reported for other viruses (Cerio et al. 2010). Thus, the developed primers were not suitable for accurate detection of the LdMNPV in insect tissues. Hypothetically, oligoBIR and oligoRING fragments could target the gene expression of homologous *L. dispar* IAP genes to induce apoptosis in insect cells. In 2008, it was a serendipitous moment when Oberemok V. decided to test the primers in an unusual way: he applied small drops of an aqueous primer solution to the surface of spongy moth larvae (Oberemok 2008; Manju et al. 2008). To his surprise, after 3-5 days, the larvae began to die in significant numbers due to the applied DNA fragments of the virus genome. This pioneering experiment marked the beginning of research into the development of a previously unknown class of contact DNA insecticides (oligonucleotide insecticides, or briefly olinscides) and the CUAD platform. The first publications of these results appeared in the Ukrainian patent (№36445) in 2008, followed by articles in Pesticide Biochemistry and Physiology (Oberemok and Skorokhod 2014; Oberemok et al. 2016). The earliest 18–20 nt long oligonucleotide insecticides based on anti-apoptotic genes demonstrated their effectiveness in LdMNPV-free larvae and were even more potent in LdMNPV-infected spongy moth larvae (Oberemok et al. 2017). Unique antisense DNA sequences of 11-20 nt can provide high selectivity in action; however, their effectiveness heavily depends on the concentration of the target RNA. As a result, CUAD biotechnology has been developed and now shows the best results on pests targeting their rRNA (which constitutes 80% of cellular RNA), utilizing the DNA containment mechanism (DNAc) to create a powerful algorithm of insect pest control (Figure 3).

