# Oligonucleotide insecticides (contact unmodified antisense DNA biotechnology) and RNA biocontrols (double-stranded RNA technology): newly born fraternal twins in plant protection

V.V. Oberemok, N.V. Gal'chinsky

Department of Molecular Genetics and Biotechnologies, Institute of Biochemical Technologies, Ecology and Pharmacy, V.I. Vernadsky Crimean Federal University, Simferopol 295007, Crimea.

* Correspondence: pcr.product@gmail.com; Tel.: +7 (978) 050-13-91

## Abstract

The rapid development of molecular genetics, methods of synthesis of nucleic acids, and bioinformatics have created fundamentally new opportunities in plant protection. DNA and RNA have become active ingredients of insecticides, going beyond their usual role as carriers of hereditary information. Now nucleic acids are increasingly acting as active tools for insect pest control using the mechanisms of DNA containment (oligonucleotide insecticides, contact unmodified antisense DNA (CUAD) biotechnology) and RNA interference (RNA biocontrols, double-stranded RNA biotechnology). Partial understanding of how antisense DNA oligonucleotides might work began in the late 1970s of XX century and for a long time (until the beginning of the XXI century) these biomolecules had not been implemented in plant protection in the form of any biotechnology based on unmodified or modified DNA fragments. RNA interference was discovered in the late 90s of XX century and also went through quite a long path of development in plant protection before the first encouraging results of its application as insecticides appeared. Both, CUAD biotechnology and double-stranded RNA technology aim to inactivate the expression of genes of insect pests using antisense DNA and double-stranded RNA that help trigger degradation of the target RNA. As two new classes of the next-generation insecticides, these products are supposed to have long operational life, low carbon footprint, high selectivity in action and rapid biodegradation in ecosystems. To date, antisense CUAD biotechnology shows the best results on sap-sucking insects while double-stranded RNA technology shows the best insecticidal potential on beetles from Tenebrionidae and Chrysomelidae families. With a high probability, oligonucleotide insecticides and RNA biocontrols, will be effective against certain groups of insects and can complement each other in complex formulations for control of wide range of pests.

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

## Introduction

Insect pest control is in constant search and development (Zheng and Xu, 2023) and some older classes of insecticides (carbamates, organophosphates, etc.) are substantially being replaced by younger classes of controlling agents (neonicotinoids, diamides, etc.) (van den Berg et al., 2021; Sparks et al., 2023). Due to the development of genetic resistance, this process never stops (Sparks et al., 2021; Mogilicherla and Roy, 2023) and it is possible to improve duration of operational life, selectivity in action, increased biodegradability, and low carbon footprint of current insecticides. Obviously, biomolecules are most likely to have such characteristics (Christiaens et al., 2020; Oberemok et al., 2022; Palli, 2023; Gal'chinsky et al., 2024). Technologies in plant protection

evolved and created opportunity of development of insecticides based on natural polymers, DNA and RNA, having the characteristics which past generations of chemical insecticides have never had (Oberemok et al., 2018a; Rank and Koch, 2021; Oberemok et al., 2023; Koeppe et al., 2023). Today, oligonucleotide insecticides (CUAD biotechnology) and RNA biocontrols (double-stranded RNA technology), homologous and at the same time different classes of insecticides, create a precedent for efficient and eco-friendly plant protection (Nitnavare et al., 2021; Gal'chinsky et al., 2024). In our opinion, these insecticides will occupy their specific niches in plant protection; presumably, oligonucleotide insecticides will be used against sap-sucking insects, and RNA biocontrols will be effective against beetles.

**Oligonucleotide insecticides**

Since the discovery by P. Zamecnik and M. Stephenson of inhibition of Rous sarcoma virus replication using 13 nucleotides (nt) long modified antisense DNA oligonucleotide (Stephenson and Zamecnik, 1978), the study of effects of antisense oligonucleotides on cells in the vast majority of cases has occurred in the paradigm of down-regulation of target genes using modified antisense DNA sequences with the participation of RNase H (Dias and Stein, 2002). For a long time, it was believed that unmodified oligodeoxyribonucleotides are rapidly cleaved by DNases and their degradation products have toxic effects on cells (Dias and Stein, 2002). In addition, it was assumed that rRNA is resistant to degradation by antisense DNA oligonucleotides (Will and Luhrmann, 2001; Bachellerie et al., 2002). Oligonucleotide insecticides (briefly, olinscides, or DNA insecticides) have shifted the old paradigm and marked the formation of a new paradigm in which unmodified oligodeoxyribonucleotides are capable of causing both, down-regulation and up-regulation of target genes during DNA containment mechanism (Oberemok et al., 2022; Gal'chinsky et al., 2024). DNA containment mechanism is comprised of 2 steps. At first step, antisense DNA oligonucleotide (oligonucleotide insecticide) complementarily interacts with target rRNA (in other words, ‘arrests’ target rRNA) and interferes with normal functioning of ribosomes; this process is accompanied with insect pest mortality. Apparently, target rRNA hypercompensation by the DNA-dependent RNA polymerase is the only way for the insect cell to fight for life in such a situation when target rRNA is ‘arrested’ by antisense DNA oligonucleotides. Both, ‘old’ blocked target rRNA in ribosomes (ribosomes apparently do not function properly in this situation) and ‘newly’ synthesized target rRNA and polycistronic rRNA transcripts, can be found by RT-PCR and, thus, we detect target rRNA hypercompensation. At second step, RNase H cleaves target rRNA and substantial decrease in its concentration occurs.

Fundamentally, unmodified antisense DNA fragments can be used as complementary DNA "tentacles" to elucidate the functioning of a specific short sequence in the target rRNA. Hypercompensation of the target rRNA gene will be a marker that the DNA "tentacle" has found a complementary site, and further elucidation of the function of a particular short rRNA sequence can be performed (e.g., expression analysis of the affected genes). The first step of the DNA containment mechanism (rRNA "arrest") is a useful tool for elucidating the functions of short rRNA sequences. Sap-sucking insects would obviously be suitable model objects for this study. It should be noted that RNA interference cannot provide this kind of fine analysis of the functioning of short rRNA sequences. Ribosomal RNAs is abundant in cell and apparently play a large number of roles in the interaction with variety of biomolecules. The DNA containment mechanism, discovered on hemipteran rRNAs, combines both modes of action of antisense DNA sequences (up-regulation and down-regulation of target genes) and opens up new horizons for understanding the functioning of eukaryotic cells and viral replication (Gal'chinsky et al., 2024).

It is obvious that DNA viruses can also take advantage of the hypercompensation of rRNA gene expression observed during the DNA containment mechanism in order to increase the number

of ribosomes in the cell necessary for their replication. Bioinformatics studies indicate that hijacking of host genes by viruses is common (Cerio et al., 2010). Indeed, during coevolution, DNA viruses could not miss the opportunity to hijack rRNA genes (Evseev et al., 2021) as key players in metabolism, since rRNA makes up four fifths of all RNA in a cell (Deng et al., 2022), and more than 60% of all energy is spent on the production and maintenance of ribosomes (Szaflarski et al., 2022). Most DNA viruses replicate in nucleus and most viruses interact with the nucleolus that plays a major role in virus life and assembly of ribosomes (Salvetti and Greco, 2014). The canonical function of nucleoli is ribosome biogenesis, where they support rRNA synthesis, processing, and assembly into pre-ribosomal subunits (Rawlinson et al., 2018). It is not difficult to imagine that a fragment of DNA virus genome can regulate host rRNA synthesis according to the ON/OFF principle, using this trigger to influence the cell (to turn on this mechanism and turn it off when required). The rRNA synthesis switchboard mechanism (ON/OFF) helps the virus initiate rRNA synthesis and the formation of new ribosomes with minimal energy consumption, using the complementary interaction of specific sequences of viral DNA and host rRNA.

