BIOLOGICAL CONTROL

Use of Serological Techniques for Determination of *Spodoptera frugiperda* (J E Smith) Predators (Lepidoptera: Noctuidae)

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ABSTRACT - *Spodoptera frugiperda* (J E Smith) is an important pest of several crops, but especially on maize in Brazil. The implementation of biological control measures hinges on the identification of its predators and other natural enemies. As a means of identifying predators, antibodies against *S. frugiperda* eggs were generated by inoculating rabbits with macerated *S. frugiperda* eggs, and the production of antibodies against *S. frugiperda* egg proteins was verified by double immunodiffusion (DID). These antibodies were then utilized in another serological technique, counterimmunoeletrophoresis (CIE), to identify insects that could have ingested *S. frugiperda* eggs. Macerates of entire insects collected in maize plantations and of individual parts of their digestive tract, including the crop, were the source of antigens in the CIE, while predators fed *S. frugiperda* eggs in the laboratory served as the control. Antibodies produced by the inoculated rabbits were effective in detecting *S. frugiperda* egg proteins, especially if crop macerates were used as antigens. Among the species of insects collected from maize plantations, *Lagria villosa* Fabricius (Coleoptera: Lagriidae) and a species of Lygaeidae (Hemiptera) were identified as possible *S. frugiperda* predators.

KEY WORDS: Fall armyworm, natural enemy, double immunodiffusion, counterimmunoelectrophoresis

The fall armyworm *Spodoptera frugiperda* (J E Smith) is considered the principal pest of maize in Brazil, causing yield losses that can vary between 15% and 60%. Insecticides are the most commonly utilized control strategy but their use has not given effective control of *S. frugiperda* (Cruz 1997) and, therefore, integrated pest management strategies using predators are under investigation (Batista Filho *et al* 2003). Direct observation (Valicente & Barreto 1999, Oliveira *et al* 2004) and the use of entomopathogenic agents (Monnerat *et al* 2007) have been used in the search for natural enemies of *S. frugiperda*.

Surveys on the natural enemy fauna associated with *S. frugiperda* in maize revealed several predators and parasitoids species, pointing to their importance in the natural regulation of the population density of this pest. The role and pervasiveness of natural enemies must be considered if the adoption of any biological control measure against *S. frugiperda* is to be successful (Silva *et al* 1997, Figueiredo *et al* 2006).

In order to implement biological control programs, information can be gathered from surveys of natural enemies by employing techniques like cages and barriers, direct observation and evidence of natural enemy feeding using serology and ELISA tests (Luck *et al* 1988). Serological techniques are based on antibody-antigen reactions between a specific antiserum generated against the species of interest, and antigens within potential enemies, in particular their digestive tracts, that may have ingested the species of interest. Such techniques have been used successfully in the determination of predators of several insect orders (Healy *et al* 1975, Serrão *et al* 1997, Taylor 2004). Although PCR-based techniques are becoming more common, serological techniques are the most accessible method for detecting natural predators (Bouchard *et al* 2003). In this study, we demonstrate the viability of using serology in identifying predators of *S. frugiperda* eggs.

Material and Methods

Obtaining antigens and antiserum. Frozen (egg-1) and fresh eggs (egg-2) of *S. frugiperda* obtained in the laboratory were used as antigens. Ninety-five mg of eggs were macerated in 1 ml of 1 M sodium phosphate buffer (PBS) (pH 7.2), centrifuged at 10,000 x *g* for 5 min and the supernatant was collected and emulsified with Freund's incomplete adjuvant

at a ratio of 1:1 (v/v).

The antigen was injected intraperitoneally into two rabbits (2.5 kg each) as two 0.5 ml doses of each antigen (equivalent to 600 μg of protein) at a 15 day interval. Before the inoculation, blood samples were collected as control. The antiserum was collected after 10 ml of blood had been drawn from each rabbit, from a cut in one of the ears, and maintained at room temperature for clotting. Afterwards, the blood clot was discarded and the serum was stored at -20ºC for further use.

