AR144

PASA ASSAY FOR DIAGNOSING INSECTICIDE RESISTANCE IN THE HORN FLY POPULATION IN RONDONIA

BRITO, L.G. 1; BARBIERI, F.S. 1; OLIVEIRA, M.C.S. 2; GUERRERO, F.D. 3
1 Embrapa Rondônia, Porto Velho, RO, Brazil. 2. Embrapa Cattle-Southeast, São Carlos, SP, Brazil. 3. Knipling-Bushland U.S. Livestock Insects Research Laboratory, USDA/ARS, Kerrville, TX, US.

Knockdown (kdr) resistance is caused by a reduction in the sensitivity of the insectnnervous system to pyrethroids. Kdr resistance in field populations of horn flies can severely limit pyrethroid usefulness in fly control programs. Early detection and characterization of kdr resistance are critical to the development of resistance management strategies. Studies at the Embrapa Rondonia experimental farm, Porto Velho, RO and at Knipling-Bushland U.S. Livestock Insects Research Laboratory, USDA/ARS, Kerrville, TX were conducted to verify the genotypes of the Embrapa Rondonia horn fly population. First, the population was assessed using cypermethrin-impregnated filter papers produced at the Embrapa Rondonia Animal Health Laboratory. Horn flies from an untreated cattle herd were caught with a sweep net and used for bioassays. Flies were exposed for two hours to filter papers treated with technical grade cypermethrin in acetone, using cypermethrin concentrations from 0.01 µg to 3,200 µg/cm^2. Control flies were exposed to filter papers treated only with acetone. Three groups of ten flies were exposed at each concentration. All flies exposed to cypermethrin concentrations between 800-3,200 µg/cm^2 died. Genomic DNA was isolated from individual adult flies that survived bioassay concentrations of 200-400 µg/cm^2 and 30 flies tested by PASA (PCR amplification of specific alleles) assay for the presence of a specific nucleotide substitution in the sodium channel gene sequence that has been associated with kdr resistance in horn flies. PASA was performed using two parallel PCRs, with each PCR containing three sets of primers, and genomic DNA to detect pyrethroid resistance-associated nucleotide differences in individual flies. Two primers, FG 234 and FG 243, provided a positive control PCR product while the products of primer FG 138 with primer FG 130 (reaction 1) or with primer FG 134 (reaction 2) produced diagnostic products for genotyping the kdr allele. Reaction products were visualized after 4% agarose gel electrophoresis followed by UV illumination after staining with Syber Green. The kdr allele was not detected in flies from the Embrapa Rondonia population, which was considered a pyrethroid susceptible homozygous (SS) population. This result was expected for this horn fly population as there is no report of treatment with pyrethroids in the last six years.
Palavras-chave: Pyrethroid resistance, Haematobia irritans, kdr, allele characterization, Rondonia.