On the other hand, when the cell attacks and the virus defends, antisense DNA oligonucleotides are able to participate in the molecular mechanisms of innate immunity against plant single-stranded DNA viruses, for which hemipteran insects are the main carriers, as well as against DNA viruses that normally infect them. It is obvious that insect nucleases cleave the DNA fragments of viruses, generating antisense DNA sequences that can trigger the degradation of viral mRNAs through the DNA containment mechanism (but, apparently, without hypercompensation of the target mRNA by the corresponding virus genes). While DNA containment mechanism has direct evidence, rRNA synthesis switchboard has indirect evidence and requires further investigation.

The DNA containment mechanism as well as the rRNA synthesis switchboard mechanism (Figure 1) cover a large number of fundamental events that can occur in the virus-host relationship and are based on overexpression of rRNA genes. At the same time, in the applied aspect, the DNA containment mechanism is already used and is the basis for the action of oligonucleotide insecticides and CUAD biotechnology in general. In turn, specific blocking of viral DNA sequences that can participate in complementary interaction with host rRNA during rRNA synthesis switchboard mechanism can lead to the creation of new therapeutic agents for the treatment of viral diseases of plants, animals and humans.

Early research work on the spongy moth using the oligonucleotide insecticides, oligoRING and oligoRIBO-11, showed that successful gene silencing mediated by unmodified antisense DNA was primarily dependent on the choice of the target gene, the target region within these genes, and amplitude of expression of the target genes (Oberemok et al., 2017; Oberemok et al., 2019). In fact, while studying the most attractive target genes of insect pests, one could not “miss the opportunity” to create oligonucleotide insecticides based on rRNA genes as key players in metabolism of eukaryotes, since rRNA accounts for 80% of the total RNA in the cell. Insect rRNA comprises 28S rRNA (~3900 nt), 18S rRNA (~1920 nt), 5.8S rRNA (~160 nt), 5S rRNA (~120 nt) and mitochondrial rRNA, which includes 16S rRNA (~1140 nt) and 12S rRNA (~600 nt) (Deng et al., 2022). For the first time, among all insect rRNAs, *Lymantria dispar* 5.8 rRNA was successfully targeted by oligonucleotide insecticides (Oberemok et al., 2019). Also, oligonucleotide insecticides have already been successfully used against several sap-sucking insect pests (suborder Sternorrhyncha) targeting 28S rRNA (*Unaspis euonymi*, *Dynaspidiotus britannicus*, *Icerya purchasi*, *Ceroplastes japonicus*, *Aonidia lauri*) (Oberemok et al., 2023; Gal'chinsky et al., 2023; Gal'chinsky et al., 2024), 18S rRNA (*Pseudococcus viburni*) (Novikov et al, 2023a), 16S rRNA (*Eriosoma lanigerum*) (unpublished data), as well as the internal transcribed spacer (ITS 2) of a polycistronic RNA transcript of *Macrosiphoniella sanborni* (Puzanova et al., 2023); and even ITS of mite *Tetranychus urticae* (Novikov et al., 2023b), showing potential of oligonucleotide acaricides. As a rule, after single contact treatment of sap-sucking insect pests with oligonucleotide insecticides

at a concentration of 100 ng/µl high mortality (in average 85-90%) is observed in 3-14 days (Oberemok et al., 2023). Although oligonucleotide insecticides have proved its potential on hemipterans and lepidopterans, on coleopterans (*Leptinotarsa decemlineata*) they showed much less pronounced insecticidal effect (Oberemok et al., 2018b). Usually, 11 nt long oligonucleotide insecticides are used to target insect pest rRNAs. Ribosomal RNA makes it possible, on the one hand, to use itself as an advantageous because it comprises majority of cell RNA, whereas, on the other hand, it is sufficiently variable among organisms to create well-tailored insecticides (Gal'chinsky et al., 2024). The length of an oligonucleotide insecticide ~ 11 nt makes it possible to create selective oligonucleotide insecticides with a uniqueness frequency equal to $1/4.19 \cdot 10^6$, obviously enough to be used in most agrocenoses (Oberemok et al., 2022). We believe that contact delivery of unmodified antisense DNA (CUADs) is much more efficient than oral delivery of unmodified antisense DNA (ODUADs) because of active DNases present in digestive tract. This is particularly key for hemipteran species where extra-oral salivary provides an additional barrier to cellular uptake (Cao et al., 2018).

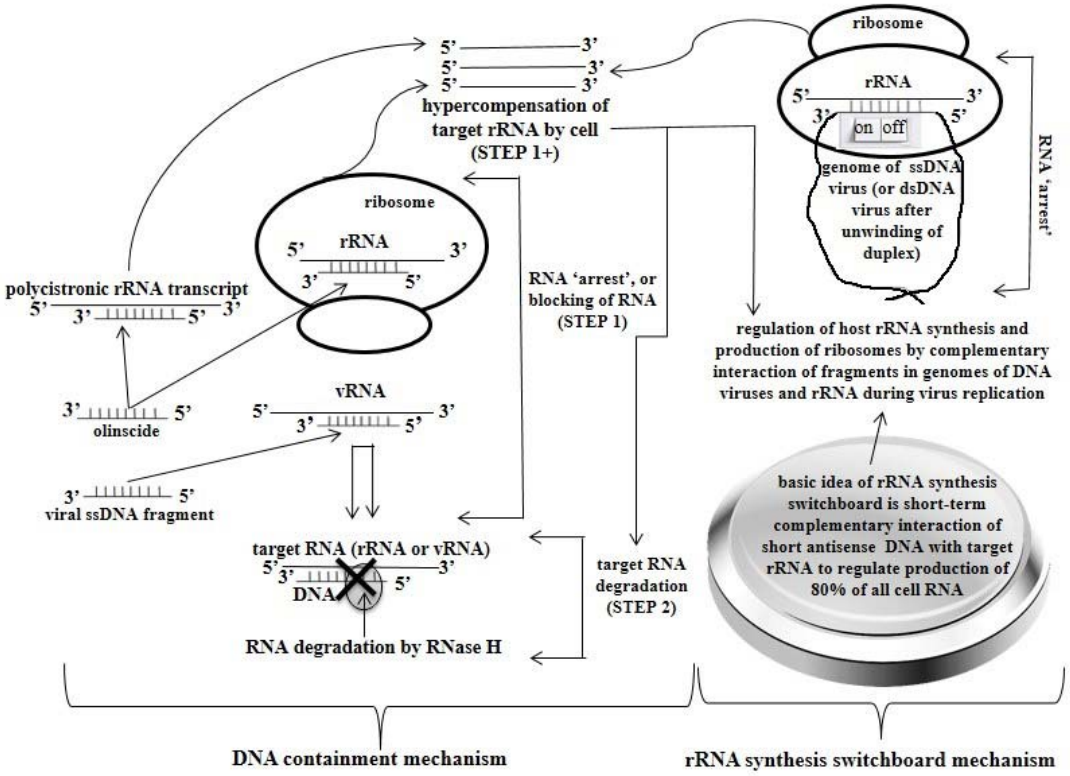


Figure 1. Mode of action of oligonucleotide insecticides and viral ssDNA fragments (DNA containment mechanism) and regulation of host rRNA synthesis by DNA viruses (rRNA synthesis switchboard mechanism).

