DEVELOPMENT OF RESISTANCE TO INSECTICIDES BY POPULATIONS OF PROSTEPHANUS TRUNCATUS (HORN) (COLEOPTRA; BOSTRICHIDAE)

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Abstract

Prostephanus truncatus (Horn), is a major pest of farm-stored maize and dried cassava in Africa. Like other Bostrichidae it is readily controlled by pyrethroid insecticides but not by organophosphates. In Tanzania a combination of permethrin and pirimiphos-methyl applied by farmers at nominal dosages of 3.3ppm and 16.6ppm respectively has been used successfully to control a spectrum of storage insect pests including *P. truncatus*. Pirimiphos-methyl is particularly effective against *Sitophilus* species and other common storage insect pests whereas pyrethroids are not.

Recent laboratory studies have demonstrated the ability of *P. truncatus* to develop resistance rapidly to permethrin and pirimiphos-methyl. Experiments were undertaken with populations from different geographical regions, from areas in which insect control measures had been absent or widely applied, in Meso America and Africa. Adults subjected to maize grain treated with increasing dosages of insecticide dusts survived application rates which would be expected produce complete kill in the field. Resistance was induced equally quickly in all strains. LD_{99}s of resistant strains were all much greater than those of parent susceptible strains in filter paper discriminating dose tests.

The ability of *P. truncatus* to develop resistance quickly has major implications for future control strategies. As it is inappropriate to use fumigation procedures at farm level it is essential to stimulate the development of alternative control procedures, especially the use of the predator *Teretriosoma nigrescens*.

INTRODUCTION

Prostephanus truncatus (Horn) is a major pest of farm-stored maize and dried cassava in Africa. Like other Bostrichidae of economic importance in the post-harvest food sector is is readily controlled by pyrethroid insecticides (Golob et al 1985) but not by organophosphates. The application of permethrin dust has become a major component in the campaign to control *P. truncatus* in East Africa.

Production of maize stored for home consumption in Tanzania necessitated a change in storage practices. Traditionally, farmers stored maize on the cob. In order to apply insecticide dust efficiently farmers were required to shell their cobs and to store grain. This change, whilst enabling *P. truncatus* to be controlled, allowed other major storage insect pests, particularly species of *Sitophilus*, to become more successful. *Sitophilus* was not controlled effectively by pyrethroids and so a second compound had to be introduced into the dust applied by farmers. In Tanzania farmers now apply a dust containing both permethrin and pirimiphos-methyl to control all on-farm storage insect pests.

In Tanzania a cocktail containing 0.3% permethrin and 1.6% pirimiphos-methyl is used by farmers, at a rate of 100g/90kg of grain, to protect maize. At the time of writing there have not been any published reports of treatment failures and evidence gained directly by questioning farmers would suggest the treatment is performing effectively. However, synthetic pyrethroids are known to select resistance in insect populations very readily (eg Denholm et al, 1983) and Golob (1988) predicted that *P. truncatus* would develop resistance to permethrin, necessitating the use of alternative control procedures.

A series of laboratory experiments were undertaken to enable predictions concerning the potential rate of resistance development of *P. truncatus* to permethrin. Similar observations were made when insects were selected with pirimiphos-methyl. Experiments were undertaken with strains of the insect of different geographical origin.

METHODS AND MATERIALS

Insect were obtained from Tabora Region, Tanzania in October 1981, from Mexico in March 1981 and from Lomé, Togo in 1985. Stocks were maintained at 27°C and 70% rh until 1987 when the temperature was changed to 30°C. The generation time at 30°C and 70% rh was approximately 28 days.

None of the three strains had been subjected to any regular insecticide selection pressure specifically designed for its control.

Selection by Admixture

Selection with permethrin or pirimiphos-methyl was applied throughout the life history by culturing beetles at 30°C and 70% rh on whole maize grain (yellow American No 3). The method employed was based on that of Dyte and Blackman (1967). The culture medium was prepared by submerging 500g lots of maize in 250ml of hexane containing the appropriate amount of insecticide. Initially technical grade insecticide was made up to a 100ml solution with a solution of petroleum ether (bp 60-80), Risella oil and acetone (3:1:1) and 1ml of this was diluted to 250ml with hexane. The hexane solution was mixed with maize in a round-bottomed flask and then rotary evaporated for 10-15 minutes to remove all the solvent. The dried maize was mixed again before use as a culture medium. Each maize concentration was made up separately and freshly prepared solutions were used for each generation of selection. The dosages applied to the grain were checked by well established GLC procedures (Golob et al 1985) after each application.

