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RAPID COMMUNICATION

A primary attempt of *Leptinotarsa decemlineata* control using contact DNA insecticide based on short antisense oligonucleotide of its CYP6B gene

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Abstract

Effective control of *Leptinotarsa decemlineata* remains an urgent problem for agriculture worldwide. Minimization of the use of non-selective neonicotinoid insecticides, such as thiomethoxam, is an actual vector of development of potato cultivation. In this rapid communication, we show the prospect of the topical use of short unmodified antisense fragment of *L. decemlineata* CYP6B gene as a DNA insecticide. Investigated parameters, namely, number of larvae per plant, aboveground biomass, yield and number of potatoes produced per plant indicate the possibility of this post-genomic approach as a safe and effective method of *L. decemlineata* control.

Key words: antisense oligonucleotide, cytochrome P450 monooxygenase gene, DNA insecticide, *Leptinotarsa decemlineata*, neonicotinoid insecticides, potato

Neonicotinoid insecticides have been the most common management tool for Colorado potato beetle, [*Leptinotarsa decemlineata* (Say)], infestations in cultivated potato for nearly 20 years (Huseth *et al.* 2014). Although the adoption of neonicotinoid insecticides has been largely beneficial to the potato industry by reducing the use of broad-spectrum insecticides (e.g. carbamates, pyrethroids, and organophosphates), the emergence of insecticide resistance to virtually every insecticide that has ever been used against *L. decemlineata* (Alyokhin *et al.* 2007) and possible non-target impacts (e.g. toxicity to pollinators and groundwater contamination) threaten the long-term sustainability of these compounds (Goulson 2013; Huseth and Groves 2014).

As an alternative, the use of unmodified nucleic acids as insecticides looks very promising, since they can work selectively, are subject to fast biodegradation in ecosystems (in contrast to the majority of conventional chemical insecticides), and the commercial synthesis of nucleic acids *in vitro* is becoming more affordable (Oberemok and Skorokhod 2014; Oberemok *et al.* 2017a). According to data from our most recent research, unmodified antisense DNA oligonucleotide (5'-CGA CGT GGT GGC ACG GCG-3') from RING (really interesting new gene) domain of the LdMNPV

Table 1. Results of the field experiment

Group A: Parameter	After	Control^{N}	oligoNorm^{N}	oligoCYP6B^{N}
Number of larvae per plant	2 weeks	41.8 ± 9.9	56.8 ± 17.5	43.2 ± 15.8
Aboveground biomass per plant [g]	2 weeks	186.8 ± 33.9	178.1 ± 16.2	174.3 ± 30.8
Yield per plant [g]	5 weeks	318.1 ± 21.3	298.5 ± 22.2	328.6 ± 31.5
Number of potatoes per plant	5 weeks	6.1 ± 0.5	6.4 ± 0.4	6.6 ± 0.5
Group B: Parameter	After	Control^{T}	oligoNorm^{T}	oligoCYP6B^{T}
Number of larvae per plant	2 weeks	74.1 ± 10.6	68.3 ± 12.7	46.8 ± 10.2*
Aboveground biomass per plant [g]	2 weeks	148.5 ± 19.2	154.8 ± 22.3	171.8 ± 21.5
Yield per plant [g]	5 weeks	304.1 ± 27.2	291.8 ± 32.3	378.3 ± 34.3*
Number of potatoes per plant	5 weeks	6.6 ± 0.4	5.7 ± 0.5	7.9 ± 0.7

Group A: ^{N} – groups treated with water (control) or oligoNorm or oligoCYP6B; scheme of the experiment: at start, after 1, 2 and 3 weeks – treatment with water (control) or oligoNorm or oligoCYP6B, after 4 weeks – pause, after 5 weeks – harvesting. Group B: ^{T} – groups treated with thiamethoxam + water (control) or oligoNorm or oligoCYP6B; scheme of the experiment: at start and after 2 weeks – treatment with thiamethoxam, after 1 week and 3 weeks – water (control) or oligoNorm or oligoCYP6B, after 4 weeks – pause, after 5 weeks – harvesting. Treatment with oligoNorm (5 nmol · ml^{-1}) or oligoCYP6B (5 nmol · ml^{-1}) – 24 ml per 16 plants of one replicate in each group; the experiment for each of three groups was repeated in triplicate. Treatment with thiamethoxam (50 nmol · ml^{-1}) – 24 ml per 16 plants of one replicate in each group; the experiment for each of three thiomethoxam-treated groups was repeated in triplicate. Means and standard errors are indicated in the table; *is marked when $p < 0.05$ (Mann-Whitney test)

(*Lymantria dispar* multiple nucleopolyhedrovirus) IAP-3 (inhibitor-of-apoptosis) gene has pronounced insecticidal effects on LdMNPV-free (Oberemok *et al.* 2016a; Oberemok *et al.* 2016b) and LdMNPV-infected gypsy moth larvae (Oberemok *et al.* 2017a; Oberemok *et al.* 2017b). Although the exact mechanism of action of DNA insecticides is currently under study, we have a lot of evidence that DNA insecticides work in a manner similar to unmodified (Dias and Stein 2002) and modified antisense oligonucleotides (Toth 2011) used in medicine, generating antisense effects through RNase H-dependent mechanism (Schultz and Champoux 2008; Mayr *et al.* 2017). Proceeding from this, we decided to apply this approach to another serious insect pest, the Colorado potato beetle in summer 2017. As a DNA insecticide, antisense oligonucleotide, 5'-TGA GAA TAC TAA CGA GA-3', from CYP6BJ1v1 (cytochrome P450 monooxygenase) gene was used (https://blast.ncbi.nlm.nih.gov/Blast.cgi). CYP6B enzymes are considered to be one of the key factors that allow insects to adapt to the poisonous host plant (Cohen *et al.* 1992) and to the use of neonicotinoids (Zhu *et al.* 2016). Accordingly, the antisense oligoCYP6B fragment may induce the degradation of target mRNA through a RNase H-dependent mechanism and lead to intoxication, dystrophy and subsequent death of the insect pest in agrocenoses.

