ASSESSMENT OF DNA INTEGRITY FROM TRAP-CAPTURED BOLL WEEVILS FOR USE IN PENDING DIAGNOSTIC TOOL

Lindsey C. Perkin
Charles P.-C. Suh
USDA-ARS ICCDRU
College Station, TX
Brenda S. Oppert
USDA-ARS CGAHR
Manhattan, KS

Abstract

Efforts are underway to develop a PCR-based diagnostic tool that can be used to rapidly and accurately differentiate the boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), from other closely related weevil species (e. g., thurberia weevils), which are commonly captured in boll weevil pheromone traps. Under typical field scenarios, weevils collected from traps may be dead, dismembered, and/or exposed to adverse environmental conditions for up to 21 days. Consequently, the integrity of DNA extracted from these weevil specimens may be too fragmented or yield insufficient amounts for identification via the PCR-based assay. In order to enhance the commercial potential and adoption of this diagnostic tool, we documented the degradation of DNA quantity and integrity in weevils and weevil body parts aged in traps up to a three-week period.

Introduction

The boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), is still a major pest of commercial cotton (*Gossypium hirsutum*) in South Texas and Mexico. Although eradication programs have greatly reduced the range of the boll weevil in these areas, remaining weevil populations continue to pose a threat as sources of re-infestation. Management of the boll weevil is further complicated by the misidentification of closely related non-pest species (e.g., *Anthonomus grandis thurberi*), which feed on wild cotton (*Gossypium thurberi*) but are commonly captured in boll weevil pheromone traps. Misidentification of weevils can lead to costly and unnecessary remedial action. Thus, there is a need for a diagnostic tool to quickly and accurately distinguish boll weevil from other non-pest species. Several diagnostic assays have been developed in an attempt to address this need, but the current method is only 94.3% accurate in distinguishing these two weevil species (Roehrdanz 2001; Barr et al., 2013).

A new PCR-based diagnostic tool is under development that uses multiple single nucleotide polymorphism (SNP) markers across the nuclear genome and is likely to provide nearly 100% accuracy. However, this tool will rely on access to DNA of sufficient quantity and quality. Based on a previous diagnostic tool that used the COI mitochondrial gene to distinguish sub-species of weevils (Barr et al., 2013), we inferred that at least a 700 base pair (bp) fragment is needed for reliable amplification of markers. However, weevils obtained from traps may be exposed to adverse environmental conditions up to 21 days, and/or only remnants of weevils may be available for analysis.

In this study we assessed the quantity and quality of DNA extracted from boll weevils aged in pheromone traps up to 21 days under field conditions. We used previously developed assay parameters as a guideline to determine fragment length and DNA quantity thresholds needed for successful amplification of SNP regions. We assayed DNA from whole weevils and individual weevil parts to determine if sample DNA quantity and integrity was sufficient for use in the pending PCR-based assay.

Materials and Methods

Weevil collection and dissections

Weevils were collected on a weekly basis from pheromone traps operated by the Texas Boll Weevil Eradication Foundation (TBWEF). Collected live weevils were maintained on bolls (15-22 mm diam.) in plexiglass cages until sufficient numbers of weevils (≥100) were obtained to initiate the experiment. At the start of the experiment, all live weevils were counted and sacrificed via CO_2 exposure for 45 to 60 min. Dead weevils were evenly distributed into ten groups. One group was used as a control to establish baseline levels of DNA quantity and integrity of freshly killed boll weevils, and the remaining groups were each placed in nine pheromone traps arranged outside of the TBWEF District Office in Harlingen, TX. Each trap was equipped with a kill strip and the cone entrance was plugged

with hot glue to minimize predator intrusion (e.g. ants or spiders). One group of weevils was collected from a trap after 0 (control), 1, 2, 3, 4, 7, 10, 14, 17, and 21 days, put into 100% ethanol, and stored at -80 until DNA was extracted. DNA was extracted from whole intact weevils, both front legs, head, and abdomen (as described below). The experiment was repeated four times (trials) between July 30, 2018 and September 30, 2018. The first trial consisted of ten weevils per trap per day (seven whole weevils and three dissected for body parts). The second, third and fourth trial consisted of 22 weevils per trap per day (18 whole weevils and four dissected for parts).

