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DIRECT DNA AMPLIFICATION FROM FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) SAMPLES

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The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), a migratory insect, is considered to be a major pest of corn, sorghum, rice, cotton and pastures in North and South America. There are at least 2 host strains of FAW commonly referred to as the rice and the corn strains. These biotypes are sympatric and morphologically identical but differ in physiological characteristics and reproductive isolation (Pashley 1988). Significant differences in susceptibility of these strains to insecticides were also found (Busato et al. 2006). In addition, both FAW strains have already been described in Argentina (Virla et al. 2008). Since the host plant is not a determinant for the identification of the colonizing strain, the molecular identification of the FAW strains is highly recommended before studying any aspect of the pest. Actually, this identification is based on genetic techniques, which involve DNA amplification. Standard protocols include several steps of DNA extraction and purification prior to PCR amplification of DNA, which require much labor when the number of samples is large. Currently available modern PCR polymerases are sufficiently robust to perform under unusual conditions, such as required for direct DNA amplification. The results presented in this brief report demonstrate that the PCR technique also serves to amplify DNA that was taken directly from samples of FAW tissues.

Specimens of FAW were obtained from a laboratory colony maintained in stable conditions (25 ± 2 °C; 70 ± 5% RH and 12:12 h L: D), and reared on an artificial diet. PCR reactions were tested using both eggs and neonate larvae. Fresh, frozen and ethanol preserved samples were evaluated. Samples were stored at -10 °C or in the presence of ethanol for at least 3 months. Samples kept in 80% ethanol were evaporated under vacuum at room temperature. Either eggs or neonate larvae were placed directly into 0.1 mL PCR microtubes. Then, the reaction mix was added. Direct PCR amplifications of FAW samples were each conducted in a 25 µL reaction mix containing 19.4 µL water (ampoule water), 5 µL 5X Taq buffer (Promega), 0.2 µL of dNTPs (25 µM), 0.1 µL of each primer JM76/JM77 (10 mM) forward JM76 -5'GAGCTGAATTAGG(G/A)ACTCCAGG3', and reverse JM77-5'ATCACCTCC(A/T)CCTGCAGGATC3' (Levy et al. 2002), along with 2 units of GoTaq ® DNA polymerase (Promega). A negative control with water instead of FAW samples was included. To prepare a pure DNA template, suitable as a positive control, DNA from a 3rd instar larva was extracted using the cetyltrimethylammonium bromide (CTAB) method (Black & Deteau 1997). The larva was homogenized in 750 µL of extraction buffer (2% CTAB, 1.4 M NaCl, 20mM EDTA, 100 mM TRIS-HCl pH 8, and 0.2% β-mercaptoethanol). Proteinase K (10 mg/mL) was added (2 µL per mL of extraction buffer) to the homogenate. Then, the mixture was incubated at 65 °C for 1 h, while the tube was inverted several times. The supernatant was extracted with 750 µL of chloroform: isoamyl alcohol (24:1) by centrifugation at 15,000 rpm during 10 min to separate the phases. The aqueous phase was transferred into a 1.5 mL tube and the chloroform: isoamyl alcohol step was repeated. DNA was precipitated by adding 500 µL of chilled isopropanol to the aqueous phase and incubated at -10 °C for 8 h. After incubation the precipitate was centrifuged at 15,000 rpm for 15 min. The isopropanol was decanted; the DNA pellet was rinsed with 500 µL 80% ethanol and centrifuged at 15,000 rpm for 15 min and repeated a second time. The ethanol was evaporated, and the pellet was resuspended in 50 µL of water. Amplifications were performed on a DNA thermocycler (Perkin-Elmer) with the following program: an initial incubation at 97 °C for 6 min, followed by 35 cycles of 94 °C (1 min), 58 °C (1 min) and 72 °C (2 min), and a final extension at 72 °C for 7 min. The amplified samples were chromatographed by gel electrophoresis on 0.8% agarose and stained with ethidium bromide.

We successfully amplified a 569-base pairs (bp) fragment of cytochrome C oxidase 1 (*CO1*) from samples containing 2 fresh eggs or 1 neonate larva either frozen or ethanol preserved (Fig. 1). In addition, these amplicons were high quality similar to that obtained using a pure DNA template (positive control). Thus, for these samples, the initial heating step at 97 °C for 6 min released sufficient target DNA to allow it to be amplified by the PCR. Furthermore, initial incubations at temperatures above and below 97 °C were also tested, but no