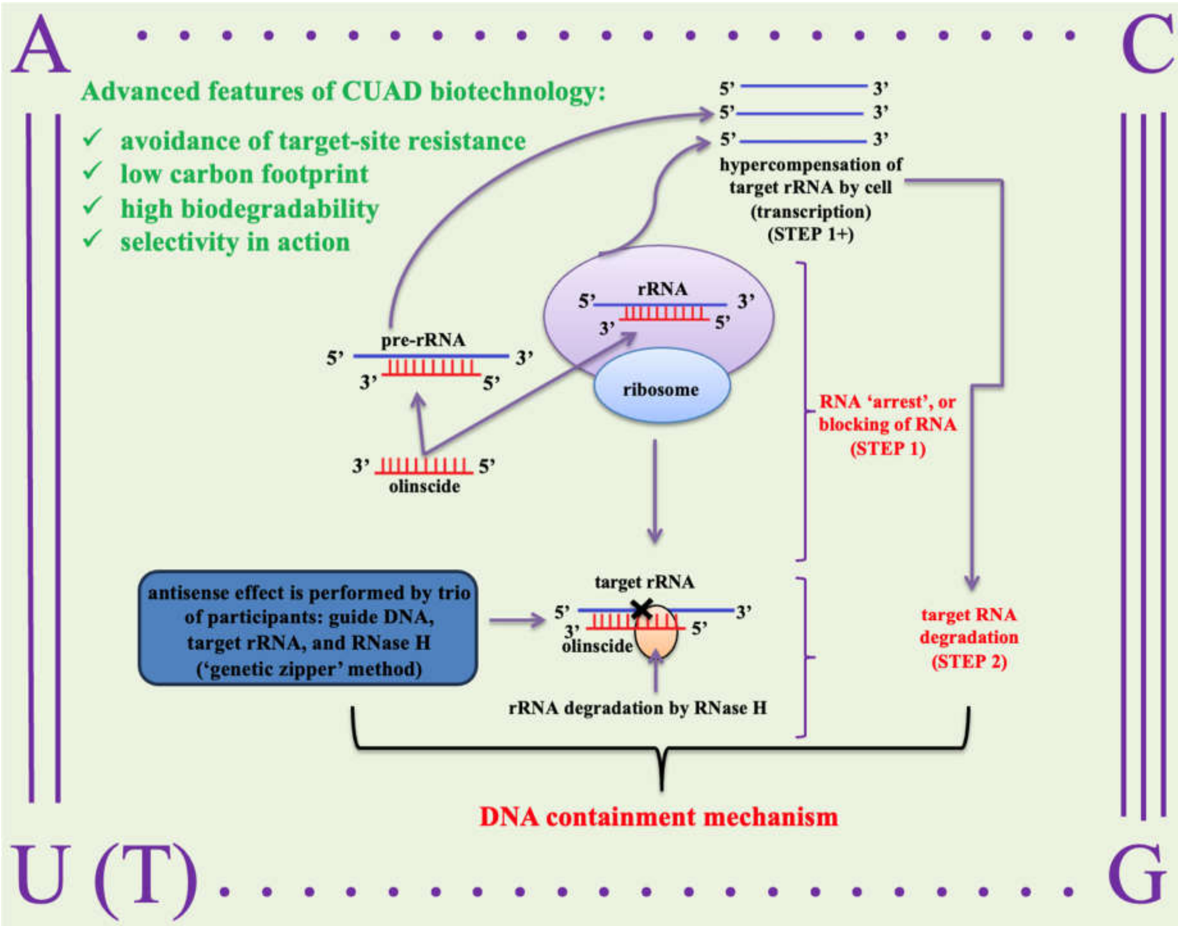

Figure 3. Advanced features of CUAD biotechnology based on oligonucleotide insecticides and DNA containment mechanism.

The discovery of oligonucleotide insecticides opened up an entirely new dimension in insect pest control using nucleic acids as contact insecticides. Scientists studying RNAi also picked up this idea three years later when Wang et al. (2011) successfully applied double-stranded RNA fragments as contact insecticides in insect pest control for the first time (Wang et al. 2011).

3.2. How It Works on Insect Pests

In 2019, Oberemok and co-workers made three key changes to substantially improve CUAD biotechnology. First, insect rRNA as a target for the action of oligonucleotide insecticides began to be used (Oberemok et al. 2019). Use of insect pest pre-rRNA and rRNA as target leads to high efficiency of oligonucleotide insecticides, since pre-rRNA and rRNA comprise 80 % of all RNA in the cell (Warner 1999). Thousands of different mRNAs constitute only 5 % of all RNA and use of pre-rRNA and rRNA for targeting substantially increases signal-to-noise ratio, ca. 100,000:1 (rRNA vs. random mRNA). In insects, cell rRNA is represented by nuclear rRNA, which includes 28S rRNA (~ 3900 nt),

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18S rRNA (~ 1920 nt), 5.8S rRNA (~ 160 nt), 5S rRNA (~ 120 nt) and mitochondrial, which includes 16S rRNA (~ 1140 nt) and 12S rRNA (~ 600 nt), and together they make up about four fifths of all RNA in the cell, representing a convenient target for the action of antisense oligonucleotides.

Secondly, the length of oligonucleotide insecticides was reduced to 10–12 nucleotides (nt). This helped to decrease the cost of oligonucleotide insecticides, since the yield of DNA synthesis (phosphoramidite method) for short DNA sequences is higher. In agrocenoses, the number of dominant insects does not exceed a dozen, including pests and beneficial insects. That is why length of an oligonucleotide insecticide 11 nt long makes it possible to create selective oligonucleotide insecticides with a uniqueness frequency equal to $1/4.19 \cdot 10^6$ for most agrocenoses (Oberemok et al. 2022). In the case of ecosystems with increased diversity, such as forests, it is possible to increase the length of oligonucleotide insecticides to 15–20 nt (Oberemok et al. 2024b).

Thirdly, it was found that the suborder Sternorrhyncha (Hemiptera) is very susceptible to unmodified antisense oligonucleotides (Oberemok et al. 2024a). To date, oligonucleotide insecticides have already been successfully used against several sap-feeding insect pests targeting 28S rRNA (*Unaspis euonymi*, *Dynaspidiotus britannicus*, *Icerya purchasi*, *Ceroplastes japonicus*, *Aonidia lauri*, *Coccus hesperidum*) (Useinov et al. 2020; Gal'chinsky et al. 2020, 2023, 2024; Oberemok et al. 2023, 2024b, c), 18S rRNA (*Pseudococcus viburni*) (Novikov et al. 2023), as well as the internal transcribed spacer (ITS2) of pre-rRNA of *Macrosiphoniella sanborni* and *Schizolachnus pineti* (Puzanova et al. 2023; Oberemok et al. 2024d) and ITS2 of pre-rRNA of bay sucker *Trioza alacris* (Oberemok et al. 2024e); and even ITS2 of pre-rRNA of spider mite *Tetranychus urticae* (Gavrilova et al. 2025; IZ 2025), showing potential of oligonucleotide acaricides. As a rule, after single contact treatment of sap-feeding insect pests with oligonucleotide insecticides at a concentration of 100 ng/μl high mortality (ca. 80 %) is observed in 3-14 days (Oberemok et al. 2023, 2024b). The first successful experiment with olinscides within suborder Sternorrhyncha was carried out on the scale insect *U. euonymi* in 2019 (Oberemok et al. 2020; Gal'chinsky et al. 2020) (Table 2).

Table 2. List of pest species successfully targeted by CUAD biotechnology.