Weevils were dissected with micro-scissors in 100% ethanol and immediately placed in individual microcentrifuge tubes with 180 µL lysis buffer (Qiagen QiaAmp Micro Kit, see below). Front legs were removed by clipping between the femur and trochanter. Heads with pro-thorax were separated from abdomens with meso- and meta-thorax (including legs). Dissecting equipment was rinsed with acetone between each sample.

DNA extractions, quantification and quality assessment

The benchtop and equipment were cleaned with DNAzap (Invitrogen, Carlsbad, CA) before and after sample preparation. DNA extractions were performed using the QiaAmp DNA Micro Kit (Qiagen, Hilden, Germany) with minor modifications. Whole weevils or individual dissected parts were ground in a microcentrifuge tube with lysis buffer using a plastic pestle. Proteinase K was added after samples were pulverized and samples were then incubated at 56°C for approximately 16 hours. The manufacturer's protocol was followed using 1 µL carrier RNA and extended elution incubation time of 5 min at room temperature to maximize DNA recovery. The final elution was with 20 µL nuclease free water (Invitrogen, Carlsbad, CA).

One microliter of each sample was used to quantify the DNA on a Quantus fluorimeter using the DNA ONE Fluorophore Kit (Promega, Madison, WI). Any samples that exceeded the measuring capacity of the kit (400 ng/µL) were diluted 1:10 and re-measured. A 1 µL aliquot of each sample was analyzed on a 2200 TapeStation using gDNA screentapes (Agilent, Santa Clara, CA) to assess the quality of DNA via fragment length measurement. Each sample was recorded as having exceeded the 700 bp requirement to be used in a PCR-based assay or not, and the largest fragment size in each sample was also recorded.

Results and Discussion

DNA quantity

DNA quantity in whole weevils remained stable throughout the 21-day period during Trials 1 and 2 (Fig. 1A). Total DNA ranged between 200-600 ng/µL. Trial 3 showed a similar trend, but DNA quantity declined sharply between day 14 and 17. Trial 4 showed a gradual decline in DNA after day 1. We speculated that rain events, which may have expedited the degradation of weevil tissue, contributed to the overall reduction in DNA quantity in Trials 3 and 4. DNA quantity in dissected body parts showed a similar trend (Fig. 1 B-D). Front legs had the lowest DNA abundance but remained consistent across the 21-day period (Fig. 1B). DNA quantity in single legs ranged from 2-16 ng/µL, whereas DNA quantities from abdomens showed a trend almost identical to that of DNA quantities obtained from whole weevils (Fig. 1C). The amount of DNA found in the head was intermediate to that obtained from abdomens and legs (Fig. 1D). Although the amount of DNA extracted from the head was lower than that obtained from the abdomen, the general trend over time was basically the same as those observed for whole weevils.

DNA integrity

We also measured the integrity of each sample by measuring the longest fragment of DNA in each sample and determined if the size was adequate for the PCR-based assay using a conservative threshold of 700 bp as described in the COI assay (Barr et al., 2013). We found that the DNA integrity in whole weevils greatly exceeded the 700 bp threshold (Fig. 2). Future work to assess the integrity of dissected weevil parts is currently under way.

