Molecular biology applied to identify targets for controlling the sugarcane weevil, *Sphenophorus levis*

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The sugarcane weevil, *Sphenophorus levis*, is an insect that feeds on the rhizome of sugarcane in its larval stage, boring channels that cause damage and death to the plant. Conventional methods of insect control have not been efficient. The aim of the present study was to generate knowledge on the biology and physiology of this insect on the molecular level using a transcriptomic approach to determine potential targets genes for the engineering of insect-resistant plants. After sequence processing and assembly using the dCAS program, 3804 sequences were grouped into 201 contigs and 1363 singlets, which were manually annotated. Several plant cell wall degrading enzymes were identified, including pectinases and cellulases. An invertase-containing contig was identified for the first time in a coleopteran. Total probable digestive enzymes accounted for 19.3% and unknown genes accounted for 28.8% of the total number of expressed sequence tags. Considering the predominance of cathepsin L enzymes among the digestive enzymes found in the transcriptome (54.3%) and their importance to the breakdown of proteins in the insect digestive process, a cDNA clone encoding a cathepsin L enzyme, denominated *Sl-CathL*, was chosen for recombinant expression in *Pichia pastoris* cells, characterization and *in vitro* inhibition by the sugarcane cystatin CaneCPI-4 (*K* < 0.196 nM). Immunolocalization assays demonstrated the production of *Sl-CathL* in the midgut epithelium and secretion into the gut lumen from vesicles containing the enzyme. *S. levis* demonstrated sensitivity in triggering RNA interference machinery induced by dsRNA injections in the body cavity and gene-specific knockdown was confirmed by real-time polymerase chain reaction. Larvae injected with V-ATPase-E dsRNA died within three weeks after injection and serpin 1-silenced larvae either exhibited delayed development, arresting in the pupal stage, or died as pharate adults. In conclusion, transcriptome analyses, together with the inhibition of the main digestive enzyme by Cane-CPI-4 and gene silencing using RNAi, are promising procedures for the establishment of insect-resistant crops and can be applied to transgenic sugarcane plants to enhance resistance to *Sphenophorus levis*.

Keywords: *Sphenophorus levis*, Insect control, Digestion, RNAi.

Supported by the Brazilian fostering agencies FAPESP (CBME, CEPID Proc. 98/14138-2) and CNPq.