

Supplementary Material for RICAÑO, J., B. GÜERRI-AGULLÓ, M. J. SERNA-SARRIÁS, G. RUBIO-LLORCA, L. ASENSIO, P. BARRANCO AND L. V. LOPEZ-LLORCA—**Evaluation of the Pathogenicity of Multiple Isolates of *Beauveria bassiana* (Hypocreales: Clavicipitaceae) On *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) for the Assessment of a Solid Formulation under Simulated Field Conditions.** Florida Entomologist 96(3) (September, 2013) at <http://purl.fcla.edu/fcla/entomologist/browse>

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ABSTRACT

A solid state formulation of *Beauveria bassiana* (Balsamo) Vuillemin has been developed for biological control of the Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier, 1790). Two kinds of bioassays (dry conidia and dipping) using 10 isolates from several coleopterans in Mediterranean environments, identified 2 RPW derived isolates (193 and 203) as most pathogenic to RPW larvae and adults (zero survival within first 4-5 d for dry conidia, and 14 and 23 d for dipping bioassays). Isolate 203 ($5.1 \times 10^8 \pm 1.9 \times 10^8$ conidia g⁻¹) was formulated with fragmented date seed and tested in palms infested with RPW under semi-field conditions in Feb, Apr/May and Jun of both 2007 and 2008. *Beauveria bassiana* significantly reduced RPW adult survival with respect to controls in May 2007 and in the Apr/Jun 2008 experiments. Total RPW adult mortality was achieved within 30 days for all *B. bassiana* treatments, and was associated with increasing numbers of insects displaying signs of mycosis in 2008 experiments. *Beauveria bassiana* formulation reduced RPW multiplication in artificially infested palms compared to controls, and a positive correlation between numbers of larvae and time post-infestation was recorded. The suppression of RPW adult populations by *B. bassiana* persisted for at least 3 months under semi-field conditions. The *Beauveria bassiana* solid formulation, which induces great adult mortality and persistence in the field, could be applied as a preventive as well as a curative treatment for the integrated management of RPW.

KEY WORDS: entomopathogenic fungi, mycoinsecticide, *Phoenix canariensis*, solid state formulation, Red Palm Weevil

RESUMEN

En el presente estudio, se describe un procedimiento para el desarrollo de una formulación en estado sólido basada en *Beauveria bassiana* (Balsamo) Vuillemin para el control biológico del picudo rojo, *Rhynchophorus ferrugineus* (Olivier, 1790). Al realizar dos tipos de bioensayos (conidios secos e inmersión directa) utilizando diez aislados de diversos coleópteros en ambientes Mediterráneos, se identificaron dos aislados derivados del picudo rojo (193 y 203) como los más patogénicos para los estadios larvarios y adultos del insecto (supervivencia nula en los primeros 4-5 días y 14 y 23 días para bioensayos con conidios secos e inmersión directa respectivamente). El aislado 203 de *B. bassiana* ($5.1 \times 10^8 \pm 1.9 \times 10^8$ conidios g^{-1}) se formuló en sólido utilizando dátiles fragmentados y se ensayó en palmeras infestadas con el picudo rojo bajo condiciones de semi-campo en los meses de Febrero, Abril/Mayo y Junio de 2007 y 2008. *Beauveria bassiana* redujo significativamente la supervivencia de los insectos adultos con respecto a los controles en los experimentos de Mayo de 2007 y Abril/Junio 2008. Se alcanzó la mortalidad completa de los adultos del picudo rojo en 30 días para todos los tratamientos con *B. bassiana* y lo que se correlacionó con el incremento del número de insectos con signos de micosis para los ensayos de 2008. El formulado de *B. bassiana* redujo la multiplicación del insecto en palmeras infestadas artificialmente con respecto a los controles, registrándose una correlación positiva entre el número de larvas y el tiempo de infestación posterior. La capacidad del formulado de *B. bassiana* para suprimir las poblaciones de insectos adultos tuvo una persistencia de al menos tres meses bajo condiciones de semi-campo. El formulado sólido de *B. bassiana*, que ha generado una alta mortalidad en adultos así como una elevada persistencia, podría utilizarse tanto en tratamientos preventivos como curativos en el manejo integrado del picudo rojo.