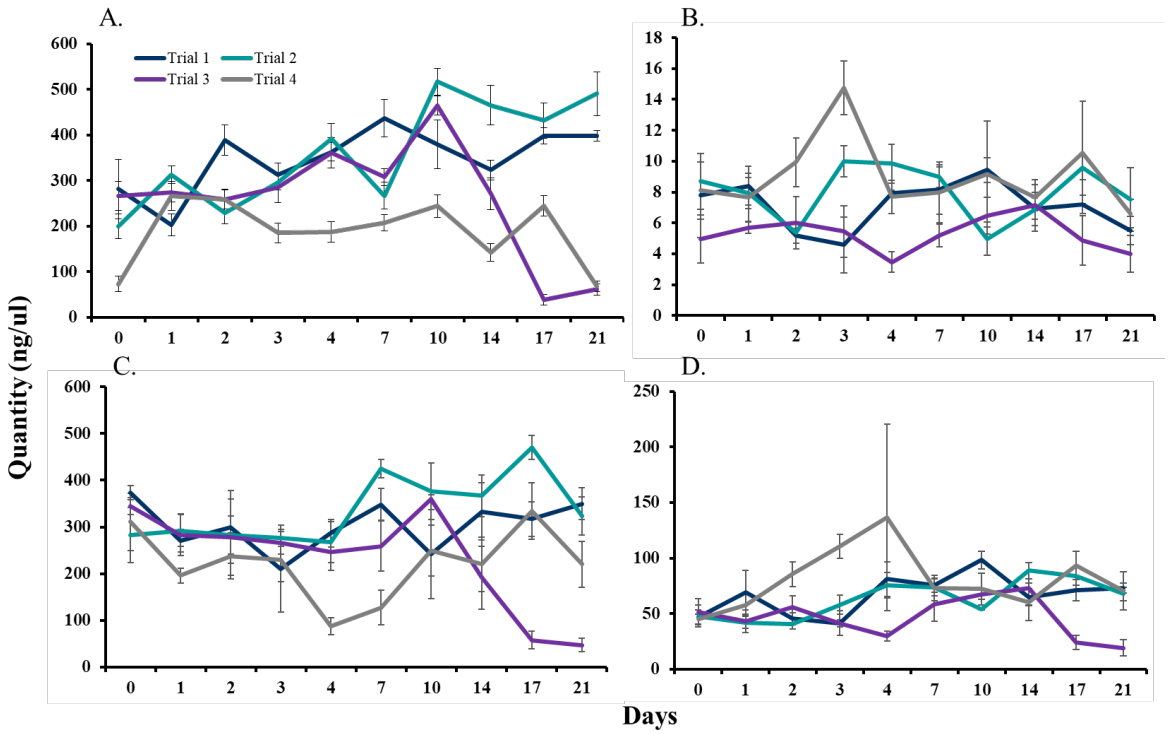

Figure 1. DNA quantity for: A. Whole weevils; B. Front leg; C. Abdomen; and D. Head. Each colored line represents an individual trial (Trial 1, blue; Trial 2, teal; Trial 3, purple; Trial 4, grey) and each data point is the average amount of DNA (ng/µL ± S.E.) found in 18 whole weevils (Trial 1 had seven weevils), and four of each body part (Trial 1 had three weevils).

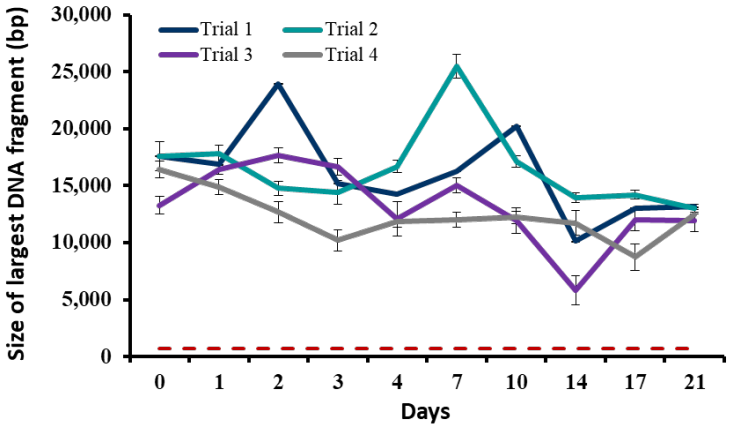

Figure 2. DNA integrity of whole weevils. Each colored line represents an individual trial (Trial 1, blue; Trial 2, teal; Trial 3, purple; Trial 4, grey) and the red dotted line shows the 700 bp threshold needed for the PCR-based assay. Each data point is the average length of DNA in base pairs (bp ± S.E.) from 18 whole weevils (Trial 1 had seven weevils).

Summary

Our results indicated that the quantity of DNA extracted from whole weevils and body parts (head, front legs, and abdomen) was sufficient for the proposed PCR-based assay, even after being aged in traps for up to three weeks. The DNA integrity found in whole weevils exceeded the required 700 bp threshold as well. We are in the progress of completing analysis of the DNA integrity of front legs, abdomens and heads. Further analysis will be conducted to

more fully understand the relationship between DNA integrity and the time weevils may be dead in traps, including analysis using the percentage of the sample with DNA fragments larger than 700 bp, and the proportion of each sample that represents the largest fragment size. We will also examine the relationship between weather events (e.g. rain) and weevil DNA degradation.

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