

ENHANCING MOLECULAR SCREENING OF HIDDEN INSECT INFESTATION IN RICE GRAINS BY COI BARCODING: PRIMER PERFORMANCE AND LIMIT OF DETECTION

Ana Maria Campos¹, Inês Gonçalves de Sousa^{1,2}, Carina Almeida^{1,3}, Carla Brites^{1,4*}

¹National Institute for Agricultural and Veterinary Research (INIAV), Oeiras, Portugal

²LEAF (Linking Landscape, Environment, Agriculture and Food) Research Center, Universidade de Lisboa, Tapada da Ajuda, Portugal

³LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, ALiCE – Associate Laboratory in Chemical Engineering, Porto, Portugal

⁴GREEN-IT Bioresources for Sustainability, ITQB NOVA, Oeiras, Portugal

*carla.brites@iniav.pt

INTRODUCTION

Infestations by *Sitophilus oryzae* and *Sitophilus zeamais* are one of the main reasons for quantity and quality loss during rice storage¹. This hidden insect infestation in rice grains poses a significant challenge to both producers and consumers across the globe and has far-reaching implications for food security, economic stability, and public health. These genetically close insect species are particularly adapted to attack rice grains and spend a considerable part of their life cycle, including the entire larval feeding period, inside them [1], representing the hidden infestation that visual inspection cannot successfully detect. They do this by creating an entrance hole, covering it after entry, and then, after the pupation process [2,3] an exit hole is made, from which it then emerges the adult insect [4,5].

