frontiers | Frontiers in Agronomy

TYPE Opinion
PUBLISHED 27 May 2024
DOI 10.3389/fagro.2024.1415314



OPEN ACCESS

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RECEIVED 11 April 2024
ACCEPTED 06 May 2024
PUBLISHED 27 May 2024

CITATION
Oberemok VV, Laikova KV and Gal'chinsky NV (2024) Contact unmodified antisense DNA (CUAD) biotechnology: list of pest species successfully targeted by oligonucleotide insecticides.
*Front. Agron.* 6:1415314.
doi: 10.3389/fagro.2024.1415314



# Contact unmodified antisense DNA (CUAD) biotechnology: list of pest species successfully targeted by oligonucleotide insecticides

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KEYWORDS

**CUAD (contact unmodified antisense DNA) biotechnology, oligonucleotide insecticides, ribosomal RNAs, rRNA hypercompensation, DNA containment mechanism, avoidance of target-site resistance, plant protection**

## Introduction

The partial possibilities of using modified antisense oligonucleotides were first found by Paul Zamechnik and Mary Stephenson in 1978 on Rous sarcoma virus (Zamecnik and Stephenson, 1978). One of the eventual mechanisms of action of antisense oligonucleotides was detected a year later when Helen Donis-Keller presented results showing that RNase H cleaves the RNA strand in RNA–DNA heteroduplexes in a site-specific manner (Donis-Keller, 1979). It took three decades for unmodified antisense oligonucleotides to be conceptually applied in the form of contact unmodified antisense DNA (CUAD) biotechnology (Oberemok, 2008) and for oligonucleotide insecticides (briefly, olinscides or DNA insecticides) to be used for plant protection (Manju et al., 2022; Gal'chinsky et al., 2024; TriLink BioTechnologies, 2024) (Figure 1). In 2008, an equal sign was put between unmodified antisense DNA oligonucleotides and contact insecticides (Oberemok, 2008). By that time, the development of phosphoramidite DNA synthesis (Hoose et al., 2023) made it possible to synthesize and test antisense DNA fragments on a large number of pests at an affordable price. Oligonucleotide insecticides were tested for the first time on the spongy moth *Lymantria dispar*. The contact application of antisense DNA oligonucleotides targeting IAP genes has shown its effectiveness on both baculovirus-free and LdMNPV-infected spongy moth caterpillars (Oberemok et al., 2016, 2017; Kumar et al., 2022).

In 2019, three important changes occurred that significantly advanced the development of CUAD biotechnology. First, rRNAs of insect pests began to be used as targets for oligonucleotide insecticides (this led to an increase in the efficiency of oligonucleotide insecticides since rRNA makes up 80% of all RNA in the cell) (Oberemok et al., 2019a). Second, the length of oligonucleotide insecticides was successfully reduced to 11

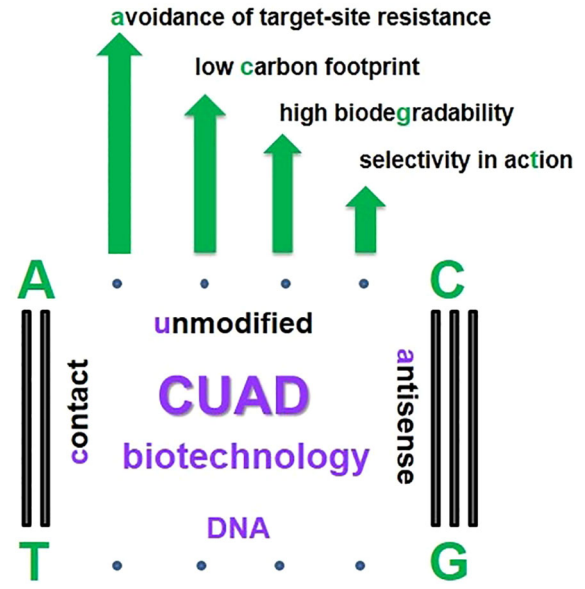


FIGURE 1
Advanced features of CUAD biotechnology.

nucleotides, providing sufficient selectivity (1/4,194,304) (Oberemok et al., 2022) in action (this helped to decrease the cost of oligonucleotide insecticides since the yield of phosphoramidite DNA synthesis for short DNA sequences is higher). Third, representatives of the suborder Sternorrhyncha, serious pests of agriculture and forestry all over the world, were found to be highly sensitive to oligonucleotide insecticides (Gal'chinsky et al., 2020; Oberemok et al., 2020; Useinov et al., 2020; Oberemok et al., 2022; Gal'chinsky et al., 2023; Oberemok et al., 2023; Puzanova et al., 2023).

