Extraction and Quantification of Peramine Present in Dalaca pallens, a Pest of Grassland in Southtern Chile

Authors : Leonardo Parra, Daniel Martínez, Jorge Pizarro, Fernando Ortega, Manuel Chacón-Fuentes, Andrés Ouiroz Abstract : Control of Dalaca pallens or blackworms, one of the most important hypogeous pest in grassland in southern Chile, is based on the use of broad-spectrum insecticides such as organophosphates and pyrethroids. However, the rapid development of insecticide resistance in field populations of this insect and public concern over the environmental impact of these insecticides has resulted in the search for other control methods. Specifically, the use of endophyte fungi for controlling pest has emerged as an interesting and promising strategy. Endophytes from ryegrass (Lolium perenne), establish a biotrophic relationship with the host, defined as mutualistic symbiosis. The plant-fungi association produces alkaloids where peramine is the main toxic substance against Listronotus bonariensis, the most important epigean pest of ryegrass. Nevertheless, the effect of peramina on others pest insects, such as D. pallens, to our knowledge has not been studied, and also its possible metabolization in the body of the larvae. Therefore, we addressed the following research question: Do larvae of D. pallens store peramine after consumption of ryegrass endophyte infected (E+)? For this, specimens of blackworms were fed with ryegrass plant of seven experimental lines and one commercial cultivar endophyte free (E-) sown at the Instituto de Investigaciones Agropecuarias Carillanca (Vilcún, Chile). Once the feeding period was over, ten larvae of each treatment were examined. Individuals were dissected, and their gut was removed to exclude any influence of remaining material. The rest of the larva's body was dried at 60°C by 24-48 h and ground into a fine powder using a mortar. 25 mg of dry powder was transferred to a microcentrifuge tube and extracted in 1 mL of a mixture of methanol:water:formic acid. Then, the samples were centrifuged at 16,000 rpm for 3 min, and the supernatant was colected and injected in the liquid chromatography of high resolution (HPLC). The results confirmed the presence of peramine in the larva's body of D. pallens. The insects that fed the experimental lines LQE-2 and LQE-6 were those where peramine was present in high proportion (0.205 and 0.199 ppm, respectively); while LQE-7 and LQE-3 obtained the lowest concentrations of the alkaloid (0.047 and 0.053 ppm, respectively). Peramine was not detected in the insects when the control cultivar Jumbo (E-) was tested. These results evidenced the storage and metabolism of peramine during consumption of the larvae. However, the effect of this alkaloid present in 'future ryegrass cultivars' (LQE-2 and LQE-6) on the performance and survival of blackworms must be studied and confirmed experimentally. **Keywords :** blackworms, HPLC, alkaloid, pest

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