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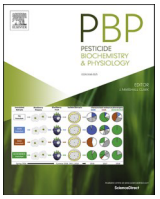

Topical delivery of dsRNA in two hemipteran species: Evaluation of RNAi specificity and non-target effects

Luca Finetti [a,*], Lorenzo Benetti [b], Jimena Leyria [a], Stefano Civolani [c], Giovanni Bernacchia [b]

[a] Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada
[b] Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy
[c] Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara, Italy.

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ABSTRACT

Double-stranded (ds) RNA-based technologies could provide novel and potential tool for pest management with efficiency and specificity of action. However, before applying this technique in the field, it is necessary to identify effective delivery methods and evaluate the non-target effects that may occur. In this article, we evaluated the effectiveness of dsRNA by topical delivery on a species of great agricultural interest, *Halyomorpha halys*. The specificity of action of the dsRNA was also investigated in *Rhodnius prolixus*, an insect phylogenetically close to *H. halys*. Of the three investigated genes (putative ATPase N2B, *ATPase*, serine/threonine-protein phosphatase PP1-β catalytic subunit, PP1, and IAP repeat-containing protein 7-B-like, *IAP*), IAP and ATPase were able to induce higher mortality in *H. halys* nymphs compared to the control, with specific concentrations for each gene targeted. However, when the same RNAs were topically delivered to both *R. prolixus* 2nd and 3rd instar nymphs, no gene silencing and mortality were observed. For this reason, to assess dsRNA application-mediated non-target effects, we injected both *H. halys* and *R. prolixus* specific dsRNA in *R. prolixus* 5th instar nymphs. When the dsRNA targeting *H. halys* IAP was microinjected into *R. prolixus* 5th instar nymphs, no mortality was observed, suggesting a strong RNAi specificity. Together, these data suggest that the topical delivery could be suitable for the dsRNA to control *H. halys* population. Furthermore, its specificity of action would allow treatments towards single harmful species with limited non-target effects.

1. Introduction

RNA interference (RNAi) technology uses an endogenous, post-transcriptional and highly conserved immune mechanism based on a double-stranded RNA molecule (dsRNA) and the highly specific degradation response against the complementary cytoplasmic mRNA (Tijsterman and Plasterk, 2004). RNAi technology is mainly used in reverse genetics studies, whereby RNAi-mediated downregulation of a gene enables the identification of its function (Pereira and Lopes-Cendes, 2013). Moreover, RNAi is applied in other fields, such as medicine (gene therapy) (Hu et al., 2020) and agriculture (RNAi-based insecticides) (Baum et al., 2007; Mao et al., 2007; Zhu and Palli, 2020).

The entire RNAi process was firstly discovered in *Caenorhabditis elegans* (Fire et al., 1998) and subsequently described in many other species, but is not yet fully characterized in insects (Singh et al., 2017). Moreover, RNAi does not always trigger the expected silencing of the target gene in insects (Cooper et al., 2019; Nitnavare et al., 2021) in particular, in some orders, such as Lepidoptera and Diptera, appear recalcitrant to RNAi-induced silencing (Terenius et al., 2011; Arraes et al., 2021). Several studies have considered the cause of this insensitivity to RNAi as being a poor expression of the core machinery genes (Swevers et al., 2011; Davis-Vogel et al., 2018; Vogel et al., 2019), but another factor that might interfere with RNAi efficiency is the delivery mechanism applied to introduce dsRNA into the organism (Zhang et al., 2013). Moreover, multiple factors might influence the RNAi efficiency. The dsRNA stability could be, indeed, affected by the action of nucleases or, in case of dsRNA delivered by feeding, by the pH present in the gut (Fan et al., 2021; Silver et al., 2021). Other factors that might play an important role in influencing the RNAi efficiency are either the poor dsRNA uptake by the cells or a defective spreading of the dsRNA throughout the whole insect. (Cooper et al., 2019). In addition, the life stage of the target insect as well as the quantity of dsRNA used might also influence the RNAi efficiency (Miller et al., 2012; Mehlhorn et al., 2021).

* Corresponding author.
E-mail address: luca.finetti@utoronto.ca (L. Finetti).

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