Quantifying the Organophosphate Resistance of Two Geographically Separate Cotton Aphid Populations by Means of a Polyacrylamide-Gel Electrophoresis (PAGE) Technique

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ABSTRACT

Analysis of isoenzymes of different aphid populations showed differences in molecular densities after the addition of different concentrations of an organophosphate. By adding the insecticide, isoenzyme bands of one population get less dense or disappear, while there were minimal changes in the density of the same bands of another geographically separate population. The surplus quantity of active esterase available for binding to the insecticide is represented by a higher density band, possibly reflecting resistance. The technique shows potential to demonstrate resistance of pest populations of red bollworm (Diparopsis castanea), American bollworm (Helicoverpa armigera) and spiny bollworm (Earias spp, probably E. biplaga), the man pests of cotton, against organophosphates. It is a potential tool to quickly monitor resistance on request of the cotton producer.

Introduction

Resistance to insecticides, especially organophosphates, is presumed to have caused an increase in aphid and bollworm populations in certain cotton- production regions of South Africa. During the latter part of the 1980's and the early 1990's reports of inadequate *A. gossypii* control began to appear. Increased insecticide dosages were necessary to achieve some measure of control. This situation has persisted and resistance to pesticides is given as the reason for this state of affairs. Inoue (1987) and Takada and Murakami (1989) found a good correlation between high esterase activity and resistance to organophosphorous compounds. In this study the polyacrylamide gel electrophoresis (PAGE) technique was used to examine the differential response of esterases to monocrotophos in two geographically separate cotton aphid populations.

Materials and Methods

Cotton leaves bearing *A. gossypii* were collected from two localities, namely Rustenburg and Settlers. After other insects and parasitised aphids were removed, the remaining aphids were collected in a petri dish by brushing the leaves with a mite brush. The aphids were massed and extraction buffer was added (Devonshire, 1975). The buffer consisted of $Na_2HPO_4.H_2O$, EDTA, triton -X-100 and sucrose. Extraction was performed by homogenising the aphids in the buffer with a pre-cooled mortar and pestle. The homogenate was then centrifuged at 11000 rpm for 10 minutes. Esterases and esterase/insecticide mixtures were run on a 7.5% polyacrylamide gel at 15mA 250V for 90 minutes. Water was constantly run through the gel to maintain a constant temperature of 10°C. Staining was at room temperature with 0.02% 1-naphtyll acetate in 0.2% Fast blue BB salt at pH 6.6. Densities of the isozyme were measured by Alpha Ease software for Windows, version 3.24.

Results

The isozyme composition of the Rustenburg population is indicated in Figure 1. Different populations exhibit different isozyme compositions. After the addition of monocrotophos, the isozyme bands became less dense (Figures 2 and 3) and migrated slower on the gel (Figure 4), depending on the molecular bonds of the insecticide molecules to the specific esterases.

Densities of bands of the Settlers population did not vary greatly after an increase in the amount of monocrotophos added (Table 2), while the density was much lower in the Rustenburg population.

Conclusion

The critical quantity of insecticide required to cause a band(s) to disappear will vary among geographically separate populations that exhibit varying degrees of resistance. Analyses of isozymes of different aphid populations showed differences in molecular densities after the addition of a range of concentrations of the organophosphate, monocrotophos. When the insecticide was added, the isozyme bands of one population became less dense or disappeared, whereas the same bands of another geographically separate population showed minimal changes in density. The surplus active esterases available for binding to the insecticide is represented by a higher density band (reflecting resistance).

This phenomenon may indicate that the Settlers population is more resistant to monocrotophos than the Rustenburg population. However, these results should be supported by probit analysis (LD_{50} and LD_{90}) and protein analysis before any control measures can be recommended. This technique could also be used to demonstrate the resistance of pest populations of red bollworm (*Diparopsis castanea*), American bollworm (*Helicoverpa armigera*) and spiny bollworm (*Earias* spp., probably *E. biplaga*), the main pests of cotton, to organophosphates. It is a potential tool which could be used to monitor resistance quickly on request of the cotton producer. Protein analysis of esterases, LD_{50} and LD_{90} must also be done to confirm the results obtained through PAGE.

