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THE SELECTIVITY OF DNA INSECTICIDES

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Abstract - Single-stranded LdMNPV iap3 gene fragments on tobacco hornworm and black cutworm, and a significant effect of single-stranded TnSNPV iap3 gene fragments on the viability of cabbage looper and their harmlessness on black cutworm was found. DNA insecticides based on LdMNPV iap3 and TnSNPV iap3 gene fragments are selective in action. Our findings emphasize the importance of appropriate concentrations of DNA insecticides used to control phyllophagous insects.

Key words: DNA insecticides; IAP genes; selectivity of pesticides; insect pest regulation

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INTRODUCTION

To preserve the balance in the environment, the selectivity and safety of pesticides has to be checked prior to their application. At the same time, about half of the crops produced in the world today are destroyed in some way by pests, with insects assuming a leading role. This dilemma presents a difficult situation for farmers who have to choose whether to make a profit and disturb an ecosystem with effective but unselective pesticides or to protect the environment with less effective but selective pesticides, paying more for less benefit.

More than 1 200 pesticides manufactured and applied today still lead to a tremendous loss of approximately 1.8 billion tons of crops each year and can hardly be counted as the solution for this problem. Generally, this situation is explained by pesticide resistance, which makes it necessary to find new types of pesticides. With the introduction of every new insecticide class – cyclodienes, carbamates, formamidines, organophosphates, pyrethroids, even *Bacillus thuringiensis* – cases of resistance appear within 2 to 20 years (Daly et al., 1998). Of course, we must not underestimate the use of many pesticides that have led to the substantial protection of crops, many of which are self-supporting and their application justified economically. Usually there is a positive correlation between the use of pesticides and the productivity of land with the increased mutagenic, carcinogenic and toxic impact on ecosystems. The challenge is to find a better pesticide or group of pesticides possessing more selective, effective features than the existing preparations.

There are three main groups of pesticides manufactured and applied today: non-organic compounds (compounds of sulfur, copper, fluorine etc.), organic compounds (DDT, dichlorvos, permethrin, etc.) and biological preparations (baculoviruses, fungi, bacteria, etc.). Biological preparations are the most selective in action and probably carry the lowest level of side effects on the surrounding environ-

ment. Viruses, bacteria, fungi, etc. usually have a significant effect on 1-2 organisms and as a rule this effect may spread to a narrow range of organisms that belong to the same genus or family, whereas chemical pesticides (non-organic or organic) can effectively kill phylogenetically distant organisms, for example mammals and insects. One of the principle reasons why biological preparations have not found wide practical application is because their production is based on the cultivation of a large number of host organisms (Moscardi, 1999; Inceoglu et al., 2006). This technology is time- and labor-intensive, factors that make it expensive to produce the quantity of viruses or bacteria required for controlling pest populations. For comparison, just 1 g of pyrethroid deltamethrin, which belongs to the group of chemical pesticides, is able to kill 20 tons of cockroaches (Tkachev, 2004). No biological preparation can provide the same efficiency today. Beyond the economic cost of production, biological preparations take longer to kill their target than chemical pesticides. For the group of phyllophagous insects, an alternative to this trend may be DNA insecticides, a technology based on the application of single-stranded viral DNA fragments possessing insecticidal activity (Oberemok, 2008, 2009, 2011; Simchuk et al., 2012). The creation of effective biological preparations based on small fragments of DNA is promising due to the mode of action of viral DNA in the host cells, the large variability and specificity of the sequences, and relatively high chemical stability. Exogenously introducing single-stranded DNA fragments of a virus that coincide with genes of a host cell should influence its biochemical activity in a manner similar to antisense molecules (Weiss et al., 1999; Dias and Stein, 2002; Lu et al., 2004) and by mechanisms that resemble those of DNA interference (Kawai-Toyooka et al., 2004) and RNA interference (Fire et al., 1998). For example, if we block anti-apoptosis genes we cause apoptosis. Thus, it is possible to create fast-acting, safe and relatively inexpensive DNA insecticides to control phyllophagous insect populations, and our recent studies with gypsy moth show this (Oberemok 2008, 2009, 2011; Simchuk et al., 2012; Oberemok and Gninenko, 2012).