Test of antiserum and determination of dilutions. The double immunodiffusion (DID) technique in agar gel was used to verify the production of antibodies against *S. frugiperda* egg antigens in those rabbits previously inoculated and to determine its titer. DID was the technique chosen due to the large availability of antigens in this procedure.

Two-fold dilutions (pure, 1:2, 1:4, 1:8, 1:16 and 1:32) of the rabbit antiserum were made in PBS and placed in wells made of 0.9% agar gel in Tris buffered saline (TSB), pH 8.4, surrounding a central well, where the *S. frugiperda* egg macerate was placed. The system was maintained for 24h in a humid chamber at room temperature for incubation and after this period the presence of precipitation lines was observed. The maximum dilution of the *S. frugiperda* egg antigens that can be detected by the antiserum was evaluated in reactions of *S. frugiperda* egg macerate with different dilutions in PBS with the rabbit antiserum.

Use of antiserum to determine *S. frugiperda* predators. The method of counterimmunoelectrophoresis (CIE) was used to determinate the predators of *S. frugiperda*. The availability of antigens present in the samples was unknown, thus CIE was the chosen technique due its sensitivity.

The antigens used in this technique were organized as i) insects collected in maize plantations macerated in 100 μl of PBS; ii) parts of the digestive tract of *Doru luteipes* (Scudder) (Dermaptera: Forficulidae) fed in the laboratory with *S. frugiperda* eggs macerated with 100 μl of PBS; iii) *D. luteipes* fed in the laboratory with *S. frugiperda* eggs macerated with 100 μl of PBS. *Dorus luteipes* is a known *S. frugiperda* predator (Reis *et al* 1988). Predatory insects fed on eggs were analyzed 48h after feeding. Whole insects or parts of their digestive tracts were macerated, centrifuged for 10 min at 1000*g* and the supernatant was collected and used as antigen.

The antigens were utilized to evaluate the antigenicity of the serum obtained from the rabbits, both before and after inoculation. Slides covered with 0.7% agar gel in 0.1 M Tris-HCl buffer pH 8.6 were prepared. Each antigen was tested with three dilutions of antiserum (pure, diluted 1:2 and 1:4) applied in the gel wells. The slides were placed in a cube filled with Tris-HCl buffer, ran at 5 mA for 2-3h, and stained with Coomassie Blue for 20 min.

Results

The DID technique showed precipitation lines up to the 1:4 dilution for antiserum produced by the rabbit inoculated with the antigen egg-1 and up to the 1:8 dilution for antiserum produced by the rabbit inoculated with the antigen egg-2, confirming the production of antibodies against *S. frugiperda* egg antigens (Fig 1).

In the CIE tests assessing *D. luteipes* fed on *S. frugiperda* eggs, the entire insect macerate was negative for all dilutions tested. However, when the antiserum was tested with macerate of the individual parts of the digestive tract, results were positive (Fig 2), mainly when the crop was used as antigen, and showed bands the same as the lines produced when *S. frugiperda* eggs were used as antigen. The gut macerates showed weaker lines when compared with crop and eggs (Fig 3).

The beetle *Lagria villosa* (Fabricius) (Coleoptera: Lagriidae) and a species of Lygaeidae (Hemiptera) collected in the field were evaluated for the presence of *S. frugiperda* eggs using the antiserum. Positive reactions were not observed when the antiserum was used to assess the macerate of the whole insect or the digestive tract of the Lygaeidae. However, for some specimens of *L. villosa*, a strong reaction was observed for parts of their digestive tract, specifically the crop, and a weak positive reaction was observed for the macerate of the whole insect (Fig 4).