In the course of research, we discovered that oligonucleotide insecticides act through the DNA containment (DNAc) mechanism, which consists of two steps. In the first step of DNAc, antisense DNA oligonucleotide (oligonucleotide insecticide) complementarily interacts with target rRNA (in other words, it "arrests" target rRNA) and interferes with the normal functioning of ribosomes ("arrested" ribosomes); this process is accompanied by substantial insect pest mortality. After that, we see target rRNA hypercompensation by the DNA-dependent RNA polymerase as the only way for the insect cell to fight for life when target rRNAs and/or polycistronic rRNA transcripts are "arrested" by antisense DNA oligonucleotides. In the second step, RNase H cleaves the target rRNA and a substantial decrease in its concentration occurs; this step is also accompanied by substantial insect pest mortality (Gal'chinsky et al., 2024; Oberemok and Gal'chinsky, 2024).

Oligonucleotide insecticides can be designed using the DNAInsector program (dnainsector.com) or using manually the sequences of pest rRNAs found in the GenBank database. Phosphoramidite solid-phase and liquid-phase methods of oligonucleotide synthesis are used to obtain olinscides. Oligonucleotide insecticides are generally dissolved in nuclease-free water, and the usual concentration is 1 mg of olinscides per 10 ml of water solution and applied per $m^2$ of plant leaves containing insect pests. We believe that contact delivery of unmodified antisense DNA (CUAD) is much more efficient (Oberemok et al., 2019b) than oral delivery of unmodified antisense DNA (ODUAD) because of active DNases present in the digestive tract of insects (Schernthaner et al., 2002; Keyel, 2017).

Oligonucleotide insecticides are applied using hand sprayers or cold fog generators. Olinscides possess high selectivity in action and safety for non-target organisms, low-carbon footprint, and rapid biodegradability and create an opportunity for elaboration of insecticides with multi-decade utility based on conservative sequences of pest ribosomal RNA genes (Oberemok et al., 2019a, 2022; Gal'chinsky et al., 2023; Puzanova et al., 2023; Gal'chinsky et al., 2024). In the case of target-site resistance, new olinscides can be easily recreated displacing the target site to the left or right from the olinscide resistance site of the target rRNA (Gal'chinsky et al., 2024).

The modern phosphoramidite method of synthesis of oligonucleotide insecticides does not lead to the accumulation of greenhouse gases such as nitrogen oxide, ozone, methane, or carbon dioxide. DNA synthesis occurs in an airless environment in an acetonitrile solution using catalysts. Compared with neonicotinoids, widely used for pest control, oligonucleotide insecticides do not have a carbon footprint although there could be a minimal amount in some cases. For example, the ratio of $tCO_2$/t for the production of neonicotinoid thiamethoxam is 0.351 (Gal'chinsky et al., 2023).

We showed that deoxyribonucleases, which are present in the cell homogenates of the spongy moth (*L. dispar* L.), Colorado potato beetle (*Leptinotarsa decemlineata* Say), cottony cushion scale (*Icerya purchasi* Maskell), and their host plants (*Quercus pubescens* Willd., *Solanum tuberosum* L., *P. tobira* Thunb.), have a high biodegradability potential for oligonucleotide insecticides and ensure their fast degradation (usually within 24 h) upon interaction with them (Oberemok et al., 2018; Oberemok et al., 2019a; Gal'chinsky et al., 2023).