Table 1. Variation in density of esterases from the Rustenburg population at different concentrations of monocrotophos.

Insecticide ai/ l	Density of bands
0.043g	85
0.049g	54
0.055g	52
0.061g	40
0.067g	40
0.072g	37

Table 2. Variation in density of esterases from the Rustenburg and Settlers populations at different concentrations of monocrotophos.

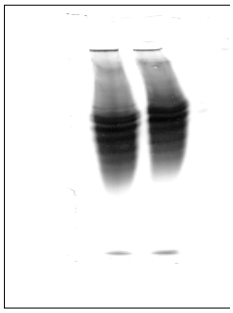
Insecticide	Density of bands	
(g ai./l)	Rustenburg	Settlers
	22	89
0.092g		
0.097g	17	88

Figure 1. Esterase patterns of Aphis gossypii from Rustenburg population without the addition of insecticide.

Lanes 1-10 5µl of aphid extract + 3 µl phosphate buffer

Figure 2. Esterase bands of the Rustenburg population at different concentrations of monocrotophos.

Lane 1 5µl of aphid extract + 3 µl phosphate buffer
Lane 2 5µl of aphid extract + 3 µl mc[1] (0.043g a.i./l
Lane 3 5µl of aphid extract + 3 µl mc (0.049g a.i./l
Lane 4 5µl of aphid extract + 3 µl mc (0.055g a.i./l
Lane 5 5µl of aphid extract + 3 µl mc (0.061g a.i./l
Lane 6 5µl of aphid extract + 3 µl mc (0.067g a.i./l
Lane 7 5µl of aphid extract + 3 µl mc (0.072g a.i./l
[1]mc = monocrotophos

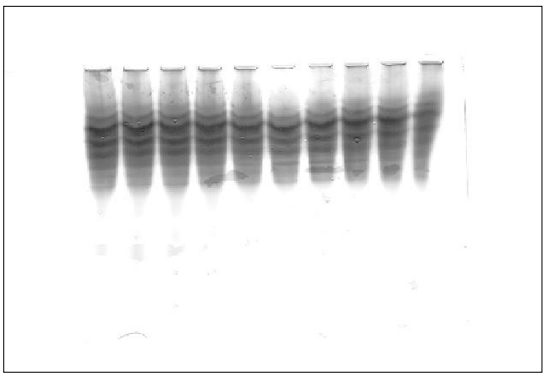
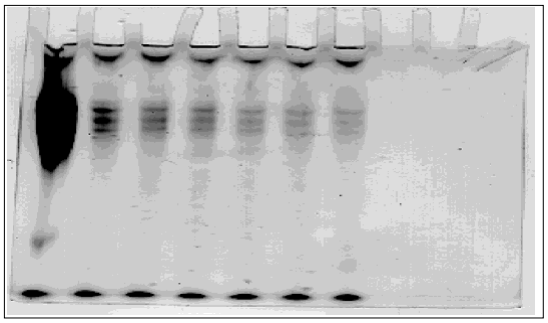


Figure 3. Esterase patterns from Rustenburg and Settlers populations.

Lane 1: 5µl Rustenburg aphid extract+ 3µl phosphate buffer



Lane 2: 5µl Rustenburg aphid extract+ 3µl phosphate buffer
Lane 4: 5µl Rustenburg aphid extract+ 3µl monocrotophos (0.092g ai./l)
Lane 5: 5µl Rustenburg aphid extract+ 3µl monocrotophos (0.097g ai./l)
Lane 7: 5µl Settlers aphid extract+ 3µl phosphate buffer
Lane 8: 5µl Settlers aphid extract+ 3µl phosphate buffer
Lane 9: 5µl Settlers aphid extract+ 3µl monocrotophos (0.092g ai./l)
Lane 10: 5µl Settlers aphid extract+ 3µl monocrotophos (0.092g ai./l)

Figure 4. Density of bands of the Rustenburg and Settlers populations relative to the migration distance of isozymes in the lanes on the gel.