Discussion

The antisera produced from newly laid and frozen eggs showed no difference in their ability to recognize antigens, demonstrating that the freezing of the sample from which

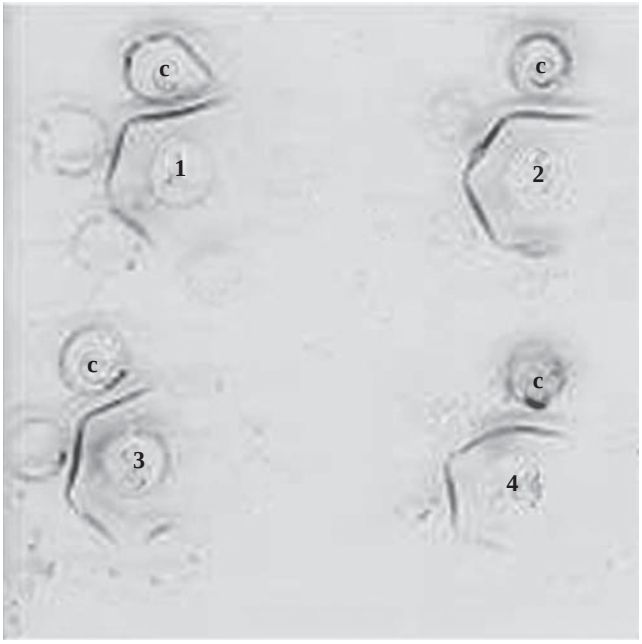

Fig 1 Double immunodiffusion in 0.9% agar gel with pure antigen in the center and decreasing dilutions of antiserum clockwise until the pure form (1 and 2) and with pure antiserum in the center and decreasing dilutions of antigen clockwise, until the pure form (3 and 4). The antigens used were egg-1 in 2 and 4; egg-2 in 1 and 3.

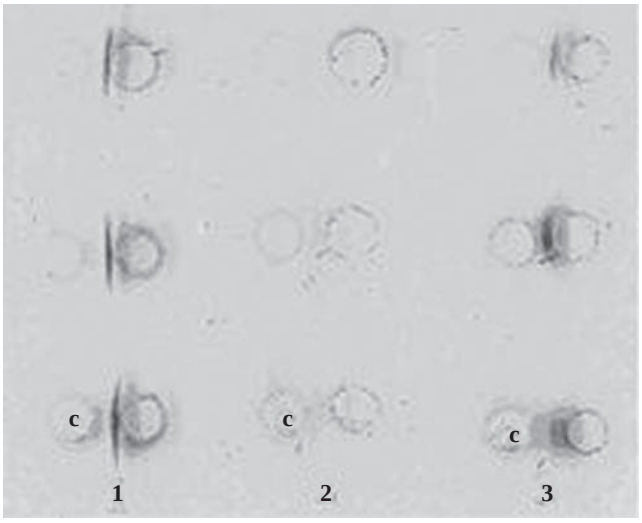

Fig 2 Counterimmunoelectrophoresis in 0.7% agar gel: decreasing dilutions of antiserum (c), treated with the following pure antigens: 1) eggs (standard); 2) extract of whole body of *Doru luteipes* and 3) extract from crop of *D. luteipes*.

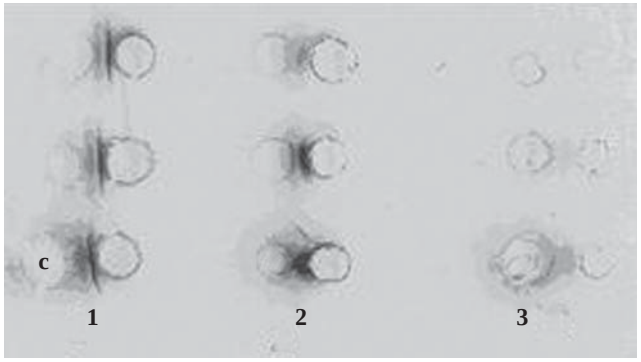

Fig 3 Counterimmunoelectrophoresis in 0.7% agar gel: decreasing dilutions of antiserum until the pure form (c) treated with the following antigens: 1) eggs (standard); 2) extract from crop of *Doru luteipes*; 3) extract from whole gut of *D. luteipes*.