The use of olinscides could solve, or at least improve, the fundamental problem of insecticide selectivity. The results of our work showed that the change of just one nucleotide at the 1st (5′-end), 6th, and 11th (3′-end) positions leads to a substantial decrease in biological efficiency of the target 11-nucleotide-long olinscides (Oberemok et al., 2019a; Puzanova et al., 2023; Gal'chinsky et al., 2024). Also many random oligos in our investigations did not cause a significant insecticidal effect on target insect pests (Oberemok et al., 2017; Gal'chinsky et al., 2020; Useinov et al., 2020; Oberemok et al., 2022). Moreover, previous studies of the effect of oligonucleotide insecticides on the biochemical parameters of the plants *Quercus robur* L., *Malus domestica* Bokh (Zaitsev et al., 2015), and *Triticum aestivum* L (Oberemok et al., 2013), and on the viability of the insects *Manduca sexta* L., *Agrotis ipsilon* Hufnagel (Oberemok et al., 2015), and *Galleria mellonella* L (Oberemok et al., 2019a), showed their safety for non-target organisms. However, we assume that non-canonical base pairing, such as A:C (C:A) and G:T (T:G) (Du et al., 2005; Luige et al., 2022), may occur between DNA olinscides and imperfect sites of rRNAs (Figure 1). Definitely, 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).

The purpose of this article is to provide a brief overview of the experiments using oligonucleotide insecticides, which will help us to show the simplicity and effectiveness of CUAD biotechnology, as well as evaluate its high potential for agronomy.

## *Lymantria dispar* (Linnaeus, 1758)

The spongy moth (formerly known as gypsy moth) *L. dispar* L. (Lepidoptera: Erebidae) is the notorious invasive polyphagous pest of the Holarctic region, infesting over 500 plant species and causing widespread loss of leaves in forests in Europe, Asia, North America, and parts of Africa (Martemyanov et al., 2019; Boukouvala et al., 2022). In Europe and North America, the preferred hosts of *L. dispar* vary by region but include the species of *Quercus*, *Salix*, *Populus*, and *Betula* (Boukouvala et al., 2022).

The oligonucleotide insecticide oligoRIBO-11 (5′-TGC-GTT-CGA-AA-3′) targeting the 5.8S rRNA at a concentration of 72 ng/μL leads to a 46.9% ± 9.3% mortality rate of the spongy moth larvae on the sixth day (Oberemok et al., 2019a).

## *Unaspis euonymi* (Comstock, 1881)

The euonymous scale *U. euonymi* Comstock (Hemiptera: Diaspididae) is the most common and serious pest found on *Euonymus* plants (Salisbury et al., 2013). It is frequently encountered in dendrological nurseries, parks, and ornamental gardens.

The oligonucleotide insecticide oligoUE-11 (5′-AGA-CCG-ACG-AC-3′) targeting the 28S rRNA at a concentration of 100 ng/μL leads to a 99.24% ± 1.32% mortality rate of the euonymous scale larvae on the 10th day (Gal'chinsky et al., 2020; Oberemok et al., 2020).

## *Dynaspidiotus britannicus* (Newstead, 1898)

The holly scale *D. britannicus* Newstead (Hemiptera: Diaspididae) is often found on the species of *Buxus*, *Hedera*, *Ilex*, and *Laurus* (Nakahara 1982) and on conifers (Ülgentürk et al., 2012; Kaydan et al., 2014) and is also a minor pest of olive trees, palms, and ornamentals.

The oligonucleotide insecticide oligoDB-11 (5′-ATA-CCG-ACG-AT-3′) targeting the 28S rRNA at a concentration of 100 ng/μL leads to an 82.44% ± 15.62% mortality rate of the holly scale larvae on the 10th day (Gal'chinsky et al., 2020).

## *Ceroplastes japonicus* (Green, 1921)

The Japanese wax scale *C. japonicus* Green (Hemiptera: Sternorrhyncha: Coccidae) is a polyphagous pest of soft and hardwood trees, fruit trees, citrus trees, and ornamentals (García Morales et al., 2016). The host plants most commonly infested by these insects are *Citrus*, *Diospyros*, *Ilex*, and *Hedera* (Pellizzari and Germain, 2010).

The oligonucleotide insecticide oligoCJ-11 (5′-CGA-CCG-ACG-AA-3′) targeting the 28S rRNA at a concentration of 100 ng/μL leads to a 78.82% ± 18.60% mortality rate of the Japanese wax scale larvae on the 10th day (Useinov et al., 2020).

