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

<table>
<thead>
<tr>
<th>Group A: Parameter</th>
<th>After</th>
<th>Controla</th>
<th>oligoNormb</th>
<th>oligoCYP6Ba</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of larvae per plant</td>
<td>2 weeks</td>
<td>41.8 ± 9.9</td>
<td>56.8 ± 17.5</td>
<td>43.2 ± 15.8</td>
</tr>
<tr>
<td>Aboveground biomass per plant [g]</td>
<td>2 weeks</td>
<td>186.8 ± 33.9</td>
<td>178.1 ± 16.2</td>
<td>174.3 ± 30.8</td>
</tr>
<tr>
<td>Yield per plant [g]</td>
<td>5 weeks</td>
<td>318.1 ± 21.3</td>
<td>298.5 ± 22.2</td>
<td>328.6 ± 31.5</td>
</tr>
<tr>
<td>Number of potatoes per plant</td>
<td>5 weeks</td>
<td>6.1 ± 0.5</td>
<td>6.4 ± 0.4</td>
<td>6.6 ± 0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group B: Parameter</th>
<th>After</th>
<th>Controlc</th>
<th>oligoNormd</th>
<th>oligoCYP6Bd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of larvae per plant</td>
<td>2 weeks</td>
<td>74.1 ± 10.6</td>
<td>68.3 ± 12.7</td>
<td>46.8 ± 10.2*</td>
</tr>
<tr>
<td>Aboveground biomass per plant [g]</td>
<td>2 weeks</td>
<td>148.5 ± 19.2</td>
<td>154.8 ± 22.3</td>
<td>171.8 ± 21.5</td>
</tr>
<tr>
<td>Yield per plant [g]</td>
<td>5 weeks</td>
<td>304.1 ± 27.2</td>
<td>291.8 ± 32.3</td>
<td>378.3 ± 34.3*</td>
</tr>
<tr>
<td>Number of potatoes per plant</td>
<td>5 weeks</td>
<td>6.6 ± 0.4</td>
<td>5.7 ± 0.5</td>
<td>7.9 ± 0.7</td>
</tr>
</tbody>
</table>

Group A: – 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: – 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 Chamboux 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 agroecoses.

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 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 group by 24.4% compared to control. The high activity of intracellular nucleases did not prevent the DNA insecticide from acting when thiamethoxam was added to the scheme of treatments. Of note, in this experiment for thiamethoxam 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 thiamethoxam, which subsequently, after 3 weeks, led to significantly higher yields in the oligoCYP6B group by 24.4% compared to control. 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,
L. decemlineata
(1.5 mg of biomass per 10 μl of TE buffer, pH 7.4)

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Acknowledgements

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
thiometoxam, 59.8 euro vs. 65.6 euro respectively. This
suggests that the use of DNA insecticides is economi-
cally justified and just around the corner if they show
high effectiveness in large-scale field experiments.

References

Alyokhin A., Dively G., Patterson M., Castaldo C., Rogers D.,
to imidacloprid and thiamethoxam in the Colorado
potato beetle. Pest Management Science 63: 40–51. DOI:
https://doi.org/10.1002/ps.1865

Goulson D. 2013. An overview of the environmental risks posed
by neonicotinoid insecticides. Journal of Applied Ecology
50 (4): 977–987. DOI: https://doi.org/10.1111/1365-
2664.12111

Huseth A.S., Groves R.L. 2014. Environmetal fate of soil ap-
plied neonicotinoid insecticides in an irrigated agroecosys-
tem. PLoS ONE 9 (5): e97081. DOI: https://doi.org/10.1371/
journal.pone.0097081

Huseth A.S., Groves R.L., Chapman S.A., Alyokhin A., Kuhar T.P.,
Mayr L., Grijalvo S., Bachl J., Pons R., Eritja R., Diaz Diaz D.
2017. Transfection of antisense oligonucleotides mediated
by cationic vesicles based on non-ionic surfactant and polyc-
ations bearing quaternary ammonium moieties. Interna-
tional Journal of Molecular Sciences 18 (12): 1139. DOI:
https://doi.org/10.3390/ijms18061139

Oberemok V.V., Skorokhod O.A. 2014. Single-stranded DNA
fragments of insect-specific nuclear polyhedrosis vi-
rus act as selective DNA insecticides for gypsy moth
control. Pesticide Biochemistry and Physiology 113: 1–7. DOI:
https://doi.org/10.1016/j.pestbp.2014.05.005

Oberemok V.V., Laikova K.V., Zaitsev A.S., Gushchin V.A., Skor-
okhod O.A. 2016a. The RING for gypsy moth control: Top-
ical application of fragment of its nuclear polyhedrosis virus
anti-apoptosis gene as insecticide. Pesticide Biochemistry
pestbp.2016.01.006

Oberemok V.V., Laikova K.V., Zaitsev A.S., Gushchin V.A., Skorokhod O.A. 2016b. Data for increase of Ly-
mantria dispar male survival after topical application
of single-stranded RING domain fragment of IAP-3 gene
of its nuclear polyhedrosis virus. Data in Brief 7: 514–517. DOI: https://doi.org/10.1016/j.dib.2016.03.007

Oberemok V.V., Laikova K.V., Zaitsev A.S., Shumskyy M.N.,
Kasich I.N., Galchinsky N.V., Bekerova V.V., Makarov V.V.,
Agranovsky A.A., Gushchin V.A., Zubarev L.V., Kuby-
shkin A.V., Fomochkina I.I., Gorlov M.V., Skorokhod
O.A. 2017a. Molecular alliance of Lymantria dispar mul-
tiple nucleopolyhedrovirus and a short unmodified an-
tisense oligonucleotide of its anti-apoptotic IAP-3 gene:
a novel approach for gypsy moth control. International
Journal of Molecular Sciences 18 (12): 2446. DOI: https:
//doi.org/10.3390/ijms18122446

Oberemok V.V., Laikova K.V., Zaitsev A.S., Nyadar P.M.,
Gninenko Yu. I., Gushchin V.A., Makarov V.V., Agra-
nozvsky A.A. 2017b. Topical treatment of LdMNPV-infected
gypsy moth caterpillars with 18 nucleotides long antisense
fragment from LdMNPV IAP-3 gene triggers higher level
of apoptosis in the infected cells and mortality of the pest.
Journal of Plant Protection Research 57 (1): 18–24. DOI:
https://doi.org/10.1515/jppr-2017-0003

Schultz S.J., Champoux J.J. 2008. RNase H activity: Structure,
specificity, and function in reverse transcription. Virus Re-
viruses.2007.12.007

Toth R.P. 2011. Antisense therapy and emerging applications
for the management of dyslipidemia. Journal of Clinical
lipid.2011.08.007

herbivore pest adaptation to xenobiotics through up-regu-
lation of Multiple Cytochrome P450s. Scientific Reports
6 (1): 20421. DOI: https://doi.org/10.1038/srep20421

Dias N., Stein C.A. 2002. Antisense oligonucleotides: Basic
concepts and mechanisms. Molecular Cancer Therapeutics

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