EXPERIMENTAL DESIGN AND MODELIZATION AS TOOLS FOR THE DEVELOPMENT OF INSECTICIDE COMBINATION OF DELTAMETHRIN, ORGANOPHOSPHOROUS COMPOUNDS AND PIPERONYL BUTOXIDE AS STORED GRAIN PROTECTANT IN TEMPERATE AND TROPICAL SITUATIONS

Jean NICOLAS(1); Patrick VINCENT(1); Henri TRALONGO(2); P. LANTERI(3); R. LONGERAY(3)

ROUSSEL UCLAF(1) (2) ESCIL(3)

(1) CEBAT - BP.1 13367 MARSEILLE CEDEX 11
(2) 31 quai A.Barbes BP.123 69583 NEUVILLE/SAONE CEDEX
(3) Université Lyon 1 69622 VILLEURBANNE CEDEX

Abstract: Pyrethroids and organophosphorous compounds have different but complementary spectrum of activity against stored grain pests. It is then natural to consider their combination for grain protection.
Concentrations of active ingredients depend on several factors i.e. storage condition, insect spectrum, length of protection period, cost factor... When different insecticides are associated,interactions (negative or positive) have also to be considered.

In order to investigate simultaneously most important factors in the laboratory,keeping experimental workload at a reasonable level, an experimental design based on Doehlert uniform network was used to apply the response surfaces methodology.

Deltamethrin, fenitrothion and chlorpyriphos methyl, three broadly applied grain protectants, with long lasting effect, were chosen for this study,as well as piperonyl butoxide,a synergist for pyrethroids.

Treated grain (wheat) was stored under tropical condition (32°C,grain infested with R.dominica, S.zeamais and T.castaneum) or under temperate condition,(25°C,grain infested with O.surinamensis, S.granarius and T.confusum.
Treatment efficacy was monitored every 4 weeks for one year.
Treatment rates have been determined within a definite experimental domain : deltamethrin ranging from 0 to 0.5 ppm, chlorpyriphos methyl and fenitrothion ranging from 0 to 7.5 ppm and Piperonyl butoxide ranging from 0 to 5 ppm.
Resulting empirical models show clearly deltamethrin and Organophosphorous compounds complementarity against grain pests. Positive interactions between pyrethroids and organophosphorous compounds reduce the synergist interest. Due to different half lifes in grain,optimum combinations at time of treatment depends both on the expected protection period, insecticide nature and storage conditions.

Although generated in the laboratory,these information allow a better understanding of phenomena. They give keys for further field development, where uncontrolable environmental factors seldom permit to get a clear picture of insecticide actions and interactions.

INTRODUCTION

Stored grain is a vulnerable material, exposed to various sources of degradation, such as physical breakage, humidity, mould, insects, rodents etc...

In the particular case of insect degradation, an important protective measure is to treat the grain directly with a residual insecticide.

There are two main insecticide families used for this purpose, i.e. synthetic pyrethroids and organophosphorous compounds. Both families have different protective performances which are a combination of intrinsic insecticidal activity, stability and broadness of insecticidal spectrum.

It is of general experience that these two insecticidal families have different and complementary activities.

In recent years, interest has been growing in favor of combination of Pyrethroids and Organophosphorous insecticides for grain protection, this allowing a reduction of treatment cost, for an improved protection.

In practice, insecticide combination studies are always complex as activities are unlikely to be only additive. Interactions, either positive or negative usually occur. They must be detected and quantified in order to enlighten technical approach, and to not loose opportunities for treatment cost efficiency optimization.

From an experimental point of view, interaction studies are extremely time consuming. They soon become unrealistic when various insects, products or storage conditions are simultaneously considered, as interactions will differ for each of these factors combinations.

Generally speaking,the object of much experimentation is to find out how a number of experimental variables affect a response and to find experimentally the optimal combination of conditions that provides the highest response.

"Experimental Research Methodology" is the whole of the methods and tools that are to be used to "drive" an experimentation and to explain the results obtained."Experimental" means that experiment is the unique way to obtain unknown information about the subject. Many fields of research are concerned: physics, chemistry, biology, economy, human sciences, ... and stored grain protection !

An experimental design consists in introducing variations of all factors simultaneously in a series of experimental runs, in order to extract the maximum amount of information from the data in the presence of noise. In general, with experimental design, only a small numbers of experimental runs are needed compared to other approachs.

Such experimental design has been used to study combination of Deltamethrin, Piperonyl butoxide and Organophosphorous insecticides (Fenitrothion and Chlorpyriphos-methyl), which are broadly used products for stored grain protection.

Mathematical modelizations of insecticidal efficacy have then been computed, and used to investigate in 6 directions:

- interaction between Organophosphorous insecticides and Piperonyl butoxide (binary interaction)

- interaction between Deltamethrin and Piperonyl butoxide

- interaction between Deltamethrin and Organophosphorous insecticides, differences among insects

- interaction between Deltamethrin, Organophosphorous insecticides and Piperonyl butoxide (ternary interaction)

- differences among Organophosphorous insecticides

MATERIALS AND METHODS

RESPONSE SURFACE METHODOLOGY

Response surface methodology (Box and Draper 1987) comprises a group of statistical techniques for empirical model building and model exploitation.