## *Diaspis echinocacti* (Bouche, 1833)

The invasive scale insect *D. echinocacti* Bouché (Hemiptera: Diaspididae) is a major and specific pest of cactus pear species worldwide, recorded from 74 countries (Asia, Europe, North America, and Africa) (García Morales et al., 2016). This pest was recorded on 58 plant species in the family Cactaceae: most commonly on *Opuntia dillenii* and *O. ficus-indica* (Imane et al., 2022; Aalaoui and Sbaghi, 2023).

The oligonucleotide insecticide Cactus-NBG (5′-ATC-GCT-GCG-GA-3′) targeting the 28S rRNA at a concentration of 100 ng/μL leads to an 84.2% ± 2.2% mortality rate of *D. echinocacti* larvae on the 14th day (Plugatar et al., 2021).

## *Coccus hesperidum* (Linnaeus, 1758)

The soft scale insect *C. hesperidum* L. (Hemiptera: Coccoidae) is a cosmopolitan and polyphagous pest species (Kapranas et al., 2007; Villanueva et al., 2020) causing significant damage to citrus crops, mango, guava, and lychee (Kapranas et al., 2007). *Coccus hesperidum* may have the capacity to affect approximately 125 plant families (García Morales et al., 2016).

The oligonucleotide insecticide Coccus-11 (5′-CCA-TCT-TTC-GG-3′) targeting the 28S rRNA at a concentration of 100 ng/μL leads to a 95.59% ± 1.63% mortality rate of *C. hesperidum* larvae on the 12th day (Oberemok et al., 2022).

## *Trioza alacris* (Flor, 1861)

The bay sucker *T. alacris* Flor (Triozidae: Hemiptera) is an oligophagous pest commonly feeding on economically valuable plants from the family Lauraceae: *Laurus nobilis*, *Laurus azorica*, *Laurus novocanariensis*, *Cinnamomum camphora*, and *Laurus indicia*, particularly in Mediterranean areas but also in North and South America (Zeity, 2018).

The oligonucleotide insecticide Alacris-11 (5′-CCA-CCG-GGT-AG-3′) targeting the ITS2 of polycistronic rRNA transcript at a concentration of 100 ng/μL leads to a 71.02% ± 5.21% mortality rate of the bay sucker larvae on the ninth day (Novikov et al., 2022).

The oligonucleotide insecticide Laura-11 (5′-GAC-ACG-CGC-GC-3′) targeting the ITS2 of polycistronic rRNA transcript at a concentration of 100 ng/μL leads to a 72.39% ± 6.48% mortality rate of the bay sucker larvae on the ninth day (Novikov et al., 2022).

## *Icerya purchasi* (*Maskell*, 1878)

The cottony cushion scale *I. purchasi* Maskell (Hemiptera: Monophlebidae) is a cosmopolitan pest native to Australia and New Zealand and is known to have affected over 200 different plant species (Kollar et al., 2016). It is a pest of several ornamentals and crops, such as *Citrus reticulata*, *Artocarpus heterophyllus*, *Magnolia denudata*, and *Ficus altissima* (Liu and Shi, 2020).

The oligonucleotide insecticide oligoICER-11 (5′-ACA-CCG-ACG-AC-3′) targeting the 28S rRNA at a concentration of 100 ng/μL leads to a 70.55% ± 0.77% mortality rate of the cottony cushion scale larvae on the 10th day (Gal'chinsky et al., 2023).

## *Macrosiphoniella sanborni* (Gillette, 1908)

The chrysanthemum aphid *M. sanborni* Gillette (Hemiptera: Aphididae) is a major destructive oligophagous pest for chrysanthemums (Zhong et al., 2022).

The oligonucleotide insecticide Macsan-11 (5′-TGT-GTT-CGT-TA-3′) targeting the ITS2 of polycistronic rRNA transcript gene at a concentration of 100 ng/μL leads to a 67.15% ± 3.32% mortality rate of the chrysanthemum aphid after a single treatment and a 97.38% ± 2.49% mortality rate after a double treatment (with daily interval) on the seventh day (Puzanova et al., 2023).

