A NEXT GENERATION SEQUENCING MULTI-LOCI DNA BARCODING APPROACH FOR *HELICOVERPA ARMIGERA* DETECTION

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Abstract

The Old World Bollworm, Helicoverpa armigera (Hubner), is a highly destructive agricultural pest that recently invaded South America and Puerto Rico. Rapid detection and response is crucial to preventing its invasion into North America. However, it is very difficult to distinguish between it and H. zea (Boddie), a closely related pest that is native to the New World. Morphological species identification is only possible through dissection of adult males; larvae and adult females cannot be distinguished. Real-time PCR techniques can identify H. armigera using variation in the rRNA internal transcribed spacer regions 1 &2 (ITS1 & ITS2). However, the reliance on single genes could overlook hybridized invaders. To improve our abilities to detect H. armigera DNA in pest surveillance material, we adapted Next Generation Sequencing (NGS) of DNA barcodes to simultaneously sequence multiple genes from bulk DNA of unknown species, which can originate from domestic trap surveys. We first extracted DNA from pooled adult legs of both species. We then PCR amplified four, species-diagnostic, gene regions: rRNA internal transcribed spacer regions 1 &2 (ITS1 & ITS2), cytochrome oxidase 1 (CO1), and a Z-linked triosephosphate isomerase (Tpi). We sequenced these on an Illumina Miseq and adapted a metagenomics bioinformatics pipeline to resolve the two species. We successfully detected trace amounts of H. armigera genes within bulk DNA mixtures. As more diagnostic gene targets are identified they can be incorporated into our technique for robust detection. Additionally, this technique can be readily adapted to detect additional cryptic pest species.