Four selection lines of the Mexican and Tanzanian strains were started by placing 80 unsexed adults in glass jars containing 200g of maize treated nominally with 0.125 and 0.25ppm permethrin and 1.25 and 2.5ppm pirimiphos-methyl respectively. The Togolese strain was selected using the same dosage of pirimiphos-methyl but having grain treated with 2.0ppm permethrin. Parent adults were removed after 4 weeks and discarded after the mortality was recorded. Progeny were removed after a further 4 weeks and live adults divided into lots of 80 which were used to set up fresh cultures in 200g lots of treated maize. Progeny derived from cultures treated with the lower of the two initial dosages were placed on grain treated at the rate of the initial higher dosage. Progeny from maize treated with the higher dosages were started on maize treated nominally with 0.5ppm or 3.5ppm permethrin or pirimiphos-methyl respectively. The Togolese progeny were placed on 2.5ppm permethrin.

This procedure was repeated with each subsequent generation. Selection continued through to the F7 generation at which time adults were selected with 7ppm permethrin and 30ppm pirimiphos-methyl.

Measurement of Resistance Levels

In order to assess the change in tolerance of the population which survived exposure to treated grain adults from the F6 generation were exposed to filter papers treated with a range of insecticide concentrations. The dose response curves produced were compared to similar curves produced when the adults from the parent population were similarly exposed.

Bioassays were conducted by exposing batches of 40 adults to 7cm Whatman No 1 filter papers treated with 0.5ml of insecticide dilution as described by Anon (1974). Dilutions were prepared in the petroleum ether, Risella oil and acetone mixture mentioned above, which was also used alone to treat controls. Insects were deprived of food for one hour before being placed on the filter paper. Batches of adults were exposed on filter paper for 5 hours. Knockdown and mortality of those placed on pirimiphos-methyl treated papers were observed immediately the exposure period terminated. At the end of the exposure period adults on permethrin-treated papers were placed in small glass vials and left for 24 hours when knockdown and mortality was assessed. This interval was to enable any knocked down individuals to recover. *P. truncatus* individuals remaining knocked down after this period did not recover and eventually died (Golob unpublished information).

RESULTS

Table 1 illustrates the percentage of adult survival of successive generations subjected to treated grain. The results are for the line which was subjected to higher dosages initially. There were large variations between some of the replicates but the trend remained the same throughout the experiment.

Survival on all the permethrin treated grain was at least 60% in all generations up to the F5. It was only the F7 generations which exhibited reduced tolerance to the 7ppm treatments and even then considerable numbers of adults survived, the F6 Togo strain was also more susceptible. The populations were equally successful in developing on pirimiphos-methyl treated grain, at 30ppm there were no indications that this tolerance was becoming reduced.

The responses of the parent adults and F6 populations to filter paper treated with permethrin and pirimiphos-methyl are illustrated in tables 2 and 3 respectively.

The slopes of the probit-regressions for the Mexico and Togo strains treated with permethrin were parallel for both parent and F6 population but those for the latter adults were moved to the right indicating resistance. The tolerance of the Togo strain was greater than the Mexican strain as demonstrated by the resistance factor. With the Tanzanian strain the F6 slope was flatter than for the parent adults and although the LD_{50}s were similar the LD_{99} was much higher for the selected population.

When selected with pirimiphos-methyl all the slopes were parallel with those of the selected strains illustrating resistance. The Tanzanian strain became more resistant upon selection than the other two strains.