Sl. No.	Insects name	Targeted Gene(s)	Affected System	References
1.	Euonymous scale, *Unaspis euonymi*	28S rRNA	Protein biosynthesis	Gal'chinsky et al. 2020; Oberemok et al. 2020
2.	Holly scale, *Dynaspidiotus Britannicus*	28S rRNA	Protein biosynthesis	Gal'chinsky et al., 2020, 2024
3.	Japanese wax scale, *Ceroplastes japonicus*	28S rRNA	Protein biosynthesis	Useinov et al. 2020
4.	Cactus scale, *Diaspis echinocacti*	28S rRNA	Protein biosynthesis	Plugatar et al. 2021
5.	Bay sucker, *Trioza alacris*	ITS2 of pre-rRNA and 28S rRNA	Protein biosynthesis	Oberemok et al. 2024e
6.	Cottony cushion scale, *Icerya purchase*	28S rRNA	Protein biosynthesis	Gal'chinsky et al. 2023
7.	Chrysanthemum aphid, *Macrosiphoniella sanborni*	ITS2 of pre-rRNA	Protein biosynthesis	Puzanova et al. 2023

8.	Mealybug, *Pseudococcus viburni*	5.8S and 28S rRNA	Protein biosynthesis	Novikov et al. 2023
9.	Laureal scale, *Aonidia lauri*	28S rRNA	Protein biosynthesis	Gal'chinsky et al. 2024
10.	Soft scale, *Coccus hesperidum*	28S rRNA	Protein biosynthesis	Oberemok et al. 2022
11.	Two-spotted spider mite, *Tetranychus urticae*	ITS2 of pre-rRNA	Protein biosynthesis	Gavrilova et al. 2025
12.	Grey pine aphid, *Schizolachnus pineti*	ITS2 of pre-rRNA	Protein biosynthesis	Oberemok et al. 2024c
13.	Large pine aphid, *Cinara pinea*	ITS2 of pre-rRNA	Protein biosynthesis	Oberemok et al. 2024c
14.	Pine needle aphid, *Eulachnus rileyi*	ITS2 of pre-rRNA	Protein biosynthesis	Oberemok et al. 2024c

Important to note, investigating sternorrhynchans, it was shown that unmodified oligodeoxyribonucleotides (oligonucleotide insecticides) are capable of causing both, up-regulation and down-regulation of target genes during DNA containment mechanism (1st step: 'arrest' of target rRNA, block of functioning of ribosomes accompanied with hypercompensation of expression of rRNA through rDNA transcription; 2nd step: degradation of target rRNA by RNase H) (Gal'chinsky et al. 2024; Oberemok and Gal'chinsky 2024; Oberemok et al. 2024a). A target rRNA and an olinscide interlock and in the presence of RNase H resemble zipper mechanism performed by DNA-RNA duplex (‘genetic zipper’ method) (Oberemok et al. 2024e). The "genetic zipper" method is not just innovation but also an algorithm which with a high degree of probability calculates the efficiency of a particular olinscide not only for the target insect pest, but also for closely related species having perfect complementarity to the developed olinscide (Oberemok et al. 2024e).

Oligonucleotide insecticides can be designed using DNAInsector program (dnainsector.com) or manually using sequences of pest pre-rRNA and rRNA found in GenBank database. Literally, now anyone can manually create any oligonucleotide insecticide complementary to pre-rRNA or/and rRNA of a sternorrhynchan and with a very high probability it will work very efficiently. For selectivity, you should consider the same sites of pre-rRNA or/and rRNA of non-target organisms, they must not coincide. The synthesis of DNA insecticides is possible by the phosphoramidite method using liquid-phase synthesis or solid-phase synthesis on DNA synthesizers such as ASM-800 (BIOSSET, Russia), OligoPilot™ (Cytiva, Sweden), 10-Column DNA Synthesizer (PolyGen, Germany), etc. (Gal'chinsky et al. 2023). Oligonucleotide insecticides are generally dissolved in nuclease-free water and usual concentration is 1 mg of olinscides per 10 ml of water solution and applied per m^2 of plant leaves containing insect pests.

3.3. Perspectives and Limitations of CUAD for Insect Pest Control

Today, DNA is revolutionizing plant protection by creating new controlling agents with advanced characteristics. Molecules of natural origin inevitably turn out to be more attractive for maintaining the balance of ecosystems, including agrocenoses. As a programmable molecule, DNA allows for the design of treatments that can induce specific effects – such as insecticidal, acaricidal, etc. Moreover, if resistance arises, various adaptive strategies can be employed. New olinscides can be developed by shifting the target site left or right of the resistance site within pre-rRNA and rRNA (Gal'chinsky et al. 2024). Oligonucleotide insecticides also offer several advantages: they have a low carbon footprint, high specificity for target organisms, rapid biodegradability in ecosystems, and minimal risk of target-site resistance. Additionally, their effectiveness can now be predicted across different insect pests based on their performance in closely related species (Oberemok et al. 2024e).

