PASA assay for diagnosing insecticide resistance in the horn fly population in Rondônia - Brito L.G.[1]*, Barbieri F.S.[1], Oliveira M.C.S.[2], Guerrero F.D.[3]

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Knockdown (kdr) resistance in field populations of horn flies can severely limit pyrethroid's 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 Rondônia and at Knipling-Bushland U.S. Livestock Insects Research Laboratory were conducted to verify the genotypes of the Embrapa Rondônia horn fly population. The population was assessed using cypermethrin-impregnated filter papers. Horn flies from an untreated cattle herd were caught with a sweep net and used for bioassays. Three groups of ten flies were exposed for two hours to filter papers treated with cypermethrin concentrations from 0.01 µg to 3,200 µg/cm^2. Control flies were exposed to filter papers treated only with acetone. 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 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 flies. The kdr allele was not detected in flies from the Embrapa Rondônia 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.

Key-words: pyrethroid resistance, *Haematobia irritans*, allele characterization

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

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Introduction

The horn fly, *Haematobia irritans* (L.), is the second most serious economic pest of cattle in Brazil, costing producers with pastured cattle an estimated US$ 865 million annually (Bianchin et al., 2006) . Horn fly control primarily has been based on the use of insecticides, and this control strategy has led to resistance to most commercially available products (Byford et al., 1985; Drummond, 1987; Kunz et al., 1991; Guglielmone et al., 1999; Bianchin and Alves, 2002). Currently, the majority of products used for horn fly control are either pyrethroids or organophosphates (OPs). The physiological and biochemical mechanisms associated with pyrethroid resistance in the horn fly include reduced target site sensitivity, reduced penetration and increased metabolism or detoxification (Byford et al., 1985; Sparks et al., 1990). Bull et al. (1988) provided direct evidence that enhanced metabolic detoxification can contribute to pyrethroid resistance in horn flies, but they acknowledged that target site insensitivity is the major factor in pyrethroid resistance in the horn fly. The target site insensitivity resistance mechanism is commonly referred to as knockdown resistance (kdr). Guerrero et al. (1997) identified two mutations (designated kdr and superkdr) in the horn fly's sodium channel gene that are associated with pyrethroid target site resistance. Knockdown (kdr) resistance is caused by a reduction in the sensitivity of the insect's nervous system to pyrethroids. The presence of kdr resistance in the field has severe consequences for sustained use of pyrethroids to control horn flies. Early detection and characterization of kdr resistance are therefore critical to the development of strategies for resistance management. The purpose of this study was to use the PCR assay to verify the target site resistance mechanism in various field populations of horn flies in the state of Rondônia and to determine if the sodium channel genotype is associated with survival of flies with different doses of pyrethroid insecticides by the impregnated filter paper method (Sheppard and Hinkle, 1987).

Materials and Methods

Studies were carried out at the experimental farm of Embrapa Rondonia, Porto Velho, RO and at Knipling-Bushland U.S. Livestock Insects Research Laboratory, USDA/ARS, Kerrville, TX to verify the genotypes of the Embrapa Rondônia horn fly population. In the first step, the population was assessed by using impregnated filter papers produced at the Embrapa Rondônia Animal Health Laboratory by using technical cypermethrin. Horn flies were caught with a sweep net from an untreated cattle herd and used for bioassays. The flies were exposed for two hours to technical grade cypermethrin diluted with acetone, presented on filters papers treated with insecticide concentrations ranging from 0.01 µg to 3,200 µg/cm². Controls flies were exposed to filter papers treated only with acetone. Three groups of ten flies were exposed to each concentration. Fly mortality was determined after two hours of exposure; flies unable to walk were considered dead. Three replicates of approximately 25 flies each were used for each insecticide concentration. Genomic DNA was isolated from individual adult flies that survived bioassay concentrations of 200-400 µg/cm², because all the flies exposed to cypermethrin concentrations between 800-3,200 µg/cm² died. Thirty flies were tested by the PASA assay (PCR amplification of specific alleles) 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. In each reaction, two control samples of flies were used (susceptible and resistant controls). Reaction products were visualized by 4% agarose gel electrophoresis followed by UV illumination after staining with Syber Green.

Results and Discussion

The kdr allele was not detected in flies from the Embrapa Rondônia population, which was considered a pyrethroid susceptible homozygous (SS) population. Synthetic pyrethroids have been used as insecticides since the early 1980s and have rapidly disseminated worldwide. According to SINDAN (2010) there are 67 products for horn fly control registered for cattle use in Brazil. About 75% of these products contain pyrethroids, including associations with other insecticides or synergists. Recently, Sabatini et al. (2009) detected horn fly resistance to pyrethroids in three populations in Rondônia (Montenegro, Ariquemes 1 and Ariquemes 2), in which kdr allelic frequencies between 7 to 20% were observed, a fact that was not observed in the Embrapa Rondônia population. One possible explanation for the absence of kdr alleles in this population may be related to the horn fly control strategy in the experimental field of Embrapa in Porto Velho. The dairy herd where the flies were collected had not received specific treatment for horn fly control in the last six years. Instead, the fly population this area was controlled exclusively by the use of the beetle *Digithontophagus gazella*. This biological control meant that resistance alleles to pyrethroid pesticides did not become established in this horn fly population.

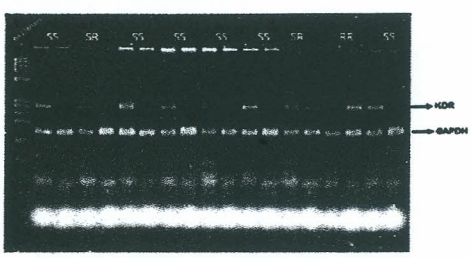

Figure 1: Agarose gel stained with 4% GelStar with the PCR products of DNA samples of flies of Embrapa Rondônia population genotyped for KDR mutation, which confers resistance to pyrethroid pesticides. Where: φX 174 Hae III = standard size pairs; SS = samples of flies with susceptibility genotype; three last flies are the sample controls: SR = control sample of heterozygous flies; RR = control sample of homozygous R-as with resistance genotype and SS = control sample for homozygous flies with resistance genotype; KDR = the band at 286 bp, which enables diagnosis of the mutation; GAPDH (Glyceraldehyde 3 - phosphate dehydrogenase) = band of about 194 bp, used as control of the PCR reaction

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