Palabras Clave: Hongos entomopatógenos, micoinsecticidas, *Phoenix canariensis*, formulación sólida, Picuro rojo de las palmeras



Fig. 1. System for evaluation of the pathogenicity of a *Beauveria bassiana* solid state formulation against the red palm weevils infesting *Phoenix canariensis* in a simulated field condition.

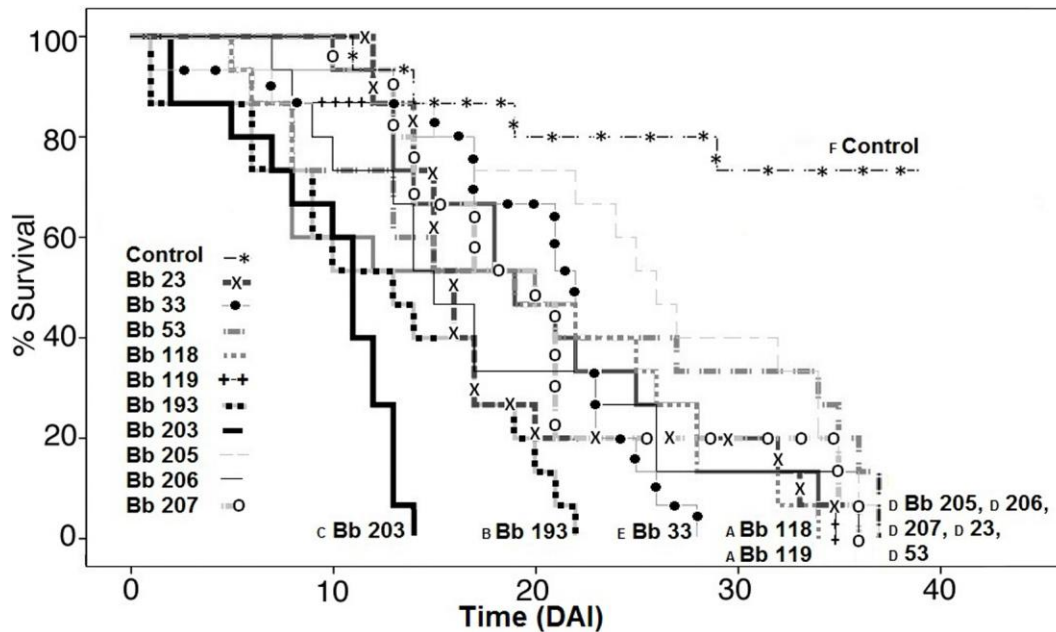


Fig. 2. Evaluation of RPW survival after exposure to dipping treatments of *B. bassiana* isolates (10^7 conidia/mL). Comparisons among treatments were performed using the logrank test ($P < 0.05$) applied to the Kaplan-Meier estimator of the survival function. Different capital letters represent significant differences. Time was measured in days after inoculation (DAI).