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Obviously, it is a matter of time to obtain this kind of end-products for plant protection. The mechanism by which DNA will act is known – DNA containment mechanism, the synthesis of DNA fragments has been developed – the phosphoramidite method of oligonucleotide synthesis, the delivery routes for such preparations have been established – contact and less perspective, by oral feeding. The cost of DNA insecticides is of some concern, however, this issue will be finally resolved in the near future, since DNA insecticides for distinct insect pests are already competing in affordability with existing chemical insecticides (Oberemok et al. 2024b).

For conifer aphids, CUAD biotechnology has achieved a significant reduction in the cost (Oberemok et al. 2024c) of nucleic acid synthesis due to liquid phase synthesis (Gal'chinsky et al. 2023). One of market leaders in liquid phase synthesis, Sumitomo Chemical Co., Ltd. (Tokyo, Japan), offers the synthesis of 1 kg of unmodified oligonucleotides 11 nt long for 25,000 USD (personal communication). In contrast, using non-optimized solid-phase DNA synthesis, which is more widely available in laboratories, the cost of synthesizing 1 kg of the same oligonucleotides can reach approximately $1 million. At an application rate of 200 L per hectare with a concentration of 0.1 mg/L (or 0.1 ng/mL), the cost of the required oligonucleotide insecticide would be approximately $0.50 per hectare when produced via liquid-phase DNA synthesis (Oberemok et al. 2024c). This affordability allows for increased treatment frequency under field conditions. However, if non-optimized solid-phase DNA synthesis is used, the cost rises to $20 per hectare. For many pests within the suborder Sternorrhyncha, effective control with DNA insecticides requires a higher concentration of 0.05 g/L of the active ingredient at the same application rate. This significantly increases the cost of olinscides to $250 per hectare. Therefore, achieving a balance between cost-effectiveness and formulation efficiency will be crucial for the widespread adoption of nucleic acid-based insecticides in the near future. Based on our current estimates, CUAD biotechnology has the potential to successfully control 10-15 % of all insect pests.

Although oligonucleotide insecticides have proven their powerful potential on hemipterans and lepidopterans, on coleopterans (*Leptinotarsa decemlineata*) they showed much less pronounced insecticidal effect (Oberemok et al. 2018). Adding of auxiliary substances (spreaders, adhesives, penetrators, and UV protectants) to formulation is possible to improve their efficiency but safety for the environment of the final formulation should be previously evaluated. Also it was found that non-canonical base pairing, such as A:C (C:A) and G:U (T:G) (Du et al. 2005; Luige et al.,2022), may occur between DNA olinscides and imperfect sites of rRNAs. Thus, non-canonical base pairing should be taken into consideration during the design of olinscides so as not to harm non-target organisms (Gal'chinsky et al. 2024; Oberemok et al. 2024c).

4. CRISPR/Cas

4.1. History of Discovery

If the history of the emergence and development of RNAi and CUAD technologies is almost completely straightforward, then CRISPR/Cas technology appeared thanks to a large number of research groups, and therefore it is not very easy to single out the most significant scientists. Basically, we will briefly focus on the generally accepted opinion in science on this issue and apologize not to name all the scientists participated in CRISPR/Cas research. In 1987, Yoshizumi Ishino and colleagues discovered a previously unknown repeat sequence in *Escherichia coli*, though they did not give it much attention (Ishino et al. 1987). In 1989, Spanish scientist Francisco Mojica, studying an archaeal microbe *Haloferax mediterranei*, also found an interesting structure – multiple copies of palindromic, repeated sequence of 30 bases, separated by spacers of ca. 36 bases – that did not resemble any family of repeats known in microbes (Mojica et al. 1993). Later Francisco Mojica and Ruud Jansen called them as CRISPR (clustered regularly interspaced short palindromic repeats) (Shmakova et al. 2022). In 2002, in the immediate vicinity to CRISPR, Cas genes were found by R. Jansen et al. (Jansen et al. 2002). In 2007, Philip Horvath and colleagues found that *Streptococcus thermophilus* required Cas7 in order to gain resistance, but those carrying a phage-derived spacer did not need the gene to remain resistant – suggesting that Cas7 was involved in generating new spacers and repeats, but not in immunity itself (Barrangou et al. 2007). In contrast, Cas9 (formerly known as Cas5, Csn1, or Csx12) whose sequence contained two types of nuclease motifs (HNH and RuvC) and whose product thus presumably cut nucleic acids (Bolotin et al. 2005; Makarova et al. 2006) – was necessary for phage resistance. Thus, the Cas9 protein was an active component of the bacterial immune system (Alaa et al. 2024). In 2011, Emmanuelle Charpantier found third-most abundant RNA in *Streptococcus pyogenes*, Trans-activating CRISPR RNA (tracrRNA), which was essential for processing CRISPR RNAs (crRNAs) and thus for CRISPR function helping Cas9 nuclease complex to cleave DNA (Jinek et al. 2012). In 2012, Šikšnys et al. in Proceedings of the National Academy of Sciences demonstrated that they could reprogram Cas9 with custom-designed spacers in the CRISPR array to cut a target site of their choosing in vitro (Lander 2016). Like Virginijus Šikšnys, Emanuelle Charpantier, Jennifer Doudna and colleagues in Science showed that Cas9 could cut purified DNA in vitro, that it could be programmed with custom-designed crRNAs, that the two nuclease domains cut opposite strands, and that both crRNA and tracrRNA were required for Cas9 to function (Shmakova et al. 2022).