A water solution with a single-stranded oligoDNA fragment (either oligoCYP6B or control oligoNorm sequence; 5'-ACGT ACGT ACGT ACGT A-3') was applied to larvae (found on potato plants) topically using a hand-held sprayer. The DNA oligonucleotides were used both without (group A) and with thiamethoxam (Syngenta, Switzerland) (group B). In group A, investigated parameters, namely, number of larvae per plant, aboveground biomass (stems, branches and leaves), yield and number of potatoes produced per plant did not have a statistical difference in oligoCYP6B^{N} cohort compared to control (Table 1). We studied the activity of intracellular nucleases of the Colorado potato beetle against oligoCYP6B and found that it is almost completely degraded after 1 hour at 27°C. As a control, the homogenate of tissues of gypsy moth larvae was used and its intracellular nucleases did not degrade oligoCYP6B noticeably (Fig. 1). Thus, the high nuclease activity of Colorado potato beetle larvae does not allow the effective use of the oligoCYP6B insecticide.

On the contrary, in group B (Table 1), a significant reduction of viability of the insect pest larvae by 36.9% resulted in increased aboveground biomass by 15.7%, which subsequently, after 3 weeks, led to significantly higher yields in the oligoCYP6B^{T} group by 24.4% compared to control. The high activity of intracellular nucleases did not prevent the DNA insecticide from acting when thiomethoxam was added to the scheme of treatments. Of note, in this experiment for thiomethoxam treatments we used 0.5 mg · m^{-2} which is lower than the standard of 1.2–15.6 mg · m^{-2} for neonicotinoids (Huseth *et al.* 2014), including thiomethoxam, thus, reducing the environmental load of the substance and increasing the yield with the help of oligoCYP6 insecticide. Use of control oligoNorm sequence did not lead to a significant decrease or increase of any investigated parameter compared to control.

This is the first report on *L. decemlineata* control using contact DNA insecticide based on short antisense oligonucleotide of its CYP6B gene. Further studies will be required for identification of the exact mechanism of its action and improvement of the DNA insecticide formula in order to control *L. decemlineata* alone, without application of neonicotinoids. Interestingly,

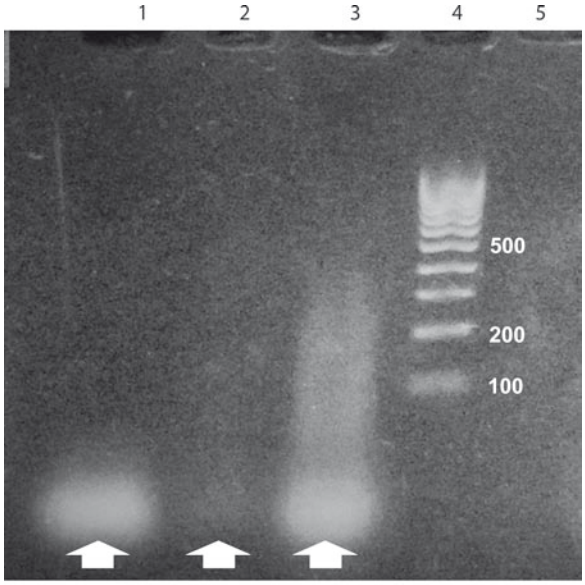

Fig. 1. Electrophoregram (1.8% agarose gel) representing activity of intracellular nucleases of *Leptinotarsa decemlineata* and *L. dispar* for 60 min at 27°C: 1 – control (10 µl oligoCYP6B at a concentration of 100 pmol · µl^{-1}); 2 – tissue homogenate of *L. decemlineata* (1.5 mg of biomass per 10 µl of TE buffer, pH 7.4) + 10 µl oligoCYP6B at a concentration of 100 pmol · µl^{-1}; 3 – tissue homogenate of *L. dispar* (1.5 mg of biomass per 10 µl of TE buffer, pH 7.4) + 10 µl oligoCYP6B at a concentration of 100 pmol · µl^{-1}; 4 – DNA ladder (100 kb); 5 – tissue homogenates of *L. decemlineata* (1.5 mg of biomass per 10 µl of TE buffer, pH 7.4) and *L. dispar* (1.5 mg of biomass per 10 µl of TE buffer, pH 7.4) without treatments

our calculations suggest that already today in Russia the cost of preparation for triplicate treatment of 1 ha of potato plants with the DNA insecticide (oligoCYP6B) is comparable to conventional triplicate treatment with thiomethoxam, 59.8 euro vs. 65.6 euro respectively. This suggests that the use of DNA insecticides is economically justified and just around the corner if they show high effectiveness in large-scale field experiments.

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