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RAPID COMMUNICATION

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

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Abstract

In this short communication describing experiments carried out on the larvae of two insects, *Unaspis euonymi* Comstock (feeding on *Euonymus japonicus* Thunb.) and *Dynaspidiotus britannicus* Newstead (feeding on *Laurus nobilis* L.), we evaluate for the first time the efficiency of using DNA insecticides in the control of sap-sucking insects, including armored scale insects. Over a period of 10 days, high insect mortality was detected in both *U. euonymi* and *D. britannicus*, accompanied by a significant decrease in the concentration of target RNAs. At the same time, no visible changes were observed when the leaves of the host plants were subjected to treatment with DNA insecticides for one month. The results show the high efficiency of DNA insecticides used against hemipteran insect pests. It is noteworthy that the high efficiency of DNA insecticides and their low cost in comparison with RNA preparations provides a safe and extremely promising potential vehicle for the control of sap-sucking insects.

Keywords: antisense oligonucleotides, DNA insecticides, insect pest control, sap-sucking insects, 28S ribosomal RNA

In 2008, working with lepidopterans (primarily the gypsy moth *Lymantria dispar* L.), our research team was the first to show that antisense DNA oligonucleotides could be used as contact insecticides in crop protection (Oberemok 2008). Although on average just under half of the insecticide-treated insect pests died in our experiments, the availability, speed of action, and selectivity of antisense oligonucleotides confirmed the viability of this research vector (Oberemok *et al.* 2017; Oberemok *et al.* 2019a). Twelve years later, our research continues to confirm many aspects of the promise of developing DNA insecticides from antisense oligonucleotides. Resistance to an antisense oligonucleotide corresponding to a highly conserved region of a gene develops slowly; for this reason, it is hard to ignore the enormous possible benefits of DNA insecticides. Insecticide resistance can be slowed by 'basing' DNA insecticides on very conservative regions of functionally important genes, such as the genes encoding ribosomal RNA. This approach is of immense value and further developments in this field may lead to safer, less expensive forestry and agriculture

sustained by DNA insecticides. In both of these fields, there exists a competition between DNA insecticides and RNA preparations based on unmodified oligonucleotides. Unlike dealing with an infection or an infestation in a single organism, gaining control over insect pests requires ensuring that a large environmental area receives a significant portion of the preparation being used. The use of unmodified oligonucleotides seems to be one of the safest ways of doing this, since cells contain ubiquitous nucleases that can neutralize them. The longest lasting insecticidal effect occurs only in the case of an insect pest with the mRNA of the target gene. In our opinion, RNA preparations rank behind DNA insecticides in almost all respects, including affordability and selectivity in action (Oberemok *et al*. 2018).

In a recent paper in the Journal of Plant Protection Research discussing our work with the gypsy moth, we mentioned that we had achieved 90–100% mortality in *Unaspis euonymi* Comstock (Diaspididae) using a DNA insecticide based on an antisense fragment of 28S ribosomal RNA gene of this insect pest (Oberemok *et al*. 2019b). In this short communication, we will expand on this issue and describe our experiments with *U. euonymi* Comstock and *Dynaspidiotus britannicus* Newstead in more detail.

The euonymous scale *U. euonymi* Comstock (Hemiptera: Diaspididae) is a pest frequently encountered not only in dendrological nurseries, but also in parks and ornamental gardens. When an outbreak occurs, the management program needs to be reevaluated to prevent the future appearance of viral or harmful organisms. Euonymus is one of the most commonly planted genera of shrubs and surveys have found that up to 68% of euonymus plants are infested with euonymus scale. Feeding by euonymus scale causes leaf discoloration and abscission, stunted growth, branch dieback, and plant death. For example, the yearly replacement cost of plants that die from euonymus scale damage has been estimated as $355,568 in Massachusetts, USA alone (Driesche *et al*. 1998). Generally, armored insect pests are among the most invasive insects in the United States (Miller *et al*. 2005) and are responsible for considerable agricultural damage, estimated to cost roughly $1–2 billion USD in damage and management expenses each year (Miller and Davidson 2005). Unfortunately, the possibilities available to combat this pest are limited; one must take into consideration the plants' placement (parks, public areas, playgrounds, rest areas, isolated bushes, green fences, private gardens), the existence of few products with reduced toxicity, the lack of biological products, application difficulties, and treatment costs (Gutue *et al*. 2012; Frank 2012).