The employment of molecular genetic engineering and insect transformation through CRISPR/Cas9 in multiple species has overcome many previously intractable problems using traditional methods which mainly relied naturally occurring genetic mutations or elements. Unfortunately, today it is almost impossible to predict the outcome from gene editing of a particular insect pest as well as which gene is better to target by this approach to gain maximum effect.

4.2. How It Works on Insect Pests

Over the last couple of years, CRISPR-based gene-editing technology sector has achieved tremendous growth as a result of their popularity in different fields of life sciences (Li et al. 2024c). In the beginning, CRISPR system was discovered in bacteria and archaea, working as an adaptive immune system that defend against invading phages and foreign genetic elements (Hossain et al. 2021). Later on, the CRISPR system has been adapted for gene editing in different field of biological sciences. Based on the structure and function of Cas proteins, the CRISPR/Cas system is categorized into Class I (type I, III, and IV) and Class II (type II, V, and VI). Class I comprises multi-subunit Cas protein complexes (4–7 proteins), whereas Class II relies on a single Cas protein. Class I is predominant in bacteria and archaea, accounting for 90% of all identified CRISPR-Cas loci, however, the remaining 10% belong to Class II type, which is exclusive to bacteria and employs a single multi-domain effector protein (Asokan et al. 2022).

Among both classes, Type II CRISPR/Cas9 system has been extensively studied due to its simple structure, making it widely used in genetic engineering. It consists of two key components: engineered single guide RNA (sgRNA) and the Cas9 protein. The Cas9 enzyme, originally isolated from *Streptococcus pyogenes*, is a large (1368 amino acids) multi-domain DNA endonuclease responsible for creating double-stranded breaks in target DNA by recognizing a protospacer adjacent motif (PAM). The RuvC and HNH domains cut single-stranded DNA, while the PAM-interacting domain ensures specificity and initiates DNA binding.

CRISPR/Cas9 system was simplified by pioneers of CRISPR system that it consists of two main components: Cas nuclease, which introduces targeted double-stranded breaks in DNA, and a sgRNA, which directs the nuclease to the specific DNA sequence (Wiedenheft et al. 2012; Lotfy and Hsu 2022; Bhatia and Yadav. 2023). The engineered sgRNA is formed by the fusion of two RNA molecules i.e., crRNA and tracrRNA (Deltcheva et al. 2011; Liao and Beisel 2021). In bacterial cells, Cas proteins process these RNAs into mature guide RNA, which forms a complex with Cas9 to recognize and cleave DNA sequences near a PAM; three nucleotides upstream (Khan et al. 2023; Wang and Doudna 2023; Wang et al. 2022; Asokan et al. 2022) (Figure 4).