It is important to note that if sequences from conservative parts of insect pest genes are used for creation of oligonucleotide insecticides, then target-site resistance to such insecticides will develop more slowly. This is due to the fact that mutations in the conserved regions of the gene occur less frequently, and therefore the target region of the rRNA will change much less frequently

as well (Sharma et al., 2014). If target-site resistance occurs new oligonucleotide insecticides can be created displacing target site to the left or to the right from the site of resistance of the target rRNA genes (Gal'chinsky et al., 2024).

The fate of oligonucleotide insecticides for the environment is "painless" since they are natural molecules that are quickly biodegraded in soils, water bodies and plants, as well as under the influence of abiotic factors such as water temperature, pH, salinity and ultraviolet (UV) radiation, and biotic factors, such as microbes and extracellular enzyme activity (Barnes and Turner, 2016). It was shown that deoxyribonucleases of *L. 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 h at 27°C (Oberemok et al., 2018b, Oberemok et al., 2019, Gal'chinsky et al., 2023, Gal'chinsky et al., 2024); and even faster, nucleases of *Macrosiphoniella sanborni* can degrade olinscides within 1 hour (Puzanova et al., 2023). Oligonucleotide insecticides do not show substantial negative effects on non-target organisms such as *Triticum aestivum* (Oberemok et al, 2013; Nyadar et al., 2019), *Quercus robur*, *Malus domestica* (Zaitsev et al., 2015), *Manduca sexta*, *Agrotis ipsilon* (Oberemok et a., 2015), *Galleria mellonella* (Oberemok et al., 2019). Moreover, it has been shown that when one nucleotide is replaced in the sequence of an oligonucleotide insecticide, its effectiveness dramatically decreases, which indicates unprecedented selectivity in action (Oberemok et al., 2019; Puzanova et al., 2023).

The use of oligonucleotide insecticides is also effective against mixed insect pest populations (Gal'chinsky et al., 2024) and resolve problem of climate change associated with production of insecticides. Modern solid-phase and liquid-phase synthesis of oligonucleotide insecticides do not lead to the accumulation of greenhouse gases such as nitrogen oxide, methane, or carbon dioxide (Gal'chinsky et al., 2023).

In conclusion, considering the widespread emergence of pesticide resistance, it seems that there is no question about the need for new molecular classes of pesticides, preferably with increased specificity to mitigate negative impacts on the environment. Oligonucleotide insecticides deserve credit for taking a bold step toward unmodified antisense oligonucleotides as one possibility. In its infancy, antisense oligonucleotide therapeutics faced numerous challenges and many critics, but after decades of research and huge investments, it is now a fascinating and rapidly developing approach (TriLink BioTechnologies, 2024). Oligonucleotide insecticides work selectively and biodegrade swiftly, and commercial nucleic acid in vitro synthesis is becoming more cost effective, the qualities of using unmodified nucleic acids as pesticides appear to be very promising (Kumar et al., 2022).

## RNA biocontrols

A. Fire and C. Mello discovered that double-stranded RNA is a key substance for RNA interference (RNAi) (Fire et al., 1998). This discovery was awarded Nobel Prize in Physiology or Medicine in 2006 and inspired many biotechnological applications. Since then, RNAi has become a widely used tool for many agricultural and forestry researchers to protect plants from insect pests. RNAi is the process of suppressing gene expression at the stage of transcription and translation using a double-stranded RNA copy (most often more than 200 bp in length) of the corresponding gene (Tomoyasu, 2008; Svoboda, 2020). At the early stages of RNA interference, the double-stranded RNA fragment is cut by the enzyme dicer (RNase III) into shorter fragments, small interfering RNAs (siRNAs) 20–30 bp long (Carmell et al. 2004; Obbard et al. 2009; Koeppe et al. 2023). These short fragments are then combined with proteins from the argonaute family (Parker et al. 2006; Kobayashi et al. 2022). Then, RISC (RNA-induced silencing complex) and RITS (RNA-

induced transcriptional silencing complex) complexes are formed, containing a short fragment of antisense RNA. These complexes block gene expression at the stage of transcription and translation, joining complementarily and blocking or degrading pre-mRNA and mRNA. According to recent findings, piRNAs are longer relatives of siRNAs and miRNAs, ranging in length from 23 to 36 nt (Wang et al., 2015), and have a germline-specific RNA silencing mechanism like other canonical RNA interference (RNAi) pathways consisting of protein from the P-element-induced wimpy testis (PIWI) subfamily. This pathway relies on the effector function of the PIWI protein, and the specificity provided by the piRNAs to repress transposable elements activity (Chattopadhyay et al., 2022). In insects and other arthropods different RNAi pathways can play crucial roles during ontogenesis (Figure 2).