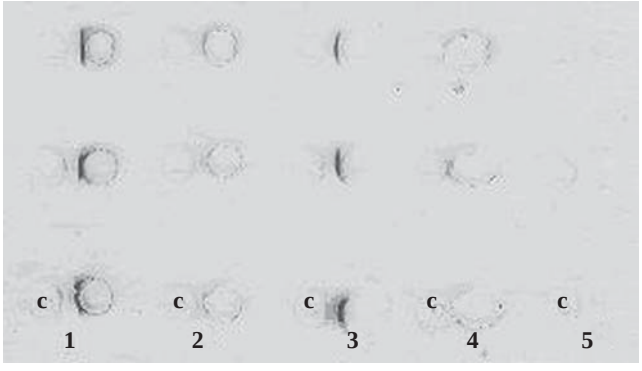

Fig 4 Counterimmunoelectrophoresis in 0.7% agar gel: decreasing dilutions of antiserum, until the pure form (c), with the following antigens: 1) eggs (standard); 2) extract of *Lagria villosa* using control antiserum; 3) extract from crop of *L. villosa*; 4) extract from Lygaeidae using control antiserum and 5) extract from digestive tract of the Lygaeidae.

antiserum will be produced has no impact on the antiserum produced. The antiserum produced in rabbits had excellent strength considering the positive results observed up to 1:8 dilution, thus demonstrating that the antiserum contains a reasonable amount of antibodies that are able to recognize *S. frugiperda* antigens.

Predator identification should be preferentially performed using parts of the preadtors's digestive tract. Tests with the gut showed weak precipitation, probably because the food had already suffered enzymatic action at in this region (Terra 1988, Guedes *et al* 2007). Once the ingested eggs are exposed to the proteases in the digestive tract of the predator, antibodies in the antiserum are unable to recognize the antigenic proteins from the eggs. The crop, which corresponds to a storage organ of the gut of the predator, gave the best results.

The tests carried out with entire insects were generally negative, perhaps because the specimens used in the tests had not fed on the source from which the antiserum was obtained. Another possible explanation is that when macerated with the whole predator body, the antigen is diluted beyond the detection limits of the antiserum. A third possibility is that the large protein diversity present in the macerate of the entire animal had clogged the pores of the gel, hindering the immune reaction.

The detection of a prey insect in the predator gut depends on the size, the size of and time after the meal, the digestion rate, the feeding strategy (sucking *versus* chewing), the abundance of closely related prey (taxonomically), and the sensitivity of the test (Luck *et al* 1988). The serological techniques used in this study have been established for other species and showed satisfactory results in identifying predators (Serrão *et al* 1997, Hoyt *et al* 2000).

Lagria villosa has not been previously described in the literature as a predator of *S. frugiperda*. It is unlikely that the positive results found in this study could be explained by cross reactivity of antibodies since some individual predator specimens obtained from the field showed negative results against the antiserum tested, suggesting that the antibody was not subject to an unspecific reaction.

The techniques described in this study provide an alternative approach for the detection of *S. frugiperda* proteins in macerated parts of the digestive tract of potential predators. Biochemical, molecular and immunoenzymatic methods have been described for the detection of pest species (Agustí *et al* 1999, Rosel & Kocher 2002, Symondson 2002). The techniques described here, although less sensitive and only qualitative, have the advantage of being simple, with minimal methodological limitations, and allow for a quick analysis of a large number of samples (Taylor 2004). This study shows that the CIE technique is efficient in the identification of insects that fed on eggs of *S. frugiperda*, and is especially advantageous when crop macerates were used as antigens. Our data confirm that *D. luteipes* is a predator of *S. frugiperda* eggs, and demonstrate, for the first time, that *L. villosa* may be a natural predator of *S. frugiperda* eggs in maize. Further work is needed to explore the possibility of using these predators in biological control of *S. frugiperda* in maize.

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