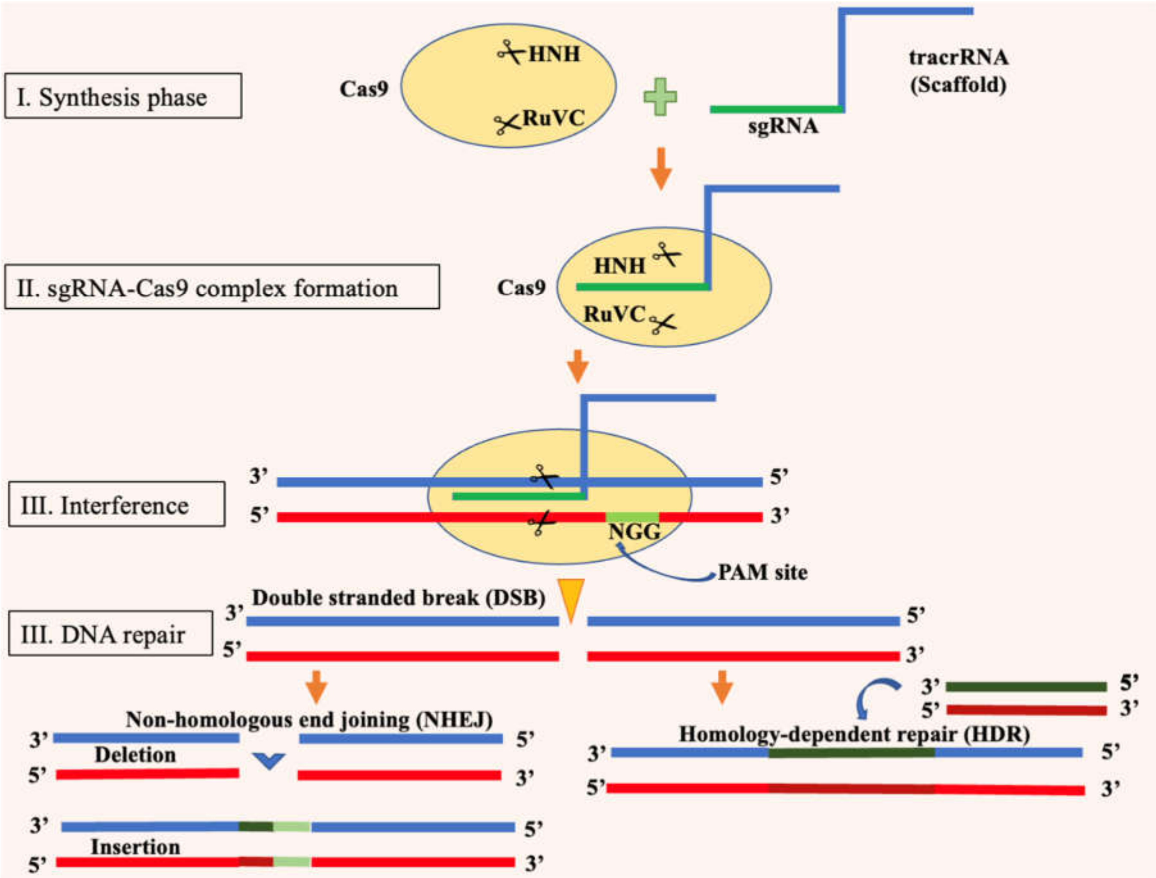

Figure 4. The CRISPR-Cas system relies on two main components: a single guide RNA (sgRNA) and CRISPR-associated (Cas) nuclease.

CRISPR-based genome editing proved to be more efficient and stable in comparison to other techniques. For example, CRISPR/Cas13-mediated knockdown of the homeobox gene Scr in silkworms resulted in developmental defects in larvae and malfunctioning of adult structures which demonstrate that it could be a better alternative to RNAi for gene editing in Lepidoptera (Huynh et al. 2020; Xu et al. 2020). Since its initial adoption in Drosophila, CRISPR/Cas9 has advanced quickly over the last 10 years into diverse insect species.

The aim of use of genome editing is to introduce desired engineered traits into wild insect pest populations which presents a promising solution for overcoming resistance issues in pests and managing invasive species. Additionally, genetic control strategies aimed at mitigating vector-borne diseases have positioned gene editing as a key area of ongoing research (Xu et al. 2019). For instance, disrupting genes in the sex-determination pathway to impair vector competence, induces lethal recessive mutations (which only take effect when both alleles are recessive), or skewing the sex ratio in targeted insect populations could help reduce the prevalence of vector-borne diseases (Ranian et al. 2022; Zulhussnain et al. 2023).

The science of CRISPR/Cas has emerged as a powerful tool for agricultural pest management. By modifying target DNA sequences, researchers can address insecticide resistance and introduce new traits that can restore susceptibility. One strategy involves releasing gene-edited insects to diminish resistant populations, demonstrating potential in global pest management efforts. Furthermore, gene drives the propagation of genetic traits, enhancing the effectiveness of other pest management strategies (Ying et al. 2023; Zahoor et al. 2024). As one of the effective insect pest management tools, CRISPR/Cas can be utilized by gene disruption rendering sterility, gene drive to propagate deleterious traits, RNA-guided pest resistance, interruption of vector competence in disease-transmitting insects, and modifying reproductive genes. These strategies offer sustainable, accurate, and environment friendly alternatives to usual pest control methods.