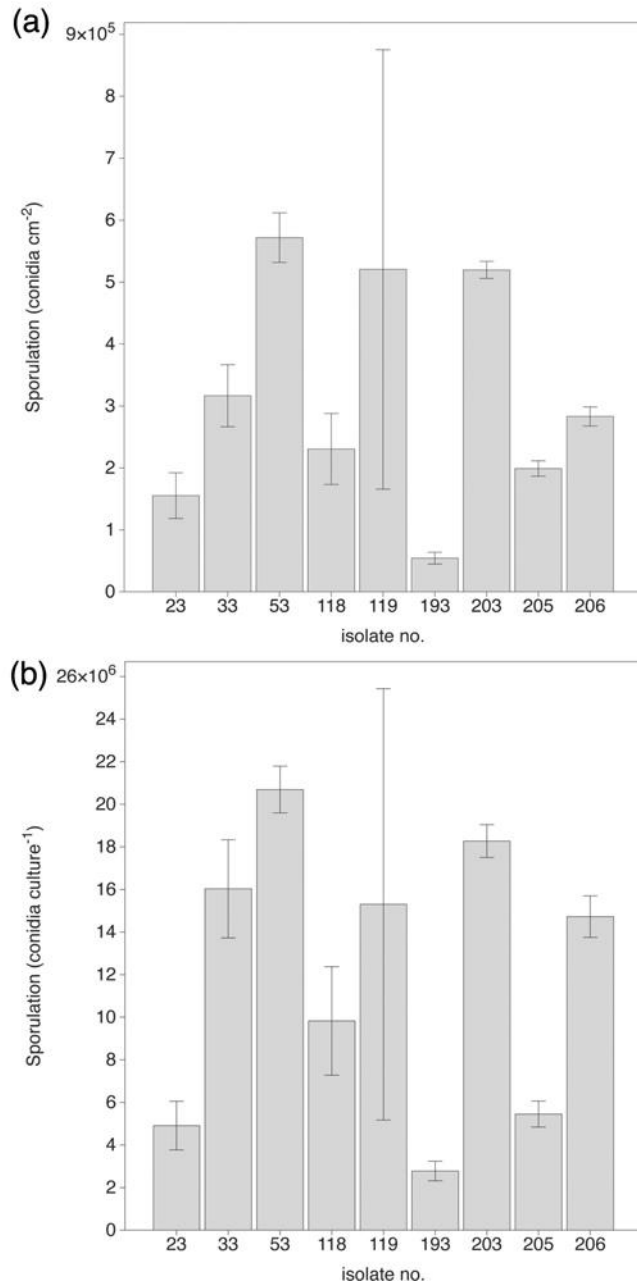


Fig. 3. Sporulation of *B. bassiana* isolates used for pathogenicity bioassays with RPW in this study. a) Density of conidia (as conidia cm^{-2}) of *B. bassiana* cultures on corn meal agar (CMA). b) Number of conidia per CMA 24-day culture of each isolate.