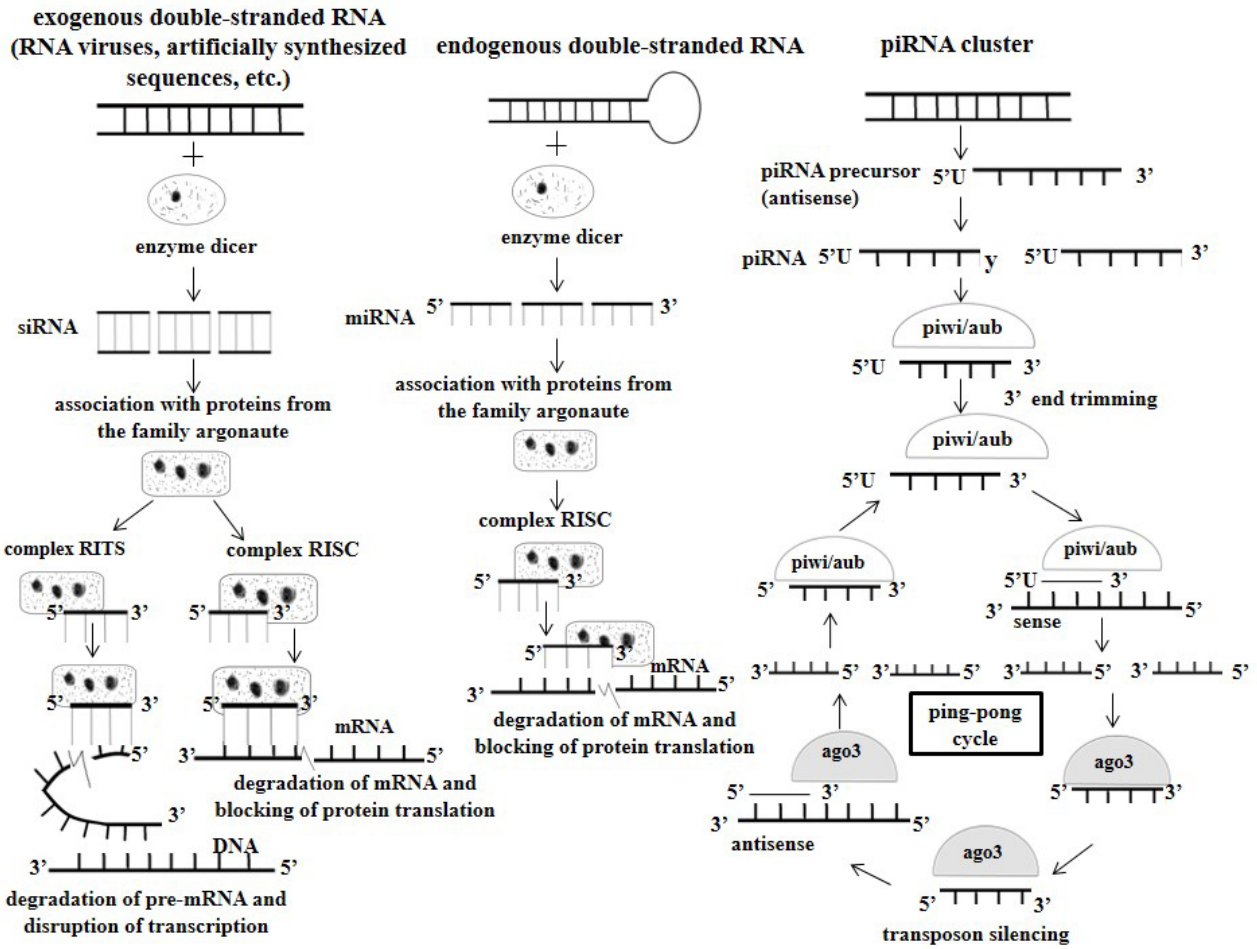


Figure 2. RNAi in insects.

The result of RNA interference is the interruption of the biosynthesis of a certain protein molecule. RNAi is highly efficient and systemic in coleopterans (Zhu and Palli, 2020) but is absent in representatives of the order 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 the use of this approach in plant protection. It took 25 years to create and register the first dsRNA-based insecticides. Calantha™ is the first sprayable dsRNA-based biopesticide that targets the Colorado potato beetle (CPB) and currently the only, was made

commercially available by GreenLight Biosciences in 2023 (Pallis et al., 2023; GreenLight Biosciences, 2024). Calantha™ ledprona, is a new class of insecticide — Group 35. This novel mode of action works by triggering a natural process in CPB after ingestion. The active ingredient prevents the production of an essential protein, leading to the death of the pest. CPB will completely stop feeding within 2-3 days after application. As a result, with ledprona treatment of small larvae CPB (1st and 2nd) at a concentration of 9.4 g/ha leads to high mortality (in average of 90 to 95% observed in 11-26 days) (GreenLight Biosciences, 2024). This first registered biopesticide based on dsRNA deserves credit for showing a precedent in plant protection, however, for dsRNA-based Calantha™ we see a lot of conventions (works best against small larvae, requires at least 2 successive applications, rotation with other insecticides having different modes of action is desirable) for this product to work properly, while, for example, for any sap-sucking insect pest oligonucleotide insecticides and current chemical insecticides (neonicotinoids, pyrethroids, etc.) can be used according to usual scheme of application without substantial conventions and limitations.

It is well known that insect genomes contain around 10□000–20□000 genes of which several hundred could be highly effective pest management targets, based on previous RNAi screens (Ulrich et al., 2015). However, not all insects are equally susceptible to dsRNA, with enormous differences between insects of different orders but also within closely related species. Moreover, there is no consensus on the best genes to be targeted (Buer et al., 2024). Additionally, variable silencing efficiency in the same insect is commonly obtained depending on development stages, target tissues and/or delivery systems. Although progress has been made in recent years to unravel the molecular determinants of the RNAi efficiency, being a set of highly interrelated factors, they are still not fully understood. Despite several exceptions reported, generally orthopteran, blattodean and coleopteran insects are considered to be very susceptible to exogenous dsRNAs, while in hemipteran (especially sap-sucking insects), the RNAi efficiency is low, and lepidopterans and dipterans usually have much lower efficiencies. Also, screening for more effective target genes may prove rewarding. In our opinion, spray-induced gene silencing (SIGS), which have recently gained great popularity in plant protection when used against insect pests, can also affect pests through contact route of delivery. We have not found convincing evidence that insect pests die from SIGS treatment solely due to feeding on a plant that has accumulated dsRNA. If known what determines SIGS efficiencies, it may have a starting point for developing solutions that demonstrate broad perspectives. Elucidating the molecular mechanisms of uptake, processing and translocation of spray-delivered RNAs is crucial for making RNA sprays realistic and achievable in future field applications. Moreover, to achieve broad applicability, which is often used as a selling point over RNAi strategies (Host-induced gene silencing, or HIGS), need to clarify the question of why RNA sprays work well for some pathosystems and not for others (Dalakouras and Vlachostergios, 2021).

There is a general expectation that by careful selection of the length and sequence of dsRNA, RNAi can be limited to target insect pests without any direct, deleterious effect on non-target species, including beneficial insects such as pollinators and biological control agents (Zotti et al., 2018; Cagliari et al., 2019). However, these predictions of dsRNA specificity can be confounded by two issues (Arora et al., 2021). First, RNAi susceptibility to heterologous dsRNA can be obtained with a single matching 21-mer sequence, require several or many matching 21-mers, be achieved with as few as 15 contiguously matching bases (Powell et al., 2017; Chen et al., 2021). Second, animals, including insects, can respond to dsRNA in a sequence-non-specific manner, i.e., independent of the sequence of the dsRNA (Hirai et al., 2004; Flenniken and Andino, 2013;

Brutscher et al., 2017; Chen et al., 2021). Obviously, some additional research should be carried out in this direction to provide sufficient level of safety of RNA biocontrols.

A determinant of RNAi efficiency is dsRNA degradation, partially explaining the differences between delivery methods, as in some species, effective silencing by injection is correlated with oral insensitivity. This is especially relevant in lepidopterans, hemipterans and dipterans. Lepidopterans have the highest degradation capacity in insects and express specific nucleases not seen in any other order, reducing the efficiency of RNAi in these insects (Guan et al., 2018), while hemipterans are the only reported order to degrade dsRNA in saliva (Lomate and Bonning, 2016). The environmental fate of dsRNA is short both in soil and aquatic environments (Dubelman et al., 2014; Bachman et al., 2020). Microbial nucleases present in the soil and on leaves, UV radiation and run-off due to dew and rain can significantly limit the availability of dsRNA to the pest (Parker et al., 2019; Bachman et al., 2020). A number of studies of insect *Spodoptera litura*, aphid *Acyrthosiphon pisum*, tobacco hornworm *Manduca sexta* nuclease activity serum and saliva has shown that dsRNA is degraded by nucleases within 0.3–3 hours (Garbutt et al., 2013; Christiaens et al., 2014; Peng et al., 2018).

Double-stranded technology has become very affordable recently (Taning et al., 2020). GreenLight Biosciences has developed large-scale cell-free biomanufacturing (cell-free dsRNA production) (Taning et al., 2020; Vilkhovoy et al., 2020) that uses endogenous cellular RNA to synthesize the desired dsRNA. Briefly, the endogenous RNA is depolymerized into nucleoside monophosphates (NMPs) with nucleases and then phosphorylated with kinases to form nucleotide triphosphates (NTPs). These NTPs are then polymerized into the target dsRNA using the corresponding DNA template and RNA polymerases (Cunningham et al., 2020).