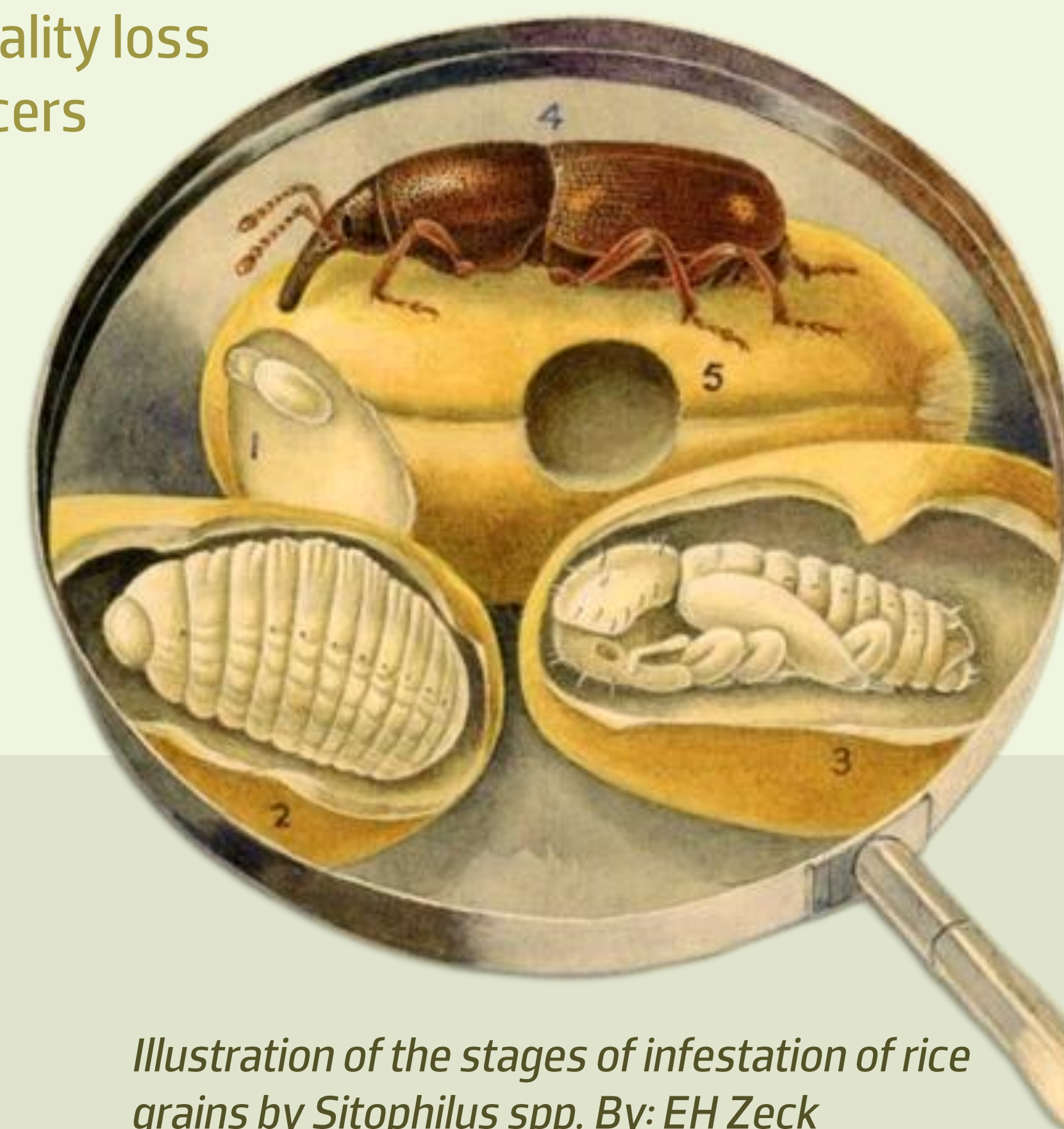


Illustration of the stages of infestation of rice grains by *Sitophilus* spp. By: EH Zeck

OBJECTIVES

The main goal of this study is to develop a fast molecular detection method, such as a multiplex real-time polymerase chain reaction (qPCR) to detect specifically and efficiently hidden infestation in stored rice for monitorization purposes and two activities were performed:

1. To verify primer performance, by analyzing its efficiency;
2. Determine the Limit of Detection (LoD) of the qPCR alone.