## *Pseudococcus viburni* (Signoret, 1875)

The mealybug *P. viburni* Signoret (formerly known as *Pseudococcus affinis*) (Hemiptera: Pseudococcidae) is a polyphagous insect recorded from 236 host-plant genera in 89 families (da Silva et al., 2017). It is a pest of several temperate fruits, including grapes (Dapoto et al., 2011; Correa et al., 2012), apples (Ciampolini et al., 2002), and pears (Dapoto et al., 2011).

The oligonucleotide insecticide Alpha-11 (5′-GGT-CGC-GAC-GT-3′) targeting the 28S rRNA at a concentration of 100 ng/μL leads to a 63.42% ± 3.1% mortality rate of the mealybug larvae on the 14th day (Novikov et al., 2023a).

The oligonucleotide insecticide Beta-11 (5′-GGA-ATC-GAA-CC-3′) targeting the 18S rRNA at a concentration of 100 ng/μL leads to a 78.31% ± 4.5% mortality rate of the mealybug larvae on the third day (Novikov et al., 2023a).

The oligonucleotide insecticide Gamma-11 (5′-CCT-CAG-ACA-GG-3′) targeting the 5.8S rRNA at a concentration of 100 ng/μL leads to a 66.96% ± 2.9% mortality rate of the mealybug larvae on the 14th day (Novikov et al., 2023a).

## *Aonidia lauri* (Bouche, 1833)

The laurel scale *A. lauri* Bouché (Hemiptera: Diaspididae) occurs in almost all regions of the world, except Australia and the Pacific Islands (Danzig and Pellizzari, 1998). It is a monophagous species that infests *Laurus* sp. and causes serious damage in all laurel-growing areas (Miller and Davidson, 1990).

The oligonucleotide insecticide oligoAL-11 (5′-ATG-CCA-ACG-AT-3′) targeting the 28S rRNA at a concentration of 100 ng/μL leads to a 98.19% ± 3.12% mortality rate of the laurel scale larvae on the 14th day in mixed insect pest populations (Gal'chinsky et al., 2024).

## *Tetranychus urticae* (Koch, 1836), a case for oligonucleotide acaricides

The two-spotted spider mite *T. urticae* Koch (Acari: Tetranychidae) is a polyphagous pest recorded from 1,275 host plants from 70 genera representing several dozen botanical families (Migeon and Dorkeld, 2022), either wild or cultivated, including vegetables, ornamental plants, crops, fruit trees, and shrubs (El-Sayed et al., 2022).

The oligonucleotide acaricide Tur-3 (5′-AAA-ACA-TCA-AG-3′) targeting the ITS2 of polycistronic rRNA transcript at a concentration of 100 ng/μL leads to a 72.85% ± 4.55% mortality rate of the mite on the third day (Novikov et al., 2023b).

The oligonucleotide acaricide Turka (5′-AGC-GAC-GTC-GC-3′) targeting the 28S rRNA at a concentration of 100 ng/μL leads to a 77% ± 0.4% mortality rate of the mite on the third day (Novikov et al., 2023b).

## Conclusion

For the first time in the 155-year history of deoxyribonucleic acid, we can confidently say that DNA is also a contact insecticide with unique and advanced characteristics for plant protection. The simplicity, flexibility, and effectiveness of the CUAD platform for sap-feeding pests (aphids, psyllids, soft scales, armored scales, mealybugs, etc.) are amazing. Using the unique conservative sequences of target rRNAs of insect pests will minimize the occurrence of target-site resistance in insect pests. Certain combinations of nitrogenous bases in an oligonucleotide insecticide will make it well-tailored to a single pest. The use of unmodified DNA as a natural polymer will minimize the toxicity load on ecosystems. Liquid-phase synthesis of DNA makes CUAD biotechnology very cheap already today. Obviously, more complex formulations of oligonucleotide insecticides with auxiliary substances will help enhance the effect of oligonucleotide insecticides on representatives from other orders of insects. If premarket environmental risk assessment for the approval of new active substances succeeds with oligonucleotide insecticides for plant protection, we will get a new class of insecticides with highly adaptable structure and selective mode of action.

## Author contributions

VO: Conceptualization, Funding acquisition, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. KL: Writing – original draft, Writing – review & editing. NG'c: Writing – review & editing, Writing – original draft, Supervision, Project administration.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The research results were 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.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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