Transcriptomic Analysis of a Susceptible and a Resistant Strain of the Lesser Grain Borer, *Rhyzopertha dominica*, and Laboratory Evaluation of s-metheprene and the Synergist Piperonyl Butoxide - Abstract

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Summary

One of the newer active ingredients (a.i.) that have been registered in many countries for the control of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) is the juvenile hormone analogue (JHA) s-methoprene. Piperonyl butoxide (PBO) is well-known as a synergist in combination with a specific group of a.i., such as pyrethroids, that exhibits toxicity through mixed function oxidases. The mechanism through which PBO inhibits P450s is mostly unknown. Moreover, resistance to s-methoprene has not been analyzed yet in *R. dominica*, largely due to the lack of genomic resources for this species. In the current study we investigate, for the first time, the mechanisms underlying s-methoprene resistance in *R. dominica* using transcriptome analysis to identify the potential role of a set of differentially expressed genes related to cytochrome P450s in resistance to s-methoprene against two strains, a resistant and a susceptible of *R. dominica*. Moreover, laboratory bioassays were performed in order to evaluate the efficacy of s-methoprene alone and in combination with PBO.

For the transcriptome analysis we sequenced the transcriptomes of both strains and identified the Cytochrome P450 (CYP) genes. Larvae of *R. dominica* (both strains) were pooled respectively and preserved in RNA later, and total RNAs of each was extracted using the GeneJet RNA Purification kit (ThermoScientific), according to the manufacturer's protocol. For the bioassays, treated wheat grains with s-methoprene, in presence and absence of PBO, were used for laboratory bioassays. S-methoprene concentrations of 0, 0.01, 0.03, 0.1 and 0.3 mg kg^{-1} for susceptible and 0, 1, 3, 10 and 30 mg kg^{-1} for resistant strain, were used. For PBO, the recommended label rate for combinations, 0.013 lt per 45.3 kg wheat, was used.

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The results of the present study indicate that resistance to s-methoprene is potentially mediated by cytochrome CYPs. MET gene sequence analysis on the resistant and the susceptible strains clearly identified P489L substitution in the open reading frame (ORF) of resistant strain, conferring resistance to s-methoprene. For the bioassays the progeny production varied. In the case of the susceptible strain, similar results had the treated wheat with s-methoprene alone or in combination with PBO. Moreover, progeny production was higher in the lowest dose of s-methoprene, regardless the existence of PBO. In contrast, for the resistant population, when s-methoprene was applied alone, progeny production was significantly lower than that in the control vials. However, there was a considerably high offspring emergence, regardless of the concentration. Similarly, when s-methoprene was applied with PBO, the increase in the concentrations reduced progeny production. Furthermore, for the two lowest s-methoprene concentrations, progeny production was not affected, regardless of the presence of PBO. Nevertheless, for the two higher concentrations, progeny production was considerably lower when s-methoprene was applied in combination with PBO, than for the application of s-methoprene alone.

Subsequently, we sequenced the transcriptomes of s-methoprene-resistant and susceptible strains and identified the CYP genes. Interestingly, their analysis revealed that a number of them were significantly upregulated in the s-methoprene-resistant strain and are thus worth of further investigation to determine their role in insecticide resistance.

Keywords: piperonyl butoxide; s-methoprene; Rhyzopertha dominica; transcriptome analysis.

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