Since every species has its own unique DNA sequence it seems possible to create the most effective and selective DNA insecticides that will manifest the highest effect on a target organism and be harmless to other members of an ecosystem. This research paper is devoted to the investigation of the influence of LdMNPV iap3 gene fragments on tobacco hornworm and black cutworm and TnSNPV iap3 gene fragments on the viability of cabbage looper and black cutworm in order to evaluate the selectivity of the DNA insecticides.

MATERIALS AND METHODS

Two DNA fragments each from relatively conserved baculovirus inhibitor of apoptosis repeat (BIR) (sense chain) and a highly conserved new gene (RING) (antisense chain) domains of the anti-apoptotic gene iap3 of nuclear polyhedrosis viruses (NPV) of gypsy moth (*Lymantria dispar* (Lepidoptera: Erebidae)) – LdMNPV, and cabbage looper (*Trichoplusia ni* (Lepidoptera: Noctuidae)) – TnSNPV, were chosen for the experiments. Both domains have significant similarities, especially in the RING domain, with analogous fragments of anti-apoptotic genes of other known nucleopolyhedroviruses. We designed DNA fragments according to the viral genome sequence found in ICTVdb and synthesized them by Eurofins MWG Operon (Germany) with HPSF clearance. The sequences of the applied DNA fragments of the LdMNPV iap3 gene were: a) 5'- GCC GGC GGA ACT GGC CCA -3' (BIR domain); b) 5'-CGA CGT GGT GGC ACG GCG-3' (RING domain) (Kuzio et al., 1999). The sequences of the applied DNA fragments of the TnSNPV iap3 gene were: a) 5'-ACC CAT AGA GTT GGC AAT-3' (BIR domain); b) 5'-CGA CAT GAC CGC AAG GTA-3' (RING domain) (Willis et al., 2005). Each of the iap3 gene fragments tested consists of 18 nucleotides and were diluted in distilled water to a concentration of 100 pmol/μl. Caterpillars were treated either by 0.3 μl/caterpillar (tobacco hornworm and black cutworm) or were immersed into a solution containing DNA fragments for 5 min (cabbage looper). Caterpillars from the control groups were treated with distilled water and polyA oligonucleotide with the following

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Table 1. Percentage of surviving caterpillars of tobacco hornworm and black cutworm after 11-day period in different groups of control and experiment

	Control	polyA	BIR+RING
Tobacco hornworm	81.7	78.3	91.7
Black cutworm	73.3	73.3	70

Table 2. Percentage of surviving caterpillars of black cutworm and cabbage looper after 7-day period in different groups of control and experiment

	Control	polyA	BIR+RING
Black cutworm	90	96.7	[illegible]3
Cabbage looper (10 pmol/μl)	76.7	80	83
Cabbage looper (100 pmol/μl)	76.7	73.3	[illegible]3*

*P<0.05

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sequence: 5'-AAA AAA AAA AAA AAA AAA-3' with the concentration of 100 pmol/μl. Insects were grown on standard wheat germ-based forage. The larvae of tobacco hornworm (*Manduca sexta* (Lepidoptera: Sphingidae), black cutworm (*Agrotis ipsilon* (Lepidoptera: Noctuidae)) and cabbage looper (*Trichoplusia ni,* (Lepidoptera: Noctuidae)) were reared in laboratory conditions. Second-instar tobacco hornworm, black cutworm and cabbage looper larvae from different egg masses were randomized and used for the experiments. Each variant of experiment was performed in three replicates with 20 individuals of caterpillars per each variant.

Pearson's chi-square test (χ^2) with Yates's Correction was used to evaluate the insecticidal effect of viral IAP-3 gene fragments.

RESULTS

Experiments with LdMNPV iap3 gene fragments on tobacco hornworm and black cutworm

Results show that single-stranded fragments of LdMNPV iap3 gene did not have a significant effect on the viability of tobacco hornworm and black cutworm second-instar caterpillars after 11 days in comparison with the control, emphasizing selectivity of DNA insecticides (Table 1). First- and second-instar gypsy moth caterpillars were significantly affected during the same period by the smaller dose of DNA insecticides based on the same fragments of BIR and RING domains of the LdMNPV iap3 gene (Oberemok 2008, 2009, 2011; Simchuk et al., 2012). Gypsy moth caterpillars have comparatively bigger average body weight than the tobacco hornworm have (8 to 6 mg respectively) and approximately 4 times bigger body weight in comparison with black cutworm at this stage of development. This implies that DNA insecticides based on fragments of BIR and RING domains of the LdMNPV iap3 gene have a reliable margin of safety in action and are harmless to non-target insects.