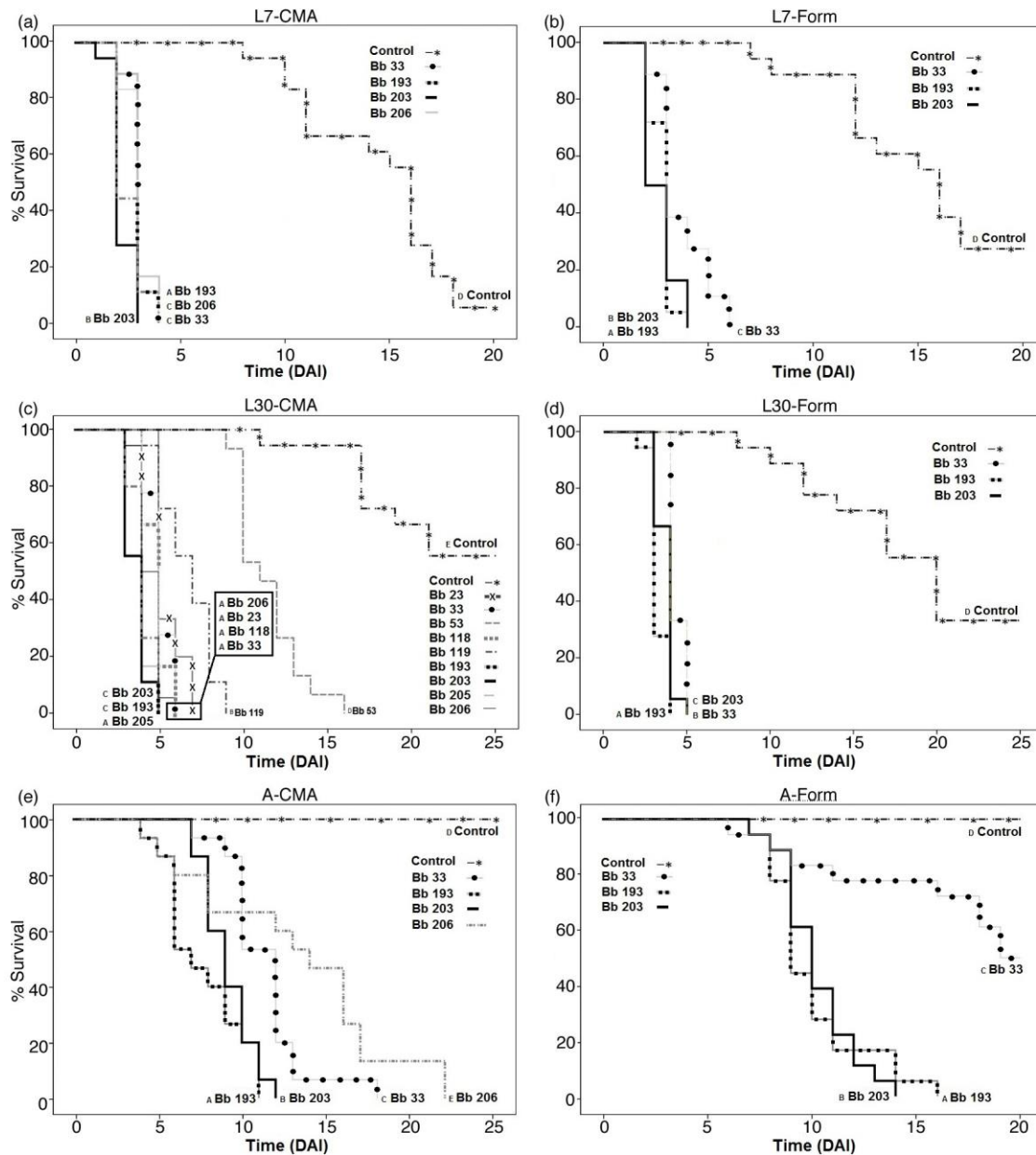


Fig. 4. Evaluation of pathogenicity of *B. bassiana* isolates on RPW in laboratory bioassays using dry conidia. Comparison between treatments were performed using logrank test ($P < 0.05$) applied to the Kaplan-Meier estimate of the survival function. 4a), b) RPW 7-10-day larvae (L7); 4c), d) 30-day larvae (L30); 4e), f) adults. In a, c and e, RPW was inoculated with corn meal agar (CMA) cultures of *B. bassiana* isolates. In b, d and f, RPW was inoculated with *B. bassiana* solid state formulations (Form). Controls were RPW mock inoculated (non-inoculated formulation or CMA plate). Different capital letters represent statistically significant differences. Time was measured in days after inoculation (DAI).