RNA biocontrols, as well as oligonucleotide insecticides, have unique characteristics that make these next-generation classes of insecticides very perspective. Since the release of the first registered insecticide Calantha™, it is obvious that RNAi as a biotechnology has the potential to be effective in the global protection of crops from insect pests; although they have a shorter operating period and slower action compared to most chemical insecticides. Whether produced endogenously in crops or applied exogenously, properly engineered dsRNA molecules can replace classical insecticides to control insect pests in a more specific, sustainable and environmentally friendly manner. However, for this to be true, we need to continue to improve our knowledge of RNAi pathways in target pests and elucidate safety of RNA biocontrols for non-target organisms.

## Conclusion

Despite the fact that CUAD biotechnology and double-stranded RNA technology use nucleic acids as insecticides, there are fundamental differences between them (Oberemok et al., 2018a). Oligonucleotide insecticides are short unmodified antisense DNA fragments and act as contact insecticides. To degrade the target rRNAs of the pest, oligonucleotide insecticides use DNA containment mechanism. Due to the deoxyribose residues present in oligonucleotide insecticides, they are less susceptible to hydrolysis in nature than RNA (Thorp, 2000), and the small length of the fragment makes the selectivity of such insecticides more manageable. For the effective action of oligonucleotide insecticides, the most convenient target is rRNA, which makes up the vast majority of all RNA in the cell (Oberemok et al., 2019; Gal'chinsky et al., 2020; Oberemok et al., 2023; Gal'chinsky et al., 2024). At the moment, oligonucleotide insecticides act most effectively on sap-sucking insects (Oberemok et al., 2023; Gal'chinsky et al., 2024). In turn, RNA biocontrols are long double-stranded RNA fragments for which contact and oral routes of delivery are used. To degrade

the target RNA, these insecticides use the machinery of RNA interference (Liu et al., 2020; Christiaens et al., 2020; Rank and Koch, 2021). Due to the ribose residues, double-stranded RNA fragments are more susceptible to hydrolysis in nature than DNA (Williams et al., 2017), and the large length of the fragment makes the selectivity of such insecticides less controllable and additionally forces one to calculate possible risks for non-target organisms (Roberts et al., 2015; Christiaens et al., 2021). For RNA biocontrols to be effective more different insect pest RNAs (Liu et al., 2020) can be chosen for degradation than with oligonucleotide insecticides (Oberemok et al., 2022). Both classes of new generation insecticides have cost-effective methods for their synthesis: liquid-phase phosphoramidite synthesis of DNA (Gal'chinsky et al., 2023) and cell-free production of RNA (Taning et al., 2020). And if RNA interference had been known long before the first successful dsRNA insecticides appeared, DNA containment mechanism was discovered after 16 years of persistent and successful applied research with antisense oligodeoxyribonucleotides in plant protection. In our opinion, these two classes of insecticides will be most effective against certain groups of insects, will have a longer operational life of action and will often be combined in complex formulations designed for a wide range of pests. Insecticides based on DNA and RNA appear so advanced in terms of safety for non-target organisms and ecosystems that it is difficult to imagine the use of other biomolecules with such an impressive set of characteristics for green agriculture in the foreseeable future.

**CRediT authorship contribution statement**

**Vladimir V. Oberemok:** Conceptualization, Funding acquisition, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Nikita V. Gal’chinsky:** Project administration, Supervision, Writing – original draft, Writing – review & editing.

**Declaration of competing interest**

The authors declare no conflict of interest.

**Acknowledgments**

We thank our many colleagues, too numerous to name, for the technical advances and lively discussions that prompted us to write this review. We apologize to the many colleagues whose work has not been cited. We are very much indebted to all anonymous reviewers and our colleagues from the lab on DNA technologies, PCR analysis, and creation of DNA insecticides (V.I. Vernadsky Crimean Federal University, Department of Molecular Genetics and Biotechnologies), from the lab on cell technologies and creation of DNA medicines (V.I. Vernadsky Crimean Federal University, Department of Molecular Genetics and Biotechnologies), and OLINSCIDE BIOTECH LLC for valuable comments on our manuscript.

**Funding:** Research results obtained within the framework of a state assignment V.I. Vernadsky Crimean Federal University for 2024 and the planning period of 2024–2026 No. FZEG-2024–0001.

**Reference**

1. Arora, A.K., Chung, S.H., Douglas, A.E., 2021. Non-Target Effects of dsRNA Molecules in Hemipteran Insects. Genes. 12, 407. https://doi.org/10.3390/genes12030407.
2. Bachellerie, J.P, Cavaille, J., Huttenhofer, A., 2002. The expanding snoRNA world. Biochimie. 84, 775–790.
3. Bachman, P., Fischer, J., Song, Z., Urbanczyk-Wochniak, E., Watson, G., 2020. Environmental Fate and Dissipation of Applied DsRNA in Soil, Aquatic Systems, and Plants. Front. Plant Sci. 11, 21.
4. Barnes, M.A., Turner, C.R., 2016. The ecology of environmental DNA and implications for conservation genetics. Conserv. Genet. 17, 1–17. doi: 10.1007/s10592-015-0775-4.
5. Buer, B., Dönitz, J., Milner, M., Mehlhorn, S., Hinners, C., Siemanowski-Hrach, J., Ulrich, J.K., Grossmann, D., Cedden, D., Nauen, R., Geibel, S., Bucher, G., 2024. Superior target genes and pathways for RNAi mediated pest control revealed by genome wide analysis in the red flour beetle *Tribolium castaneum*. bioRxiv. doi: https://doi.org/10.1101/2024.01.24.577003.
6. Cagliari, D., Dias, N.P., Galdeano, D.M., dos Santos, E.Á., Smagghe, G., Zotti, M.J., 2019. Management of pest insects and plant diseases by non-transformative RNAi. Front. Plant Sci. 10.
7. Cao, M., Gatehouse, J.A., Fitches, E.C., 2018. A Systematic Study of RNAi Effects and dsRNA Stability in Tribolium castaneum and Acyrthosiphon pisum, Following Injection and Ingestion of Analogous dsRNAs. Int. J. Mol. Sci. 19, 1079. https://doi.org/10.3390/ijms19041079.
8. Cerio, R.J., Vandergaast, R., Friesen, P.D., 2010. Host insect inhibitor-of-apoptosis SfIAP functionally replaces baculovirus IAP but is differentially regulated by its N-terminal leader. J. Virol. 84, 11448–11460.
9. Chattopadhyay, T., Biswal, P., Lalruatfela, A., Mallick, B., 2022. Emerging roles of PIWI-interacting RNAs (piRNAs) and PIWI proteins in head and neck cancer and their potential clinical implications Biochim. Biophys. Acta Rev. Cancer. 1877(5), 188772. doi: 10.1016/j.bbcan.2022.188772.
10. Chen, J., Peng, Y., Zhang, H., Wang, K., Zhao, C., Zhu, G., Reddy Palli, S., Han, Z., 2021. Off-target effects of RNAi correlate with the mismatch rate between dsRNA and non-target mRNA. RNA Biol. 1–13. doi: 10.1080/15476286.2020.1868680.
11. Christiaens, O., Sweet, J., Dzhambazova, T., Urru, I., Smagghe, G., Kostov, K., Arpaia, S., 2022. Implementation of RNAi-based arthropod pest control: environmental risks, potential for resistance and regulatory considerations. Journal of Pest Science. 95. doi. 10.1007/s10340-021-01439-3.
12. Christiaens, O., Swevers, L., Smagghe, G., 2014. DsRNA Degradation in the Pea Aphid (*Acyrthosiphon pisum*) Associated with Lack of Response in RNAi Feeding and Injection Assay. Peptides. 53, 307–314.
13. Christiaens, O., Tardajos, M.G., Martinez Reyna, Z.L., Dash, M., Dubruel, P., Smagghe, G., 2018. Increased RNAi Efficacy in via the Formulation of dsRNA With Guanylated Polymers. Front. Physiol. 9, 316. doi: 10.3389/fphys.2018.00316.
14. Christiaens, O., Whyard, S., Vélez, A.M., Smagghe, G., 2020. Double-Stranded RNA Technology to Control Insect Pests: Current Status and Challenges. Front. Plant Sci. 11, 451. doi: 10.3389/fpls.2020.00451
15. Cunningham, D. S., MacEachran, D., Abshire, J. R., Dhamankar, H., Iwuchukwu, I., Gupta, M., et al. 2020. Methods and compositions for nucleoside triphosphate and ribonucleic acid production. Washington, DC: U.S. Patent and Trademark Office.