The holly scale *D. britannicus* Newstead (Hemiptera: Diaspididae) has been recorded on hosts belonging to 23 genera in 18 plant families (Davidson *et al.* 1990). It is often found on species of *Buxus*, *Hedera*, *Ilex* and *Laurus* (Nakahara 1982), on conifers (Zahradnik 1990a; Ülgentürk *et al.* 2012; Kaydan *et al.* 2014), and as a minor pest of olive trees (Argyriou 1990) and of palms and ornamentals (Zahradnik 1990b).

Initial chemical control of scale insects began with fumigation using hydrogen cyanide (HCN). Other available metallic pesticides (such as arsenates) or natural botanical pesticides, such as nicotine, rotenone, or pyrethrum are not effective against scale insects (Mangoud *et al.* 2012). Controlling scale insects with non-selective insecticides is often expensive and can take several years to produce results. Moreover, broad-spectrum contact insecticides such as pyrethroids may not be effective and could make infestations worse by killing off the scale insects' natural enemies (Raupp *et al.* 1992).

Cover sprays and residual insecticides are tactics used by landscapers and arborists to control arthropod pests on trees and shrubs in urban settings. Trees in residential landscapes that received three cover sprays annually for at least 4 years harbored a greater diversity of scale insect pests. They were also much more likely to be infested with scales than trees in landscapes treated with cover sprays for shorter periods of time (Raupp *et al*. 2001).

This experiment was performed in triplicate between September and October 2019 within the grounds of the Nikita Botanical Garden (Republic of Crimea, Yalta). We designed two 11 nt long antisense oligonucleotides (5'-AGACCGACGAC-3' – UE-11; 5'-ATACCGACGAT-3' – DB-11) from the *U. euonymi* and *D. britannicus* 28S ribosomal RNA genes, respectively, and applied them to the target plants (1 mg of DNA per m^2 of plant leaves using 100 ng · µl concentration of DNA in water solution). In the groups treated with water (control), oligoC-11 (control group – 5'-$(C)_{11}$-3'), and UE-11, we observed larval deaths of 19.99, 30.56, 58.93%; 25.9, 35.1, 93.4%; and 26.86, 33.16, 99.24%, respectively, on the 4th, 7th, and 10th days after treatment (UE-11 vs. control: $\chi^2 = 2327.865$, $p < 0.001$, $N = 6380$, $df = 1$; $\chi^2 = 1180.5$, $p < 0.001$, $N = 2506$, $df = 1$; $\chi 2 = 2141.816$, $p < 0.001$, $N = 5113$, $df = 1$) (Table 1 – Group A). In the groups treated with water, oligoC-11, and DB-11, we observed larval deaths of 24.45, 31.74, 51.36%; 27.8, 37.3, 79.1%; and 25.81, 35.01. 82.44%, respectively, on the 4th, 7th, and 10th days after treatment (DB-11 vs. control: $\chi^2 = 137.415$, $p < 0.001$, $N = 1774$, $df = 1$; $\chi^2 = 407.1$, $p < 0.001$, $N = 1589$, df = 1; $\chi^2 = 478.511$, $p < 0.001$, N = 1751, $df = 1$) (Table 1 – Group B).

Total RNA was isolated from *U. euonymi* and *D. britannicus* larvae using ExtractRNA Reagent (Evrogen, Russia) according to the manufacturer's instructions. To produce the replicates for each treatment, three independent extractions were carried out. The quality

Table 1. Mortality of *Unaspis euonymi* and *Dynaspidiotus britannicus* larvae (shown as a percentage)

Group A: *Unaspis euonymi*			
Day	Control	Poly C	UE-11
4th	19.99 ± 7.08	30.56 ± 7.18	58.93 ± 30.98*
7th	24.93 ± 3.87	28.78 ± 7.36	83.37 ± 14.96*
10th	26.86 ± 4.07	33.16 ± 1.17	99.24 ± 1.32*
Group B: *Dynaspidiotus britannicus*			
Day	Control	Poly C	DB-11
4th	24.45 ± 4.14	31.74 ± 3.71	51.36 ± 20.92*
7th	27.13 ± 3.60	31.40 ± 4.17	62.63 ± 23.03*
10th	25.81 ± 4.34	35.01 ± 3.88	82.44 ± 15.62*

