

The lethal effect of clathrin heavy chain silencing in *Leptinotarsa decemlineata* larvae traces a potential candidate in crop protection

Oliveira, C.F.R.¹; Cappelle, K.²; Van Eynde, B.²; Christiaens, O.²; Macedo, M.L.R.¹; Smagghe, G.²

¹Department of Food Technology, University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil, ²Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

The overwhelming insect pest resistance occurrences against the current crop protection techniques have prompted research for new pest control strategies. RNA interference (RNAi) has been proven to be a useful tool in entomology and this work presents promising results of applying this technique for the control of Colorado potato beetle (CPB) *Leptinotarsa decemlineata*. Primers were designed targeting the CPB clathrin heavy chain gene to synthesize double-stranded RNA (dsRNA) using a commercial kit. This gene has an essential role in clathrin-mediated endo- and exocytosis, which is necessary for many biological functions, such as the uptake of macromolecules and the secretion of enzymes. As negative control, we fed larvae with dsRNA targeting a GPF fragment. The bioassays were conducted with neonate larvae fed with a piece of potato leaf containing 400 ng of dsRNA. The gene expression levels were measured at different time intervals (0, 24, 48 and 72h) by qPCR. A marked reduction of clathrin expression level was observed within 24h (76%) and remained in subsequent intervals, reaching a silencing rate of 92% at 72h. At this time, the clathrin-silenced larvae showed an average weight 60% lower than control larvae and a lethal phenotype was revealed after 120 h in 100% of dsRNA clathrin fed-larvae. These data demonstrate that the impairment of viability, provoked by clathrin silencing in CPB is effective and robust, proposing further studies to apply this technology in the field, through transgenic potato plants or spraying crops.

Keywords: Clathrin, *Leptinotarsa decemlineata*, RNAi

Supported by: CNPq, Special Research Fund (BOF) of Ghent University (Belgium), the Fund for Scientific Research (FWO-Vlaanderen, Belgium)