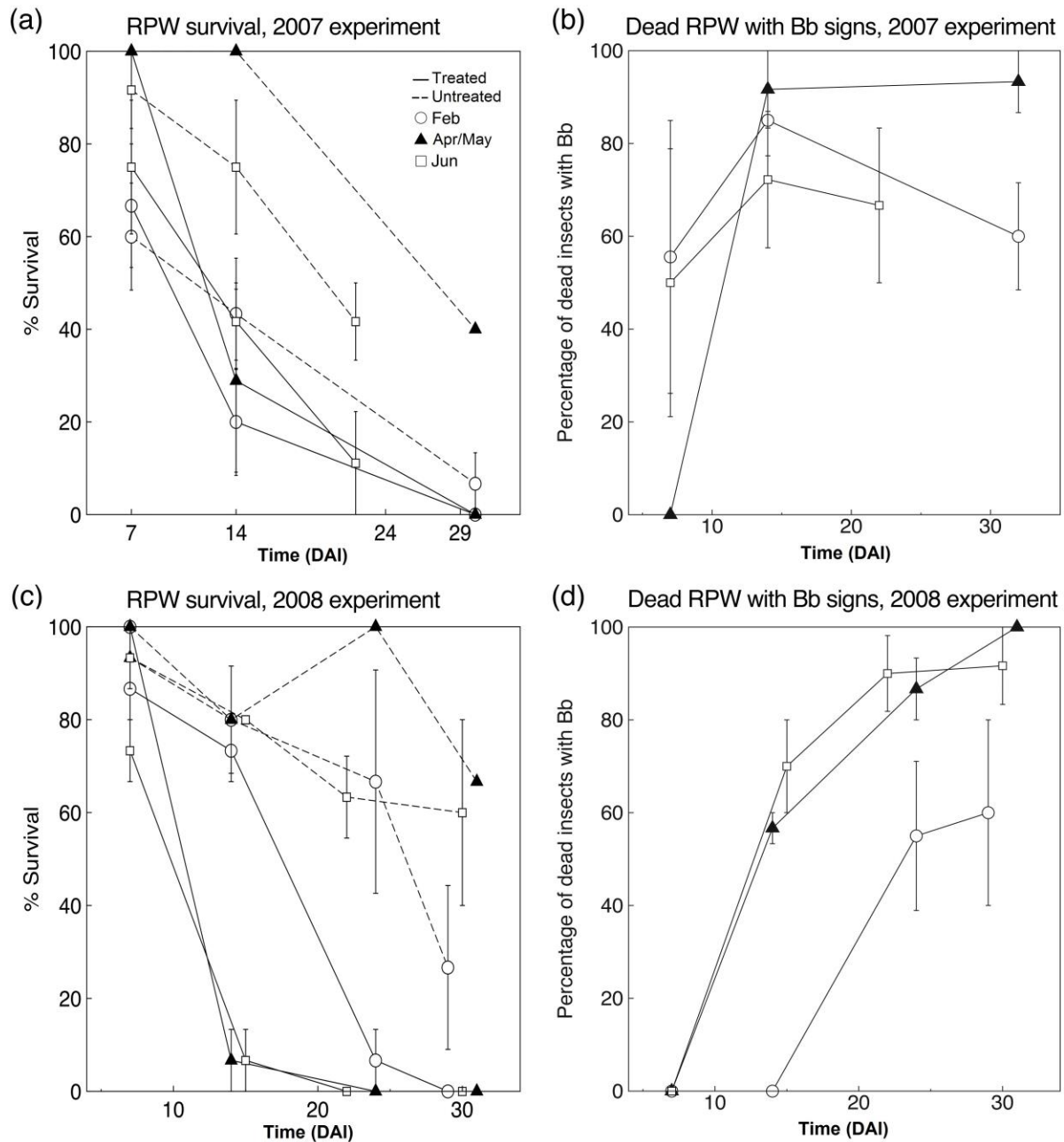


Fig. 5. Effect of *B. bassiana* on RPW survival in artificially infested palms under semi-field conditions. a) RPW survival, 2007 Experiment, b) Dead RPW with signs of *B. bassiana* infection, 2007 Experiment, c) RPW survival, 2008 Experiment, d) Dead RPW with signs of *B. bassiana* infection, 2008 Experiment. Solid line (*B. bassiana* treatments). Dashed line (controls). Feb (○), Apr/May (▲), Jun (□) experiments. Each value represents the mean of 3 replicates. Time was measured in days after inoculation (DAI).



Fig. 6. Signs of *B. bassiana* infection and colonization of RPW in artificially infested palms under semi-field conditions. a) RPW infected adults with *B. bassiana* on the surface of the potting mixture. b) RPW infected adults with *B. bassiana* buried in the substrate mixed with palm roots. c) Restricted *B. bassiana* development from RPW antennae clubs (arrows). d) *B. bassiana* development from intersegmental regions (arrowhead) and body dismembration (circle). e and f) Extensive mycelium growth and sporulation of *B. bassiana* in RPW infected adults. f) Sporodochia of *B. bassiana* in RPW infected adults.