*significant difference for $p < 0.001$

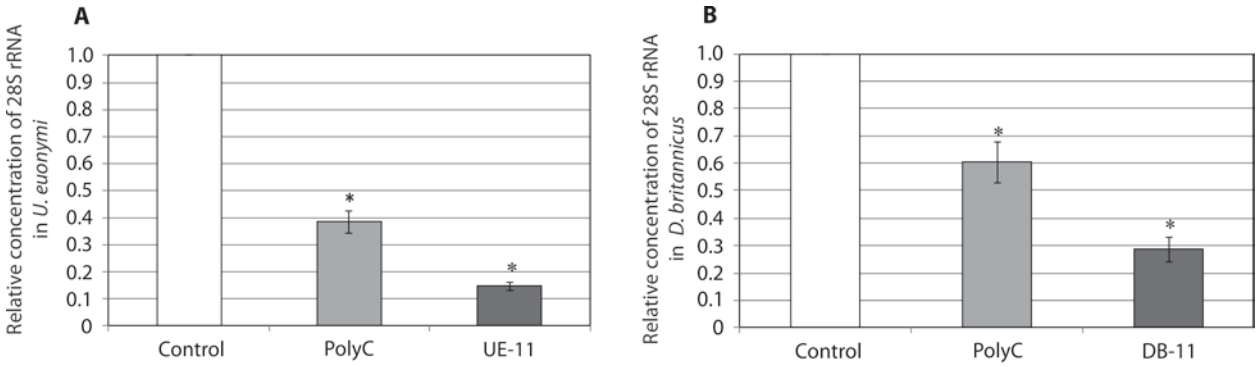

Fig. 1. Relative concentration of 28S rRNA in *U. euonymi* 7 (A) and *D. britannicus* (B) 10 days after treatment with the oligoDNAs. Data represent the means and standard errors of ribosomal RNA concentrations for 3 replicates relative to the control (water-treated) group. Values for the control equal 1 (100%)

of the extracted total RNA was assessed by loading 5 µl of the eluted volume onto a 1.8% agarose gel and running the gel in TBE (Tris-borate-EDTA) buffer (10 V/cm) for 40 min. The quantity, intensity, and pattern of RNA bands were equal in all experimental groups, confirming the quality and reproducibility of RNA extraction from the insect material. For reverse transcription, the total RNA of *U. euonymi* (0.5 µg) and *D. britannicus* (0.02 µg) was annealed with UNASPIS-R primer (5'-GGTACCAACGTGCACG-3') and BRITAIN-R primer (5'-ACGACTGTCCGCAT CAGC-3') using a MMLV Reverse Transcriptase kit (Evrogen, Russia) according to the manufacturer's instructions. The cDNA of the insect pests and following primers, forward 5'-GTCTCAATGGCTCGAC-3' and reverse 5'-GGTACCAACGTGCACG-3' for *U. euonymi,* and forward 5'-GCGAAACCCGTACATGTC-3' and reverse 5'-ACGACTGTCCGCATCAGC-3' for *D. britannicus*, were used for quantitative real time PCR studies and amplification with gene specific primers to quantify the *U. euonymi* and *D. britannicus* 28S rRNA. 28S and 5.8S rRNAs constitute about 85–90% of total cellular RNA, and are very useful as internal controls (Paule and White 2000). The concentration of the 28S rRNA in the UE-11-treated insects was significantly lower (6.82 fold) than that of the controls (water-treated) (Fig. 1A) ($p < 0.001$). Similarly, the concentration of the 28S ribosomal RNA in DB-11-treated insects was significantly lower (3.51 fold) than that of the controls (water-treated) (Fig. 1B) ($p < 0.001$). We also detected a significant decrease in the concentration of 28S rRNA in both PolyC groups (by 2.61 and 1.63 fold for *U. euonymi* and *D. britannicus*, respectively). Taking into consideration the slightly increased mortality rates of larvae in the PolyC groups, we believe that PolyC may non-specifically regulate the concentration of 28S rRNA. While the oligonucleotides caused many larvae to die, no visible changes to the leaves of *E. japonicus* and *L. nobilis* were observed during the month that the plants were subjected to treatment with DNA insecticides.

Plant health, water solubility, and the location of the scale insect on the plant and its feeding activity, along with other environmental influences, will influence whether a lethal dose of insecticide is acquired or not. Armored scales feed on parenchymal cells or vascular bundle tissue through a stylet bundle (Juárez-

-Hernández *et al.* 2014). This intracellular method of feeding may make armored scales relatively less susceptible to systemic insecticides that preferentially accumulate in the phloem, unlike soft scales, aphids, and other pests that feed on the phloem (Xiao *et al.* 2016). As a promising alternative, following certification, DNA insecticides will occupy a niche for well-tailored and affordable preparations against scale insect pests, including armored scales, on the current plant protection product market.

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