Experiments with TnSNPV iap3 gene fragments on cabbage looper and black cutworm

Experiments with single-stranded fragments of BIR and RING domains of TnSNPV iap3 gene revealed different susceptibility of cabbage looper and black cutworm to the applied DNA insecticides (Table 2).

Black cutworm was resistant whereas cabbage looper was significantly susceptible (χ^2=5.625; P<0.05) to TnSNPV iap3 fragments at the concentration of 100 pmol/μl. The highest mortality of caterpillars was detected on the first day of the experiment in all groups, reaching the highest death rate in the "BIR+RING" group (Fig. 1). This indicates that immersion of caterpillars into water solution some-

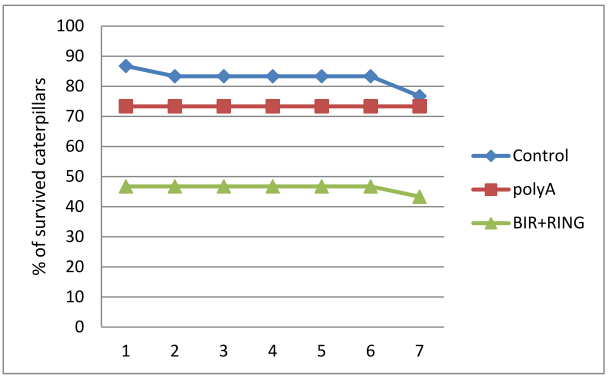

Fig. 1. Dynamics of mortality of cabbage looper caterpillars during a 7-day period in different groups of control and experiment (the concentration of DNA fragments was 100 pmol/μl)

how increases their mortality, probably by blocking normal respiration. Since 2nd instar cabbage looper caterpillars are very small, it is impossible to treat them with 0.3 μl drops containing DNA fragments. Thus, we cannot claim that the same dose of DNA fragments penetrated into the organisms of cabbage looper and black cutworm. Therefore, we should talk more about the trend rather than the fact of selectivity of TnSNPV iap3 fragments. PolyA fragments at concentration 100 pmol/μl did not have significant effect on cabbage looper in comparison with the control, thereby emphasizing that only specific DNA fragments can cause significantly elevated mortality of the insect.

The concentration of 10 pmol/μl of TnSNPV iap3 gene fragments was observed to be harmless for cabbage looper caterpillars, indicating the importance of the appropriate concentrations of DNA insecticides that could be used to control the quantity of phyllophagous insects.

DISCUSSION

This research has revealed that DNA insecticides have selective action. Applying molecular mechanisms such as DNA interference, RNA interference, antisense oligonucleotides, efficient insecticides which provide effective and safe methods of pest control because can be devised. Belles (2010) says that RNA interference itself could be envisaged as an insect control tool through targeting vital genes, although efficient systems of double-stranded RNA formulation and delivery must be developed. The approach of administering double-stranded RNAs by feeding, as seen in *E. postvittana* (Turner et al., 2006) and *R. prolixus* (Araujo et al., 2006), paves the way in this field, and the possibility of delivering the dsRNA by soaking, as in *C. elegans* (Tabara et al., 1998), should also be considered, for example, in the particularly permeable stages of aquatic insects. While the idea of DNA insecticides may resemble mechanisms characteristic of antisense oligonucleotides (Weiss et al., 1999), DNA interference (Kawai-Toyooka 2004) and RNA interference (Fire et al., 1998), it has its own peculiar features such as an external way of application, small size of oligonucleotides, single-stranded DNA molecule. The perspective of such an approach in practice is seen clearly because it provides the same effect with less effort. For example, instead of a baculovirus preparation, we could use small parts of viral genome in high concentrations and get the same effect.

Thus, DNA insecticides could provide the best characteristics of current insecticides: the speed and low cost of chemical pesticides with the safety of biological preparations. The main disadvantages of DNA insecticides are the necessity of contacting the insect cuticle and the relatively high cost of oligonucleotide production technology. Another important development in the creation of DNA is to reduce the concentration of DNA fragments in the end product through the adding of DNA carrier molecules to the preparation.

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