Table 1 **Survival of adult *Prostephanus truncatus* exposed to treated maize grain**

A. <u>PERMETHRIN</u> **Dosage in mg/kg**

	0.25 (Parents)	0.42 (F1)	1.01 (F2)	1.95 (F3)	3.60 (F4)	4.02 (F5)	4.6. (F6)	7.3 (F7)
Strain								
Mexico	81.7	81.8	78.4	68.2	68.4	72.2	69.9	54.7
Tanzania	90.0	77.9	75.5	77.1	60.6	71.3	80.1	25.5
	2.3 (Parents)	2.02 (F1)	2.60 (F3)	3.70 (F3)	4.12 (F4)	4.90 (F5)	5.7. (F6)	7. (F7)
Togo	46.5	75.7	64.2	86.8	80.4	81.2	32.3	17.8

B. <u>PIRIMIPHOS-METHYL</u> **Dosage in mg/kg**

	1.98 (Parents)	2.66 (F1)	4.82 (F2)	9.37 (F3)	9.43 (F4)	14.20 (F5)	20.45 (F6)	30.50 (F7)
Strain								
Mexico	65.0	74.2	91.1	71.2	91.8	69.3	90.6	82.3
Tanzania	68.6	64.4	80.3	78.2	78.6	62.8	86.9	67.0
Togo	66.2	73.2	86.1	80.3	94.0	90.4	91.1	83.4

Each datum represents mean of four replicates

Table 2
Response of susceptible parent adult *Prostephanus truncatus* and of F6 progeny reared on maize treated with permethrin to filter papers treated with permethrin.

Strain		Slope	LD_{50}* (%)	LD_{99}* (%)	Resistance Factor LD_{50}	LD99
Mexico	Parent	1.3	0.426	23.991		
	F6	1.4	1.480	64.320	x3.5	x2.9
Togo	Parent	1.8	0.321	6.370		
	F6	1.5	3.376	123.304	x10.5	x19.4
Tanzania	Parent	2.2	0.406	4.567		
	F6	1.1	0.596	96.688	x1.5	x21.2

* Expressed as % active ingredients in non-volatile solvents: W/V

A comparison of probit regressions by maximum likelihood programme provided the following analysis:

i) Mexico	CHI SQ.	d.f.
POSITION	71.85	1
PARALLELISM	0.09	1
TOTAL HETEROG.	22.46	26

ii) TOGO	CHI SQ.	d.f.
POSITION	330.39	1
PARALLELISM	1.41	1
TOTAL HETEROG.	35.97	26 1

CHI SQ at which a 5% probability is greater than CHI SQ

For 1 d.f. = 3.84
For 24 d.f. = 36.4
For 30 d.f. = 43.8

iii) TANZANIA	CHI SQ.	d.f.
POSITION	29.32	1
PARALLELISM	25.41	1
TOTAL HETEROG.	25.33	26

Table 3

Response of susceptible parent adult *Prostephanus truncatus* and of F6 progeny reared on maize treated with pirimiphos-methyl to filter papers treated with pirimiphos-methyl.

Strain		Slope	LD_{50}* (%)	LD_{99} (%)	Resistance Factor LD_{50}	LD_{99}
Mexico	Parent	2.0	1.474	21.148		
	F6	2.3	4.630	49.723	x3.1	x2.4
Togo	Parent	2.6	2.604	20.693		
	F6	2.2	8.088	46.393	x3.1	x2.2
Tanzania	Parent	2.0	1.549	22.939		
	F6	2.5	17.229	240.996	x11.1	x10.5

* Expressed as % active ingredients in non-volatile solvent: W/V

A comparison of probit lives by maximum likelihood programme provided the following analysis:

i) Mexico	CHI SQ.	d.f.
POSITION	72.15	1
PARALLELISM	0.97	1
TOTAL HERETOG.	30.23	26

ii) Togo	CHI SQ.	d.f.
POSITION	109.64	1
PARALLELISM	4.11	1
TOTAL HERETOG.	28.89	26

iii) Tanzania		
POSITION	243.01	1
PARALLELISM	0.11	1
TOTAL HERETOG.	26.84	26

DISCUSSION

Golob et al (1985) found that the mortality of *P. truncatus* was 100% when adults were exposed to maize treated with 2.5ppm permethrin for periods up to 24 weeks after application. When the application was reduced to 1ppm but used in combination with 4ppm pirimiphos-methyl the effect was the same, though the mortality was due principally to the permethrin component. The adults used in that experiment were from the same stock as those used in the experiment reported in this paper.

It is clear from the experiments reported here that *P. truncatus* has the ability to tolerate high dosages of permethrin if the selection pressure is increased gradually. Under African farm conditions the potential for misuse of insecticide is relatively high and it is not unreasonable to assume that both underdosing and overdosing occurs. In both situations residues of active ingredient will be available at different times of the year which *P. truncatus* will be able to tolerate and survive. A gradual selection of resistant populations will occur.