It is stated that CRISPR has great capability to drive entomology to different heights in the past few years. This technology has been able to ceremoniously enhance the knowledge of insect science via molecular research by focusing on insect physiology, biology, morphology, vision, and reproduction (Table 3). Indeed, this highly promising genome editing tool has sparked ongoing research, exploring the potential of CRISPR/Cas9 for the development of sustainable pest management strategies which aims at targeting key genes (Gouda et al. 2024). Most recent research which investigated chloroplast-engineered dsRNA that specifically target western flower thrips *Frankliniella occidentalis* and applied CRISPR/Cas9 to control this pest (Bulle et al. 2023; Han et al. 2024). Cadherin gene modifications were also performed to restore susceptibility in Chickpea pod borer *Helicoverpa armigera* and pink bollworm *Pectinophora gossypiella* to improve the efficacy of *Bt* cotton (Wang et al. 2016; Zahoor et al. 2021; Cheema et al. 2022).

Communication biology is a crucial aspect in insects which is highly developed and involve various sensory organs. Recently, Ashok et al. (2023b) explored CRISPR/Cas9 genome editing to disrupt the mating behavior of *S. frugiperda* by targeting the pheromone synthesis gene, fatty acyl-CoA Delta-9 desaturase, they used two sgRNAs to achieve a site-specific knockout with a larger deletion in the first exon. The mutation disrupted female pheromone production, leading to no fecundity when mutant females were crossed with wild males, while fecundity remained unaffected when mutant males were paired with wild females. Subsequently, Ashok et al. (2023c) investigated CRISPR/Cas9-mediated gene editing to disrupt mating in *S. frugiperda* via targeting the pheromone biosynthesis activator neuropeptide (PBAN) gene using a ribonucleoprotein complex comprising sgRNA and Cas9 protein. Microinjection into G0 embryos caused PBAN suppression, significantly reducing mating success. Mutant females were less attractive to wild males and showed no fecundity when crossed, while mutant males when crossed with wild females exhibited reduced fecundity. Ashok et al. (2023) also applied CRISPR/Cas9-mediated gene editing to target two spermatogenesis-related genes, testis-specific zinc finger protein (topi) and testis-specific serine protein kinase 1 (Tssk1), in *B. dorsalis* (Ashok et al. 2023a). The edited mutants exhibited reduced fecundity with significantly fewer eggs laid than the control group (6.12 and 3.60 eggs per day, respectively, compared to 11.16 in controls). Hatching rates were also lower in the mutants, with topi and Tssk1 which reported 44.51 % and 30.04 %, respectively, compared to 73.96 % in controls.

CRISPR/Cas genome editing is an important modern tool for accurate genetic modification. The technology is applied widely across agriculture, medicine, and pest management settings. A small, but nevertheless impactful cluster of studies showcased the potential with this technique in gene knockout, control of transcription, and gene replacement. The versatility and efficiency of CRISPR/Cas make it an effective tool for potential genetic engineering.

Table 3. List of pest species successfully targeted by CRISPR/Cas system in insects.

Sl. No.	Insect name	Target gene(s)	Affected system	Reference
1.	Mosquito, *Anopheles stephensi*	Kynurenine hydroxylase	Parasite-resistance	Gantz et al. 2015
2.	Fall armyworm, *S. frugiperda*	Ebony gene Doublesex (dsx) (Sfdsx) Antennapedia (Antp) Spermatogenesis-related gene, tssk2	Melanin biosynthesis Sex differentiation Insect thorax and wing development Male reproductive system	Zhu et al. 2020 Gu et al. 2022 Wang et al. 2023 Anu et al. 2024