16. Dalakouras, A., Vlachostergios, D., 2021. Epigenetic approaches to crop breeding: current status and perspectives. J. Exp. Bot. 72, 5356–5371. doi: 10.1093/jxb/erab227
17. Deng, Z.L., Münch, P.C., Mreches, R., McHardy, A.C., 2022. Rapid and accurate identification of ribosomal RNA sequences via deep learning. Nucleic Acids Res. 50(10), e60. doi: 10.1093/nar/gkac112.
18. Dias, N., Stein, C.A., 2002. Antisense oligonucleotides: basic concepts and mechanisms. Mol Cancer Ther. 1(5), 347-55.
19. Dubelman, S., Fischer, J., Zapata, F., Huizinga, K., Jiang, C., Uffman, J., Levine, S., Carson, D., 2014. Environmental Fate of Double-Stranded RNA in Agricultural Soils. PLoS ONE. 9, e93155.
20. Evseev, P.V., Landysheva, Y.G., Landyshev, N.N., Ignatov, A.N., 2021. "Presence of rRNA-like regions in Genbank viral sequences," 2021 IEEE Ural-Siberian Conference on Computational Technologies in Cognitive Science, Genomics and Biomedicine (CSGB). 310-314, doi: 10.1109/CSGB53040.2021.9496035.
21. Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., Mello, C.C., 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature. 391, 806–811.
22. Gal'chinsky, N., Useinov, R., Yatskova, E., Laikova, K., Novikov, I., Gorlov, M., Trikoz, N., Sharmagiy, A., Plugatar, Y., Oberemok, V., 2020. A breakthrough in the efficiency of contact DNA insecticides: rapid high mortality rates in the sap-sucking insects *Dynaspidiotus britannicus* Comstock and *Unaspis euonymi* Newstead. Journal of Plant Protection Research, 60(2), 220-223. https://doi.org/10.24425/jppr.2020.133315.
23. Gal'chinsky, N.V., Yatskova, E. V., Novikov, I.A., Sharmagiy, A.K., Plugatar, Y.V., Oberemok, V.V., 2024. Mixed insect pest populations of Diaspididae species under control of oligonucleotide insecticides: 3′-end nucleotide matters. Pesticide Biochemistry and Physiology. 105838 https://doi.org/10.1016/j.pestbp.2024.105838.
24. Gal’chinsky, N.V., Yatskova, E.V., Novikov, I.A., Useinov, R.Z., Kouakou, N.J., Kouame, K.F., Kra, K.D., Sharmagiy, A.K., Plugatar, Y.V., Laikova, K.V., Oberemok V.V., 2023. *Icerya purchasi* Maskell (Hemiptera: Monophlebidae) Control Using Low Carbon Footprint Oligonucleotide Insecticides. Int. J. Mol. Sci. 24, 11650. https://doi.org/10.3390/ijms241411650.
25. Garbutt, J.S., Bellés, X., Richards, E.H., Reynolds, S.E., 2103. Persistence of double-stranded RNA in insect hemolymph as a potential determiner of RNA interference success: evidence from Manduca sexta and Blattella germanica. J Insect Physiol. 59, 171–8.
26. GreenLight Biosciences, 2024. https://www.greenlightbiosciences.com/international-crop-network-validates-ledprona-as-a-new-mode-of-action-group/ (accessed 12 March 2024).
27. Guan, R.B., Li, H.C., Fan, Y.J., Hu, S.R., Christiaens, O., Smagghe, G., Miao, X.X., 2018. A Nuclease Specific to Lepidopteran Insects Suppresses RNAi. J. Biol. Chem. 293, 6011–6021.
28. Iarovaia, O.V., Ioudinkova, E.S., Velichko, A.K., Razin, S.V., 2021. Manipulation of Cellular Processes via Nucleolus Hijaking in the Course of Viral Infection in Mammals. Cells. 10, 1597. https://doi.org/10.3390/cells10071597.
29. Jain, R.G., Robinson, K.E., Asgari, S., Mitter, N., 2021. Current scenario of RNAi-based hemipteran control. Pest Manag Sci. 77(5), 2188–2196. doi: 10.1002/ps.6153.
30. Kaplanoglu, E., Kolotilin, I., Menassa, R., Donly, C., 2022. Plastid Transformation of Micro-Tom Tomato with a Hemipteran Double-Stranded RNA Results in RNA Interference in Multiple Insect Species. Int. J. Mol. Sci. 23, 3918.