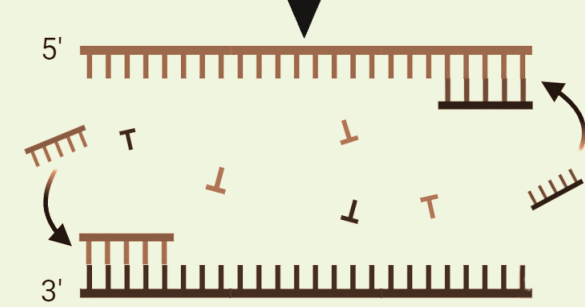
METHODS

PRIMERS AND PROBES DESIGN

S. oryzae and *S. zeamais* mtDNA

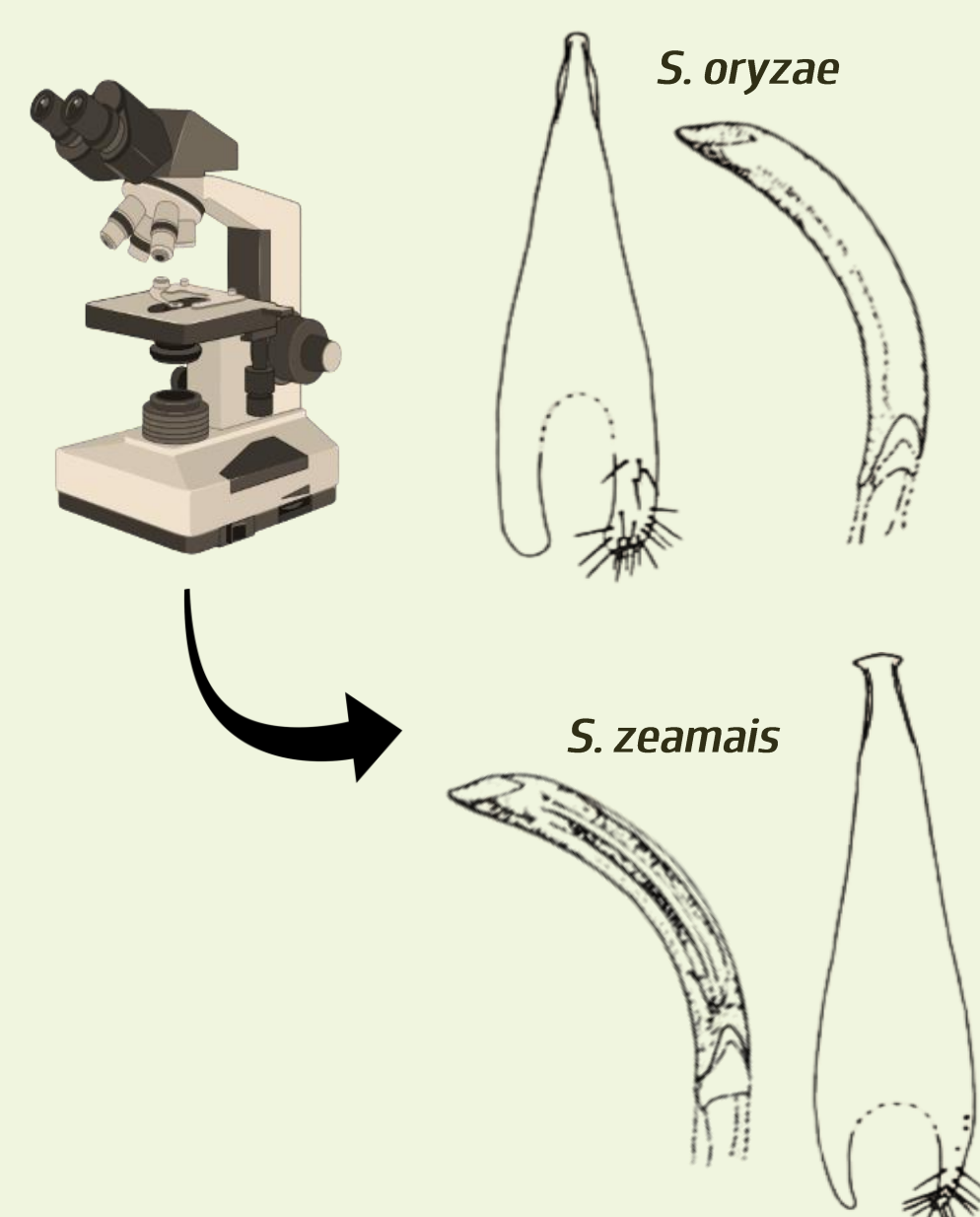
COI Barcode region

(compilation of DNA sequences from GenBank®)