Pyrethroids in general, have limited use for control of stored product insect pests. In Australia where *Rhyzopertha dominica* (Fab) is a major pest bioresmethrin has been used widely for its control. Heather (1986) obtained resistance factors of x256 and x98 for permethrin and deltamethrin respectively when *Sitophilus oryzae* was selected over 25 generations. He found that cross resistance to other pyrethroids was also potentiated.

The organophosphate compounds are known to be not very effective against *P. truncatus* but 100% mortality has been obtained with 10ppm for up to 3 weeks after application with pirimiphos-methyl (Golob et al 1985). In these experiments *P. truncatus* was able to develop a tolerance to pirimiphos-methyl very quickly. The use of a mixture of different chemical components to control insects should reduce the potential rate of a population to develop resistance. However, the effects of selection using individual components would not appear to provide confidence that a mixture would be more effective in suppressing resistance trends. This needs to be assessed.

The application rate of the mixture of permethrin and pirimiphos-methyl used in Tanzania for protection of farm stored maize provides nominal dosage of 3.3 and 17.6ppm respectively. Although these rates would provide grain residues in excess of the maximum residue limits recommended by the Joint Meeting on Pesticide Residues of the UN FAO and WHO (maxima of 2 and 10ppm respectively) considerable loss during application (Golob et al, 1985 and Golob et al in press) would result in much lower residues being achieved in practice. However, even if the nominal dosages of permethrin were achieved the experiments reported herein would indicate they would be insufficient to prevent resistance developing relatively rapidly. Examination in 1986 and 1987 of on-farm treated maize grain after several months storage highlighted the survival of *P. truncatus* even when the farmer was satisfied subjectively with the performance of the permethrin dust she applied (Golob unpublished information). These observations were obtained only 3-4 years after permethrin was introduced to farmers. It is most likely that continous exposure of *P. truncatus* to pyrethroids will result in the use of these compounds becoming less cost effective as they will have to be superceded by alternative measures. It is pertinent now to investigate methods by which reliance on pyrethroids for control of *P. truncatus* in East Africa can be reduced, by using these compounds in conjunction with biological control procedures in a more integrated pest management approach than has been previously.

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DEVELOPPEMENT DE LA RESISTANCE AUX INSECTICIDES PAR LES POPULATIONS DE *PROSTEPHANUS TRUNCATUS* (HORN) (COLEOPTERA : BOSTRYCHIDAE)

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RESUME

Prostephanus truncatus (Horn) est un des principaux ravageurs des stocks de maïs et de cassave séchée en Afrique. Comme d'autres *Bostrychidae*, il est éliminé par les insecticides pyréthrinoïdes mais pas par les organophosphorés. En Tanzanie, un mélange de perméthrine et de pirimiphos-méthyl appliqué par les fermiers aux doses respectiyes de 3,3 ppm et 16,6 ppm, a été utilisé avec succès pour éliminer un large éventail de ravageurs, dont *P. truncatus*. Le pirimiphos-méthyl est particulièrement efficace sur *Sitophilus* et les autres ravageurs communs, tandis que les pyréthrinoïdes ne le sont pas.

De récentes études en laboratoire ont démontré l'aptitude de *P. truncatus* à développer rapidement une résistance à la perméthrine et au pirimiphos-méthyl. Des expériences ont été entreprises sur des populations provenant de diverses régions géographiques, de zones dans lesquelles les mesures d'élimination des insectes étaient absentes ou, au contraire, largement répandues, en Amérique centrale et en Afrique. Les adultes soumis à du maïs traité par des doses croissantes de poudres insecticides ont survécu à des niveaux d'application qui auraient dû produire une mortalité complète. Une résistance a été induite également et rapidement dans toutes les souches. Les DL 99,9 de ces souches résistantes étaient toutes beaucoup plus élevées que celles des parents sensibles dans les essais de discrimination sur papier filtre.

L'aptitude de *P. truncatus* à développer rapidement une résistance a des implications majeures dans la mise en place de futures stratégies de lutte. Etant donné qu'il est malaisé d'employer des procédés de lutte par fumigation dans les fermes, il est donc essentiel d'encourager le développement de méthodes de lutte alternatives, l'utilisation du prédateur *Teretriosoma nigrescens*, en particulier.