31. Koeppe, S., Kawchuk, L., Kalischuk, M., 2023. RNA Interference Past and Future Applications in Plants. Int. J. Mol. Sci. 24, 9755. https://doi.org/10.3390/ijms24119755.
32. Kumar, H., Sharma, M., Chandel, A., 2022. DNA Insecticides: Future of Crop Protection. ICAR- Indian Agricultural Research Institute, New Delhi-110012. Article ID: 37444. www.agrifoodmagazine.co.in (accessed 28 Feb 2024).
33. Liu, S., Jaouannet, M., Dempsey, D.A., Imani, J., Coustau, C., Kogel, K.H., 2020. RNA-based technologies for insect control in plant production. Biotechnol Adv. 39, 107463. doi: 10.1016/j.biotechadv.2019.107463.
34. Lomate, P.R., Bonning, B.C., 2016. Distinct Properties of Proteases and Nucleases in the Gut, Salivary Gland and Saliva of Southern Green Stink Bug, *Nezara viridula*. Sci. Rep. 6, 27587.
35. Lucena-Leandro, V.S., Abreu, E.F.A., Vidal, L.A., Torres, C.R., Junqueira, C.I.C.V.F., Dantas, J., Albuquerque, É.V.S., 2022. Current Scenario of Exogenously Induced RNAi for Lepidopteran Agricultural Pest Control: From dsRNA Design to Topical Application. Int J Mol Sci. 23(24), 15836. doi: 10.3390/ijms232415836.
36. Mogilicherla, K., Roy, A., 2023. Epigenetic regulations as drivers of insecticide resistance and resilience to climate change in arthropod pests. Front Genet. 13, 1044980. doi: 10.3389/fgene.2022.1044980.
37. Nitnavare, R.B., Bhattacharya, J., Singh, S., Kour, A., Hawkesford, M.J., Arora, N., 2021. Next Generation dsRNA-Based Insect Control: Success So Far and Challenges. Front. Plant Sci. 12, 673576. doi: 10.3389/fpls.2021.673576.
38. Novikov, A., Rybareva, A., Bilyk, A., Puzanova, Y., Sharmagiy, A., Oberemok, V., 2023b. Early Attempts at Population Control of the Two-spotted Spider Mite *Tetranychus urticae* Using Antisense Oligonucleotides. In Vitro Cellular & Developmental Biology-Animal; Springer: New York, NY, USA. 59, 92–108. doi.org/10.1007/s11626-023-00795-x.
39. Novikov, A., Yatskova, E., Bilyk, A., Puzanova, Y., Sharmagiy, A., Oberemok, V., 2023a. Efficient Control of the Obscure Mealybug *Pseudococcus viburni* with DNA Insecticides. In Vitro Cellular & Developmental Biology-Animal; Springer: New York, NY, USA. 59, 92–108. doi.org/10.1007/s11626-023-00795-x.
40. Nyadar, P.M., Oberemok, V., Omelchenko, A., Kerimova, S., Seidosmanova, E., Krasnodubiets, A., Shumskykh, M., Bekirova, V., 2019. DNA Insecticides: The Effect of Concentration on Non-Target Plant Organisms Such as Wheat (*Triticum aestivum* L.). J. Plant Prot.Res. 59, 60–68.
41. Oberemok, V.V., Gal'chinsky, N.V., Useinov, R.Z., Novikov, I.A., Puzanova, Y.V., Filatov, R.I., Kouakou, N.J., Kouame, K.F., Kra, K.D., Laikova, K.V., 2023. Four Most Pathogenic Superfamilies of Insect Pests of Suborder Sternorrhyncha: Invisible Superplunderers of Plant Vitality. Insects. 14(5), 462. doi: 10.3390/insects14050462.
42. Oberemok, V.V., Laikova, K., Shumskykh, M., Kenyo, I., Kasich, I., Deri, K., Seidosmanova, E., Krasnodubets, K., Bekirova, V., Gal’chinsky, N., 2018b. A primary attempt of *Leptinotarsa decemlineata* control using contact DNA insecticide based on short antisense oligonucleotide of its CYP6B gene. J. Plant. Prot. Res. 58, 106–108. doi: 10.24425/119124.
43. Oberemok, V.V., Laikova, K.V., Gal'chinsky, N.V., Useinov, R.Z., Novikov, I.A., Temirova, Z.Z., Shumskykh, M.N., Krasnodubets, A.M., Repetskaya, A.I., Dyadichev, V.V., Fomochkina, I.I., Bessalova, E.Y., Makalish, T.P., Gninenko, Y.I., Kubyshkin, A.V., 2019. DNA insecticide

developed from the *Lymantria dispar* 5.8S ribosomal RNA gene provides a novel biotechnology for plant protection. Sci Rep. 9(1), 6197. doi: 10.1038/s41598-019-42688-8.
44. Oberemok, V.V., Laikova, K.V., Repetskaya, A.I., Kenyo, I.M., Gorlov, M.V., Kasich, I.N., Krasnodubets, A.M., Gal'chinsky, N.V., Fomochkina, I.I., Zaitsev, A.S., Bekirova, V.V., Seidosmanova, E.E., Dydik, K.I., Meshcheryakova, A.O., Nazarov, S.A., Smagliy, N.N., Chelengerova, E.L., Kulanova, A.A., Deri, K., Subbotkin, M.V., Useinov, R.Z., Shumskykh, M.N., Kubyshkin, A.V., 2018a. A Half-Century History of Applications of Antisense Oligonucleotides in Medicine, Agriculture and Forestry: We Should Continue the Journey. Molecules. 23(6), 1302. doi: 10.3390/molecules23061302.
45. Oberemok, V.V., Laikova, V.K., Zaitsev, S.A., Nyadar, M.P., Shumskykh, N.M., Gninenko, I.Y., 2015. DNA insecticides based on iap3 gene fragments of cabbage looper and gypsy moth nuclear polyhedrosis viruses show selectivity for non-target insects. Arch. Biol. Sci. 67, 785–792.
46. Oberemok, V.V., Nyadar, P., Zaytsev, O., Levchenko, N., Shiyntum, H., Omelchenko, O., 2013. Pioneer evaluation of the possible side effects of the DNA insecticides on wheat (*Triticum aestivum* L.). Int. J. Biochem. Biophys. 1, 57–63.
47. Oberemok, V.V., Useinov, R.Z., Skorokhod, O.A., Gal’chinsky, N.V., Novikov, I.A., Makalish, T.P., Yatskova, E.V., Sharmagiy, A.K., Golovkin, I.O., Gninenko, Y.I., Puzanova, Y.V., Andreeva, O.A., Alieva, E.E., Eken, E., Laikova, K.V., Plugatar, Y.V., 2022. Oligonucleotide Insecticides for Green Agriculture: Regulatory Role of Contact DNA in Plant–Insect Interactions. Int. J. Mol. Sci. 23, 15681. https://doi.org/10.3390/ijms232415681.
48. Palli, S.R., 2023. RNAi turns 25: contributions and challenges in insect science. Front. Insect Sci. 3, 1209478. doi: 10.3389/finsc.2023.1209478
49. Pallis, S., Alyokhin, A., Manley, B., Rodrigues, T., Barnes, E., Narva, K., 2023. Effects of Low Doses of a Novel dsRNA-based Biopesticide (Calantha) on the Colorado Potato Beetle. J Econ Entomol. 116(2), 456-461. doi: 10.1093/jee/toad034.
50. Parker, K.M., Barragán Borrero, V., van Leeuwen, D.M., Lever, M.A., Mateescu, B., Sander, M., 2019. Environmental Fate of RNA Interference Pesticides: Adsorption and Degradation of Double-Stranded RNA Molecules in Agricultural Soils. Environ. Sci. Technol. 53, 3027–3036.
51. Peng, Y., Wang, K., Fu, W., Sheng, C., Han, Z., 2018. Biochemical Comparison of dsRNA Degrading Nucleases in Four Different Insects. Front. Physiol. 9, 624. doi: 10.3389/fphys.2018.00624
52. Powell, M., Pyati, P., Cao, M., Bell, H., Gatehouse, J.A., Fitches, E., 2017. Insecticidal effects of dsRNA targeting the Diap1 gene in dipteran pests. Sci. Rep. 7, 1–13.
53. Puzanova, Y.V., Novikov, I.A., Bilyk, A.I., Sharmagiy, A.K., Plugatar, Y.V., Oberemok, V.V., 2023. Perfect Complementarity Mechanism for Aphid Control: Oligonucleotide Insecticide Macsan-11 Selectively Causes High Mortality Rate for *Macrosiphoniella sanborni* Gillette. Int. J. Mol. Sci. 24, 11690. https://doi.org/10.3390/ijms241411690.
54. Rank, A.P., Koch, A., 2021. Lab-to-Field Transition of RNA Spray Applications - How Far Are We? Front Plant Sci. 12, 755203. doi: 10.3389/fpls.2021.755203.
55. Rawlinson, S.M., Zhao, T., Rozario, A.M., Rootes, C.L., McMillan, P.J., Purcell, A.W., Woon, A., Marsh, G.A., Lieu, K.G., Wang, L.F., Netter, H.J., Bell, T.D.M., Stewart, C.R., Moseley, G.W., 2018. Viral regulation of host cell biology by hijacking of the nucleolar DNA-damage response. Nat Commun. 9(1), 3057. doi: 10.1038/s41467-018-05354-7.