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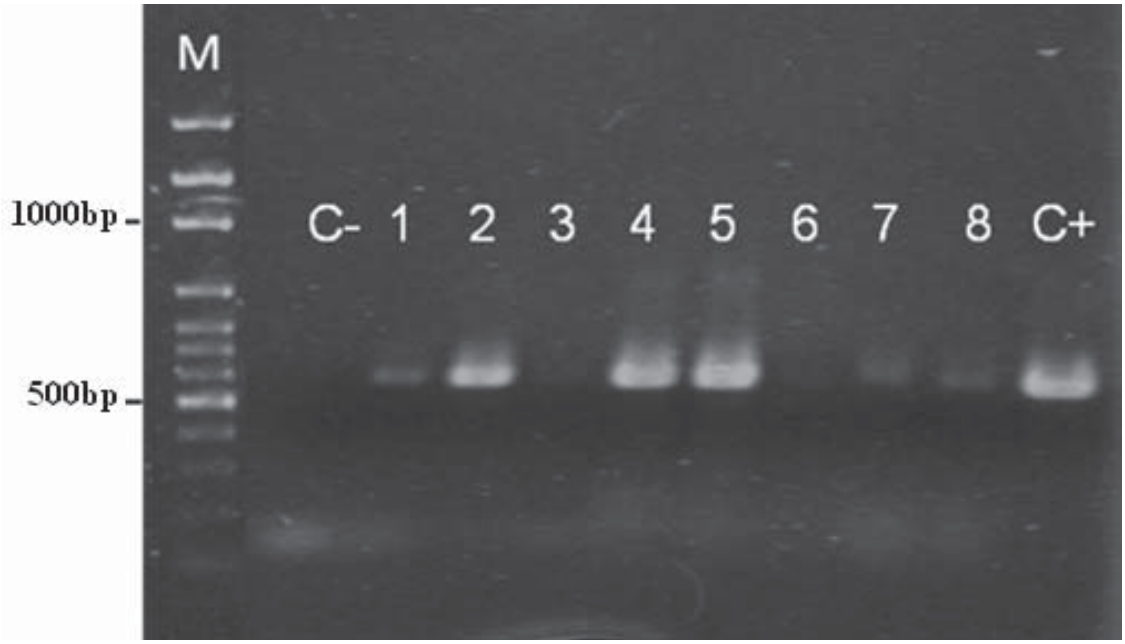


Fig. 1. Direct PCR-amplified fragment of 569 bp from FAW samples. M: Molecular marker. C (-): Negative Control. C (+): Positive control. DNA amplification from one (1), two (2) or three (3) fresh eggs. DNA amplification from a frozen neonate larva (4), an ethanol preserved neonate larva (5), and a fresh neonate larva (6). DNA amplification from two frozen (7) and two ethanol preserved (8) eggs.

amplifiable DNA fragment was detected in them. Samples containing one fresh egg, or two eggs either frozen or ethanol preserved resulted in poor amplification. In the remaining samples, there was no amplification. According to the results obtained, by maintaining both the reaction mixture volume and the Taq polymerase concentration, an increase in the number of the eggs could also be accompanied with an increase of co-extracted inhibitors. In addition, use of the methods of preserving assayed eggs resulted in a poor amplification (Fig. 1, line 7 and 8). Interestingly, the PCR response was different in the presence of neonate larva. In this case, the sample treated by freezing or ethanol not only released sufficient amplifiable DNA but also seemed to have less inhibitors (e.g. proteases, DNAases) than the fresh one.

Also PCR amplification of DNA directly from whole, undissected larvae and adults of the fruit fly *Drosophila melanogaster* was reported (Grevelding et al. 1997). Additionally, studies with non-invasive extraction protocols (Shokralla et al. 2010) or those used with ancient DNA (Thomsen et al. 2009) have demonstrated the release of DNA from tissues for direct amplification. However, there is no relevant information regarding the release of DNA from the FAW by such extraction protocols.

In summary, our results showed that DNA extraction from FAW samples was an avoidable step prior to amplification of DNA by PCR. In fact, the work described here would be useful in many protocols providing a relatively quick, easy, and inexpensive method for conducting PCR amplification and sequencing of lepidopteran genes. As an example, use of direct PCR proved to be particularly helpful to simplify the identification of FAW biotypes. Also remarkable was the high resistance to amplification of the DNA of neonate larvae that has been stored in ethanol. This feature is very important in those cases where the sample is required to maintain optimum conditions for extended periods, e.g., during the collection of insect specimens. Thus, the direct PCR approach reported here is a new alternative for DNA amplification from FAW samples.

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Summary

The direct amplification by PCR of the DNA in tissue samples of eggs and neonate larvae of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) was accomplished, and it is a new alternative for DNA amplification from fall armyworm samples. This method is simple, fast, economic, and accelerates studies on this polyphagous pest.

Key Words: DNA extraction, DNA purification, PCR amplification, sequencing, strain identification

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RESUMEN

Se aplicó la técnica de PCR para amplificar el ADN directamente a partir de huevos y de larvas neonatas de *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). Esta es una nueva alternativa para la amplificación del ADN de muestras del gusano cogollero. El método propuesto es simple, rápido, económico y agiliza los procedimientos para diversos estudios sobre esta plaga polífaga.

Palabras Clave: extracción del ADN, purificación del ADN, amplificación por PCR, secuenciado, identificación de cepa

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