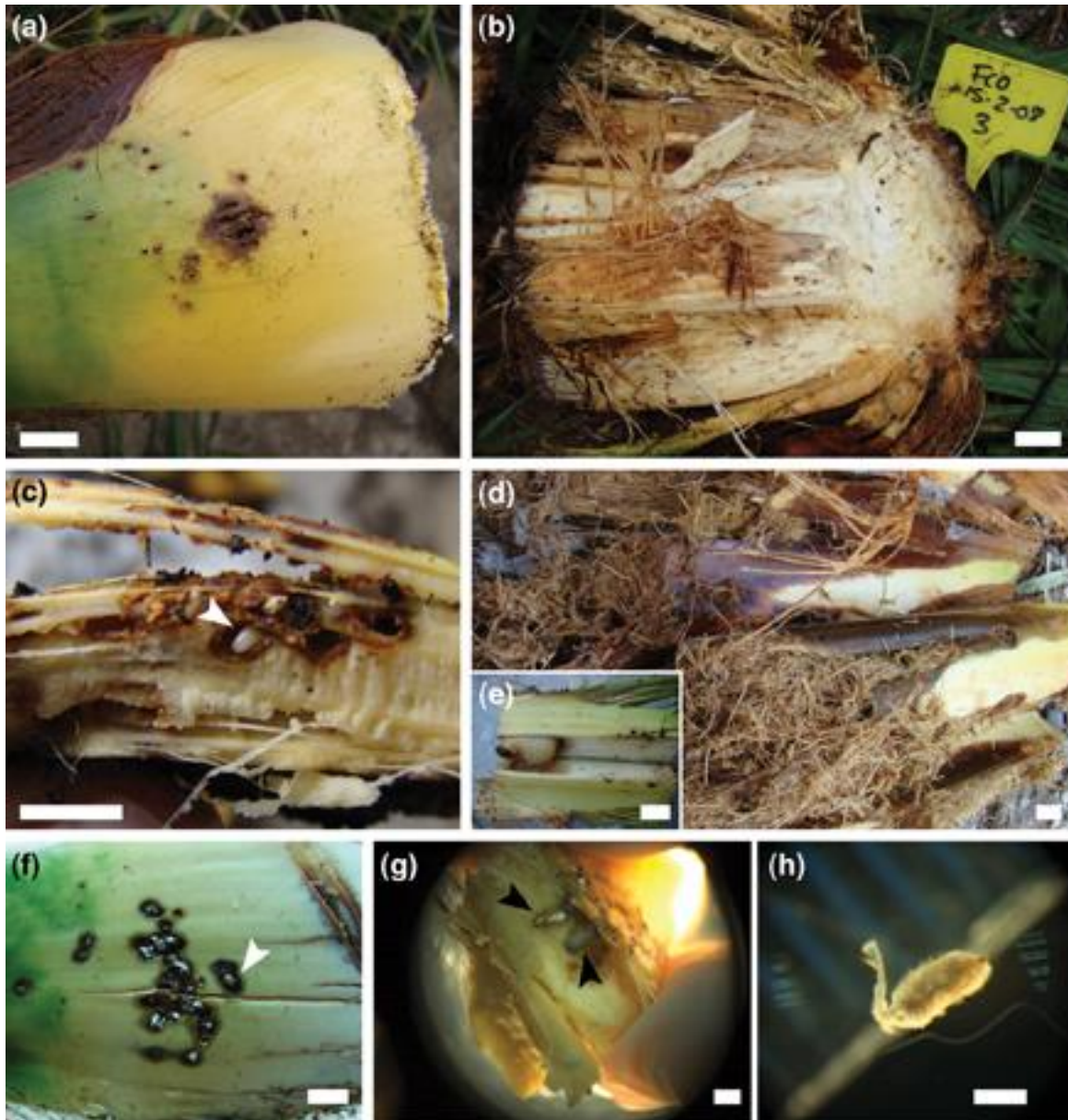


Fig. 7. RPW activity in artificially infested palms under semi-field conditions. a) RPW feeding galleries, Feb 2008 experiment (control). b) Dissection of palm from Feb 2008 experiment without signs of RPW larvae (control). c) RPW egg (arrowhead) on palm petiole Apr 2008 experiment (control). d) Palm with signs of RPW larval feeding Jun 2007 experiment (control). e) Detail of d with RPW larvae feeding on palm tissue. f) RPW feeding galleries colonized by *B. bassiana* (arrowhead). g) RPW infected egg (arrowhead) within palm tissue. h) Detail of Fig. g showing infected egg.