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3.	Diamondback moth, *P. xylostella*	Yellow gene Ebony gene LW-opsin	Body pigmentation Body pigmentation Efficiency of phototaxis	Wang et al. 2020 Xu et al. 2021 Chen et al. 2021b
4.	European bee, *Apis mellifera*	Amyellow-y gene	Melanization in cuticle	Nie et al. 2021
5.	Beet armyworm, *S. exigua*	Desaturase (SexiDES5)	Sex pheromone biosynthesis	Ahmed et al. 2021
6.	Brown planthopper, *N. lugens*	Cysteine sulfinic acid decarboxylase (CSAD)	Melanin metabolism	Chen et al. 2021c
7.	Chickpea pod borer, *H. armigera*	Wnt1 gene	Segmentation, appendage development, and pigmentation	Fu et al. 2022
8.	Asian corn borer, *Ostrinia furnacalis*	Abdominal-A (Abd-A) and Ultrabithorax (Ubx)	Anatomical structure formation	Bi et al. 2022a
9.	Black garden ant, *Lasius niger*	Cinnabar gene	Eye pigmentation	Konu et al. 2023
10.	Common cutworm, *S. litura*	Serine protease 2 Odorant-binding proteins gene	Male sterility Perception of a sex pheromone	Bi et al. 2022b Han et al. 2022
11.	Indian meal moth, *Plodia interpunctella*	ATP binding cassette (ABC) proteins	Eye pigmentation	Shirk et al. 2023
12.	Eggplant shoot and fruit borer, *Leucinodes orbonalis*	Tryptophan 2, 3-dioxygenase Vitellogenin (Vg)	Eye pigmentation Female reproductive system	Ashok et al. 2023d Ashok et al. 2025
13.	Mango fruit fly, *B. dorsalis*	White gene White locus OBP13 gene	Eye pigmentation Eye pigmentation Methyl eugenol	Ashok et al. 2023a Bhargava et al. 2024, Pradhan et al. 2023 Sujatha et al. 2024
14.	Pomace fly, *Drosophila suzukii*	Doublesex gene	Population suppression	Yadav et al. 2023
15.	Australian cotton bollworm, *H. armigera conferta*	Cadherin gene	Cry1Ac resistance	Fang et al. 2025
16.	Cricket, *Gryllus bimaculatus*	Laccase 2 (Gb-lac2) gene	Cuticle system pigmentation	Matsuoka et al. 2025

4.3. Perspectives and Limitations of CRISPR/Cas for Insect Pest Control

The CRISPR/Cas system could contribute to managing populations of invasive and migratory pest species, including locusts. CRISPR can target survival or reproductive genes, potentially compromising the stability of a population. For instance, swarming or flight genes can be targeted in locusts in order to mitigate their catastrophic movements. However, a good genetic understanding

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of these complex behaviors will be essential for proper targeted results. CRISPR/Cas technology provides a revolutionary approach to insect pest control, holding great potential for sustainable and precise methods. This technology enables precise alterations in the insect's genome, opening up potential pathways to disrupt essential biological functions. One potential approach to achieving this goal could involve disrupting a gene that targets reproduction, such as those involved in gametogenesis. Alternatively, it may also target genes responsible for determining the sex of the insect, hence affecting the fertility of the respective insect or decreasing their population. By targeting the genes responsible for pheromone production or perception, it would be possible to disrupt insect communication, thereby hindering mating and aggregation behaviors as already reported and discussed above. Moreover, CRISPR could serve as an effective tool in combating insecticide resistance by targeting genes linked to resistance mechanisms in insects, thereby restoring the insects' sensitivity to insecticides that were previously ineffective. This technology offers a precise approach to controlling insect pests and harmful insects, causing less environmental damage as compared to the broad-spectrum insecticides.

CRISPR/Cas genome editing is widely used for its effectiveness and simplicity. Different Cas proteins like Cas3, Cas12a, and Cas13a are now used to expand gene editing, natural and engineered, for precise editing, base editing, prime editing, and gene regulation. Recent advances enabled DNA-free editing, allowing genome modification without inducing double-stranded breaks. The use of CRISPR/Cas technology in pest control also brings up several ethical concerns, particularly with gene drive technology, as it can quickly spread modified genes throughout a population. Careful risk assessment and responsible implementation are crucial to ensure the safe and sustainable use of CRISPR/Cas for insect pest management. Off-target effects, which lead to unintended genetic changes, and resistance remain significant challenges standing between the invention and its successful application. Thus, the necessity for rigorous research and development remains.

5. Conclusion

Antisense technologies are based on natural mechanisms that regulate cell life. Complementary interactions between nucleic acids are fundamental to cell division, metabolism and defense. The creation of practical tools using antisense technologies is a highly relevant and promising area of scientific research. In our view, dedicating decades to advancing this field is justified to create effective and environmentally friendly solutions. The application of antisense technologies (RNAi, CUAD and CRISPR/Cas) in insect pest control has already demonstrated significant potential. Notably, RNAi, CUAD and CRISPR/Cas yield optimal results for specific groups of insect pests, highlighting the potential for combining these approaches to maximize their effectiveness across broader pest populations. While competition exists among modern antisense technologies, the physiological and genetic characteristics of different pest groups may prevent any single approach from becoming a universal solution. Currently, antisense technologies (RNAi, CUAD and CRISPR/Cas) for insect pest control are in the development stage. Data on both their successes and limitations continue to accumulate, while major companies work toward commercializing end-products. Meanwhile, legislative framework governing the implementation of antisense technologies are gradually evolving in various countries. Rapid breakthroughs may not be imminent, but progress in this field is inevitable, ultimately contributing to improvements in both human and environmental health.

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