Sequences alignment and design of primers and probes using Geneious Prime

MORPHOLOGICAL IDENTIFICATION OF ADULT INSECT SPECIES BY GENITALIA OBSERVATION



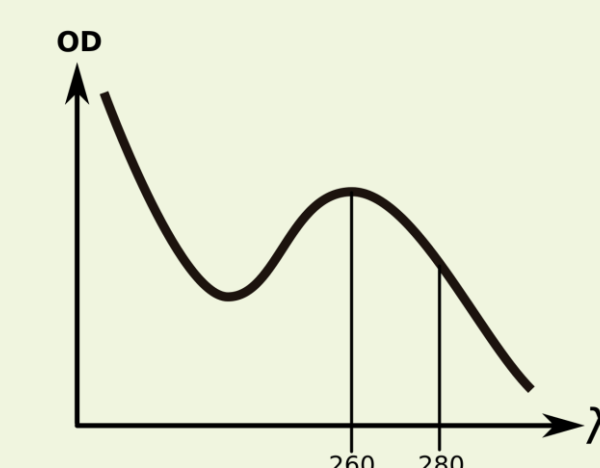
DNA EXTRACTION



Grinding in a mortar

Purified DNA

RT-PCR - PRIMER EFFICIENCY AND LOD ASSAYS

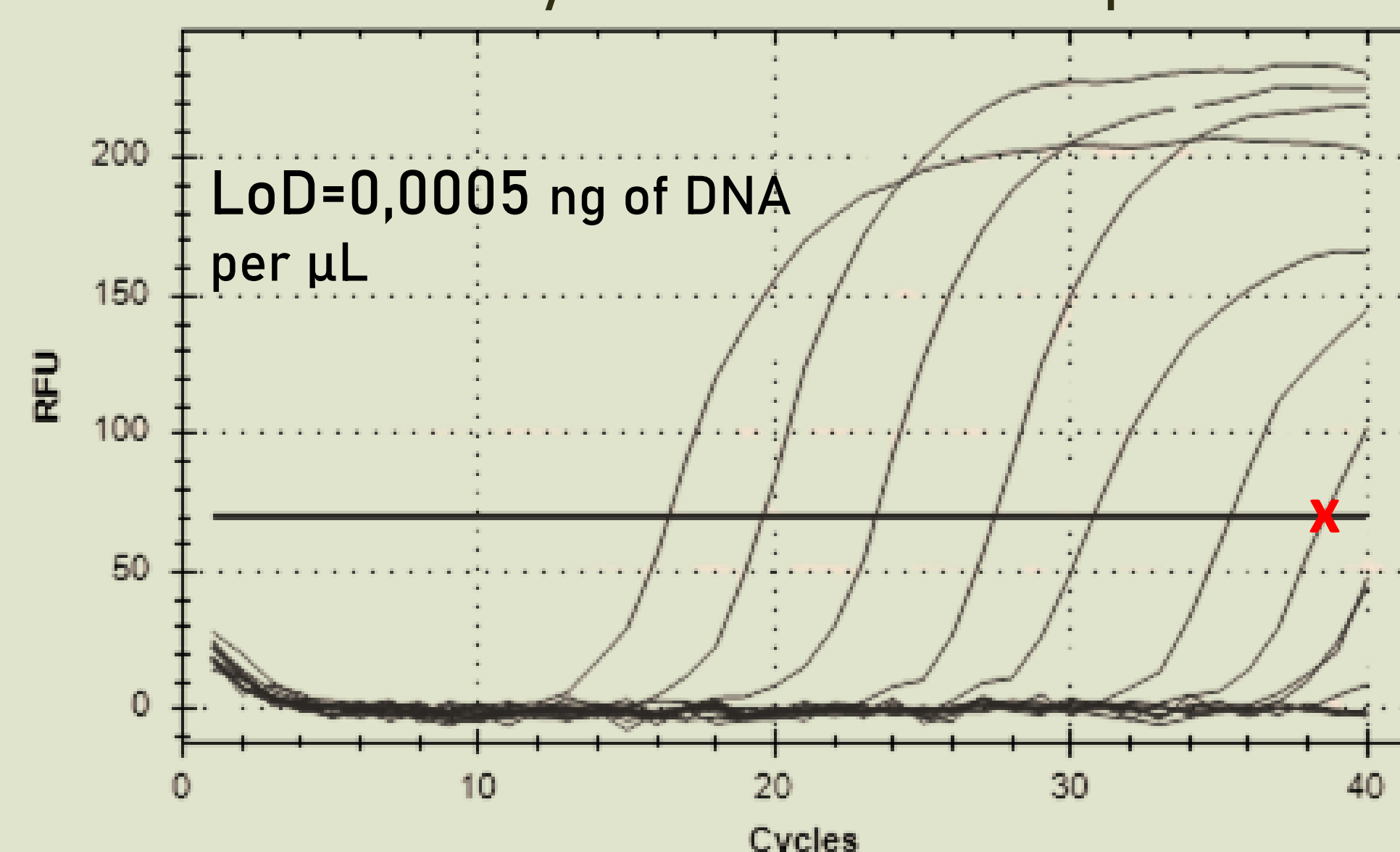