56. Roberts, A.F., Devos, Y., Lemgo, G.N.Y., Zhou, X., 2015. Biosafety research for non-target organism risk assessment of RNAi-based GE plants. Front. Plant Sci. 6, 958. doi: 10.3389/fpls.2015.00958.
57. Salvetti, A., Greco, A., 2024. Viruses and the nucleolus: the fatal attraction. Biochim Biophys Acta. 1842(6), 840-7. doi: 10.1016/j.bbadis.2013.12.010.
58. Sharma, V.K., Sharma, R.K., Singh, S.K., 2014. Antisense oligonucleotides: modifications and clinical trials. Med. Chem. Comm. 5, 1454–1471.
59. Sparks, T.C., Storer, N., Porter, A., Slater, R., Nauen, R., 2021. Insecticide resistance management and industry: the origins and evolution of the Insecticide Resistance Action Committee (IRAC) and the mode of action classification scheme. Pest Manag Sci. 77(6), 2609-2619. doi: 10.1002/ps.6254.
60. Stephenson ML, Zamecnik PC. Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. Proc Natl Acad Sci U S A. 1978, 75 (1), 285-8. doi: 10.1073/pnas.75.1.285.
61. Svoboda, P., 2020. Key Mechanistic Principles and Considerations Concerning RNA Interference. Front. Plant Sci. 11, 1237. doi: 10.3389/fpls.2020.01237.
62. Szaflarski, W., Leśniczak-Staszak, M., Sowiński, M., Ojha, S., Aulas, A., Dave, D., Malla, S., Anderson, P., Ivanov, P., Lyons, S.M., 2022. Early rRNA processing is a stress-dependent regulatory event whose inhibition maintains nucleolar integrity. Nucleic Acids Res. 50(2), 1033-1051. doi: 10.1093/nar/gkab1231.
63. Taning, C.N.T., Arpaia, S., Christiaens, O., Dietz-Pfeilstetter, A., Jones, H., Mezzetti, B., Sabbadini, S., Sorteberg, H., Sweet, J., Ventura, V., Smagghe, G., 2020. RNA-Based Biocontrol Compounds: Current Status and Perspectives to Reach the Market. Pest Manag. Sci. 76, 841–845. doi: 10.1002/ps.5686.
64. Terenius, O., Papanicolaou, A., Garbutt, J.S., Eleftherianos, I., Huvenne, H., Kanginakudru, S., Albrechtsen, M., An, C., Aymeric, J.L., Barthel, A., Bebas, P., Bitra, K., Bravo, A., Chevalier, F., Collinge, D.P., Crava, C.M., de Maagd, R.A., Duvic, B., Erlandson, M., Faye, I., Felföldi, G., Fujiwara, H., Futahashi, R., Gandhe, A.S., Gatehouse, H.S., Gatehouse, L.N., Giebultowicz, J.M., Gómez, I., Grimmelikhuijzen, C.J., Groot, A.T., Hauser, F., Heckel, D.G., Hegedus, D.D., Hrycaj, S., Huang, L., Hull, J.J., Iatrou, K., Iga, M., Kanost, M.R., Kotwica, J., Li, C., Li, J., Liu, J., Lundmark, M., Matsumoto, S., Meyering-Vos, M., Millichap, P.J., Monteiro, A., Mrinal, N., Niimi, T., Nowara, D., Ohnishi, A., Oostra, V., Ozaki, K., Papakonstantinou, M., Popadic, A., Rajam, M.V., Saenko, S.,Simpson, R.M., Soberón, M., Strand, M.R., Tomita, S., Toprak, U., Wang, P., Wee, C.W., Whyard, S., Zhang, W., Nagaraju, J., Ffrench-Constant, R.H., Herrero, S., Gordon, K., Swevers, L., Smagghe, G., 2011. RNA interference in Lepidoptera: an overview of successful and unsuccessful studies and implications for experimental design. J. Insect Physiol. 57, 231–245.
65. Thorp, H.H., 2000. The importance of being r: greater oxidative stability of RNA compared with DNA. Chem Biol. 7(2), 33-6. doi: 10.1016/s1074-5521(00)00080-6
66. Tomoyasu, Y., Miller, C., Tomita, S., Schoppmeier, M., Grossmann, D., Bucher, G., 2008. Exploring systemic RNA interference in insects: a genome- wide survey for RNAi genes in Tribolium. Genome biology. 9(1).
67. TriLink BioTechnologies, 2023. Feasibility of Antisense Oligonucleotides as DNAInsecticides. https://www.trilinkbiotech.com/blog/feasibility-of-antisense-oligonucleotides-as-dna-insecticides/ (accessed 12 March 2024).

68. Ulrich, J., Dao, V.A., Majumdar, U., Schmitt-Engel, C., Schwirz, J., Schultheis, D., Ströhlein, N., Troelenberg, N., Grossmann, D., Richter, T., Dönitz, J., Gerischer, L., Leboulle, G., Vilcinskas, A., Stanke, M., Bucher, G., 2015. Large scale RNAi screen in *Tribolium* reveals novel target genes for pest control and the proteasome as prime target. BMC Genomics. 16, 674.
69. van den Berg, H., da Silva Bezerra, H.S., Al-Eryani, S., Chanda, E., Nagpal, B.N., Knox, T.B., Velayudhan, R., Yadav, R.S., 2021. Recent trends in global insecticide use for disease vector control and potential implications for resistance management. Sci Rep. 11(1), 23867. doi: 10.1038/s41598-021-03367-9.
70. Vilkhovoy, M., Adhikari, A., Vadhin, S., Varner, J.D., 2020. The Evolution of Cell Free Biomanufacturing. Processes. 8, 675. https://doi.org/10.3390/pr8060675.
71. Wang, W., Han, B.W., Tipping, C., Ge, D.T., Zhang, Z., Weng, Z., Zamore, P.D., 2015. Slicing and binding by Ago3 or Aub trigger Piwi-bound piRNA production by distinct mechanisms. Mol. Cell. 59, 819–30.
72. Will, C.L., Luhrmann, R., 2001. Spliceosomal UsnRNP biogenesis, structure and function. Curr Opin Cell Biol. 13, 290–301.
73. Williams, J.S., Lujan, S.A., Kunkel, T.A., 2016. Processing ribonucleotides incorporated during eukaryotic DNA replication. Nat Rev Mol Cell Biol. 17(6), 350-63. doi: 10.1038/nrm.2016.37.
74. Zaitsev, A.S., Omel'chenko, O.V., Nyadar, P.M., Oberemok, V.V., 2015. Influence of DNA oligonucleotides used as insecticides on biochemical parameters of *Quercus robur* and *Malus domestica*. Bull. Transylvania Univ. Bras. Ser. II For. Wood Ind. Agric. Food Eng. 8, 37–46.
75. Zheng, J., Xu, Y., 2023. A Review: Development of Plant Protection Methods and Advances in Pesticide Application Technology in Agro-Forestry Production. Agriculture. 13, 2165. https://doi.org/10.3390/agriculture13112165.
76. Zhu, K.Y., Palli, S.R., 2020. Mechanisms, Applications, and Challenges of Insect RNA Interference. Annu Rev Entomol. 65, 293-311. doi: 10.1146/annurev-ento-011019-025224.
77. Zotti, M., dos Santos, E.A., Cagliari, D., Christiaens, O., Taning, C.N.T., Smagghe, G., 2018. RNA interference technology in crop protection against arthropod pests, pathogens and nematodes. Pest Manag. Sci. 74, 1239–1250.