

#1: Transposon-based germ-line transformation of the Coffee Berry Borer, *Hypothenemus hampei*: Opportunities for functional genomics
Acevedo, Flor E.; Barrera, Carlos; Aluvihare, Channa; Harrell, Robert A.; Benavides, Pablo.

The coffee berry borer (CBB), *Hypothenemus hampei* (Coleoptera: Curculionidae), poses a serious threat to worldwide coffee production, causing annual losses of about \$500 million. This invasive beetle is a very aggressive pest with ecological, behavioral and genetic characteristics that make effective control difficult to achieve. As an effort to improve our understanding of the genetic regulation of the insect's basic biology we developed a transposon-based germ-line transformation protocol. The location of presumptive germ cells as well as main embryological events was documented in CBB by observing alive embryos under a composed microscope. Embryogenesis of CBB is typical of long germ-band insects and took approximately 144 hours at 27°C. The syncytial blastoderm stage was observed at approximately 8.5 hours after egg laying, and pole cells were visible at the posterior end of the embryo. To transform the insect's germ cells two sets of injections were performed: In the first set, a mixture of two vectors and two transposase expressing plasmids (pPB (piggyBac) 3xP3ECFP + PBhs¹SST & pMos (Mos) 3xP3DsRed + PKhsp82Mos) was applied. From 495 injected embryos, 50.7% G0 adults were recovered and one transgenic line containing pPB 3xP3ECFP was obtained. In the second set a different mixture of vectors and transposase-expressing plasmids was injected (pMinos (Minos) 3xP3DsRed + PHss6hs1LMi20 & pHermes (Hermes) Actin5CEGFP + pBCHsHH) resulting in 144 (82.7%) G0 adults. Three of these adults produced transgenic progeny containing pMinos 3xP3DsRed. These transgenic tools along with transcriptomic and genomic analysis would be useful in functional genomics studies and would potentially lead to the development of new insect control techniques.