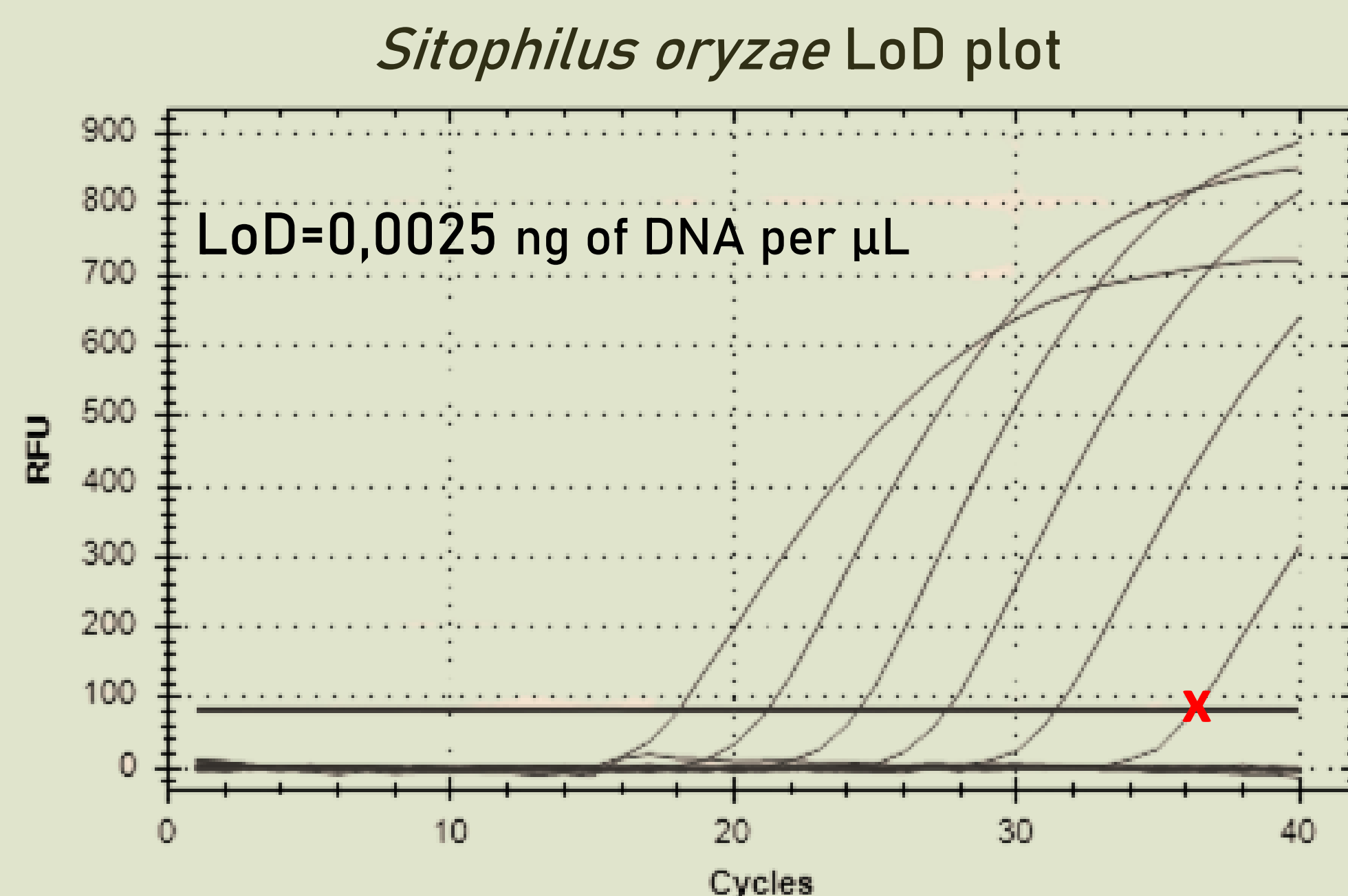
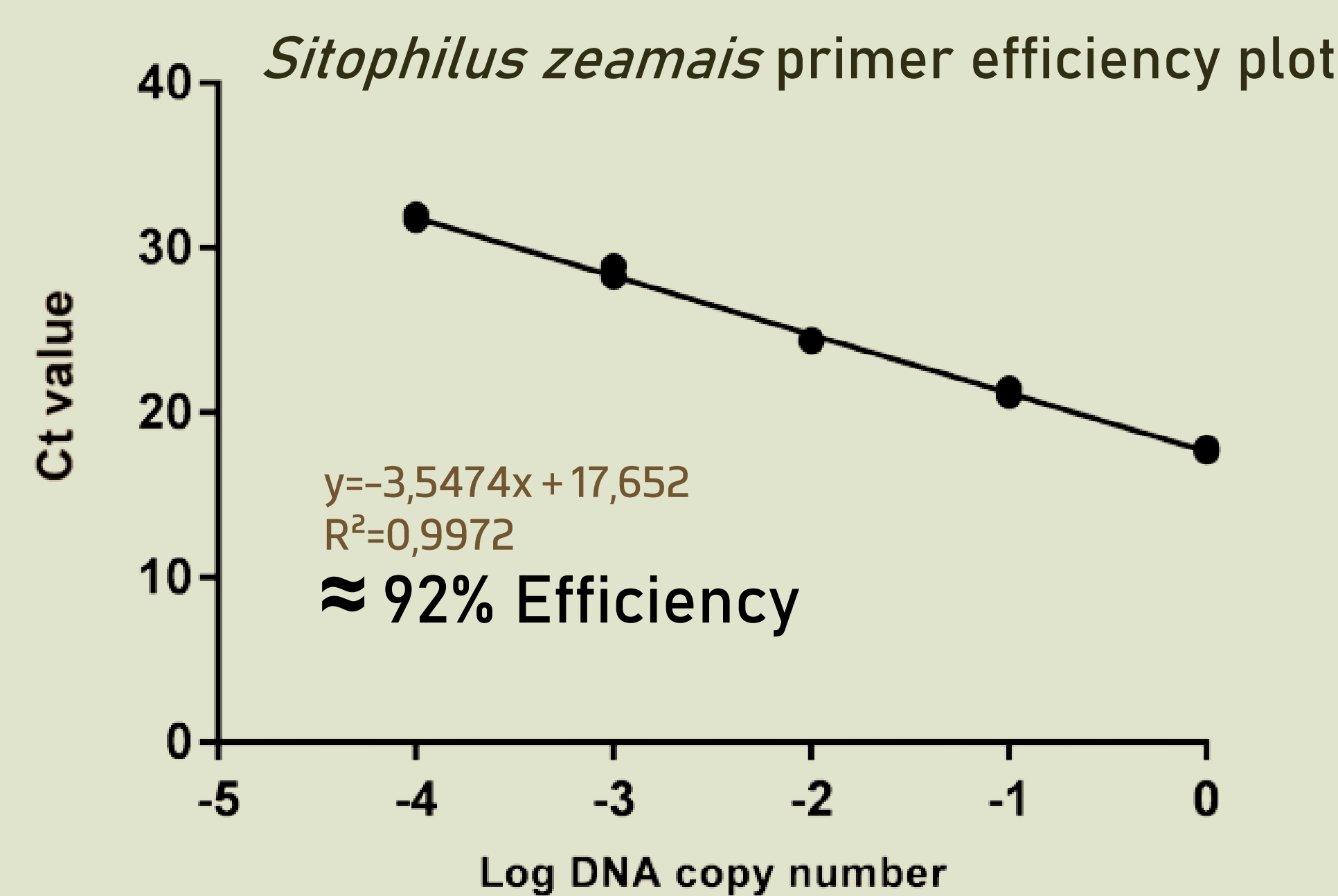
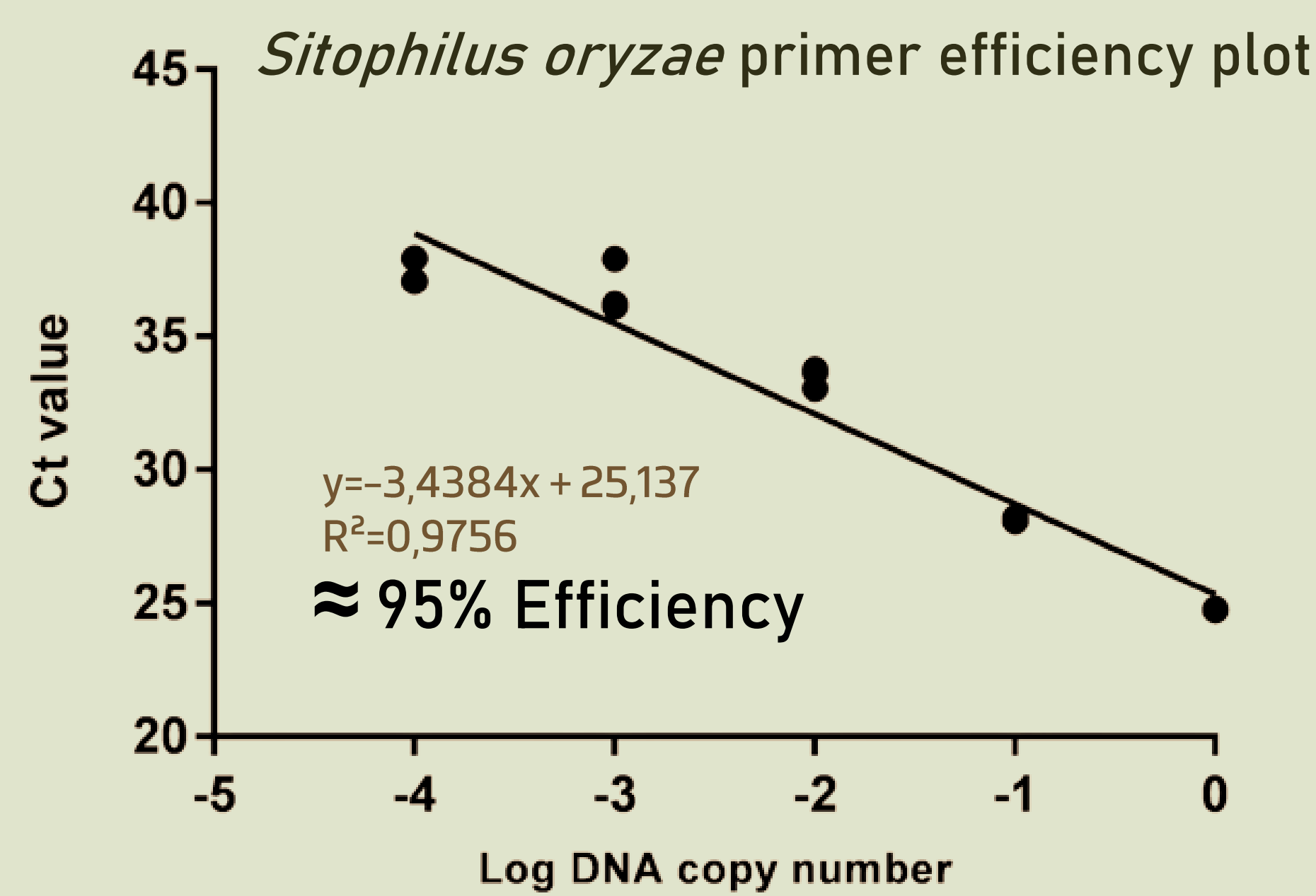


DNA quantification (Nanodrop)



DNA amplification and analysis of results

RESULTS AND DISCUSSION



Most of the current methods to detect hidden infestation in grains are obsolete and time-consuming² so is important to develop an efficient and sensitive qPCR method. These results led us to conclude that the designed primers were efficient, and the method is highly sensitive. However, more studies are needed to quantify the LoD that reflects the early stages of hidden infestation in rice grains.

ACKNOWLEDGMENTS

Graça Barros from ISA-UL for tutoring in insect species identification. Funding from TRACE-RICE with Grant nº 1934, (call 2019, section 1 Agrofood) that is part of the PRIMA Programme supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation. C. Brites from UIDB/04551/2020 and UIDP/04551/2020. C. Almeida from LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC). I. Sousa from FCT PhD grant 2023.02433.BDANA.

REFERENCES