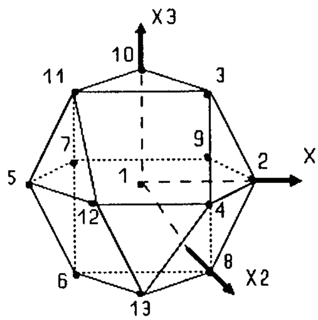

Figure 1: Hyper-sphere showing experimental points distribution in a uniform shell design

TABLE II- Natural values of insecticides concentrations in the 13 experimental points
(order different from Tab I)

DOSE N°	DTM	OP	PBO
1	0.50	3.75	2.5
2	0.00	3.75	2.5
3	0.375	7.5	2.5
4	0.125	0.00	2.5
5	0.375	0.00	2.5
6	0.125	7.5	2.5
7	0.375	5.0	5.0
8	0.125	2.5	0.0
9	0.375	2.5	0.0
10	0.250	6.25	0.0
11	0.125	5.00	5.0
12	0.250	1.25	5.0
13	0.250	3.75	2.5
CONTROL	0.0	0.0	0.0

DTM:Deltamethrin, OP: Fenitrothion or Chlorpyriphos methyl,PBO: Piperonyl butoxide

TREATMENT

Insects used in this study were from colonies of susceptible strains cultured in ROUSSEL UCLAF Research Center of Marseilles (CRBA).
Test species were *Oryzaephilus surinamensis (L.), Sitophilus granarius (L.) and Tribolium confusum (Duval)* for storage under temperate condition (25°C), and *Rhyzopertha dominica (F.), Sitophilus zeamais (Motschulsky) and Tribolium castaneum (Herbst)* for storage under tropical condition (32°C).

Wheat to be treated was stored for several months to detect any natural infestation; absence of insecticide residues was chemically and biologically checked. Before treatment, 10 % in weight were grinded to a size between 1 and 2 mm, to create an appropriate environment for secondary pests (*Tribolium sp.* and *O.surinamensis*).

A 4 ml aqueous solution of formulated insecticide was spraid (Fisher sprayer model JRP3, pressure 2 bars, outflow 6l/mn) onto 2kg of wheat being tumbled in a 20 l stainless steel drum rotating at 60 rpm, with its axis set at an angle of 45°with vertical.

Tumbling was continued for 5 minutes. Treated wheat was splited in two samples of 1 kg kept in alimentary plastic containers closed with a punched lid to allow air exchanges. One container was kept in tropical climatic chamber (32 °C, 70 % RH), the other in temperate climatic chamber (25 °C, 60 % RH).

BIOASSAYS

Bioassays have been performed one day and 1, 2, 3, 4, 5, 6, 9, 12 months after treatment..

A 100 g sample was taken from each container, placed in a plastic vessel and infested with 50 adults of each species representative of respective storage conditions, i.e. tropical and temperate.

These vessels were sealed with a punched lid and stored in climatic chambers.

After 14 days, grain was sifted to remove adults, and dead and alive insects were recorded.

Treatment efficacy was expressed

as % of dead insects in the total population, corrected by the natural mortality (Abbott 1925).

RESULTS AND DISCUSSION

An exemple of experimental results is given in Table III.

Following discussion will be based on 6 months results, considered as representative of an average practical situation. However, the same data analysis could have been performed for any other storage period, from 1 to 12 months.

From these values, have been computed mathematical models relating factors levels (i.e. concentrations of products expressed in coded values) with responses (i.e. insect mortality).These models will be called "activity equations".

By keeping constant one factor level in these equations, it is then possible to calculate mortality as a fonction of the two other products concentrations in the grain. Isomortality curves can then be drawn from these equations to facilitate interpretation.

INTERACTION BETWEEN ORGANOPHOSPHOROUS INSECTICIDES AND PIPERONYL BUTOXIDE:

Antagonism between several Organophosphorous insecticides and Piperonyl butoxide has been studied by various authors. Although few works have been done regarding antagonistic action in the specific case of stored grain protectants Piperonyl butoxide effect on Organophosphorous insecticides activity is reviewed in SNELSON (1987).

In activity equations, it is possible to analyse interactions between Organophosphorous insecticides and Piperonyl butoxide by setting Deltamethrin concentration at 0 ppm (-1 in coded value in activities equations).

For instance, with Chlorpyriphos-methyl associated with Piperonyl butoxide against *Tribolium castaneum*, an antagonism is clearly noticeable (Figure 2), as an increase in Piperonyl butoxide concentration will result in a reduced mortality of *Tribolium castaneum*, for any level of Chlorpyriphos methyl.

TABLE III : Adulticidal efficacy of 6 months stored samples of the Deltamethrin-Fenitrothion-Piperonyl Butoxyde interaction study.

DOSE N°	INSECTS					
	O.SURIN.	S. GRAN.	T.CONF.	R.DOMIN.	S.ZEAM.	T.CASTAN
1	100	100	100	100	88	100
2	100	100	36	0	100	8
3	100	100	100	100	100	78
4	28	36	10	100	12	0
5	78	56	52	100	38	26
6	100	100	100	100	100	16
7	100	100	100	100	100	88
8	100	50	6	100	58	0
9	100	66	28	100	62	6
10	100	100	64	100	100	10
11	100	100	70	100	100	14
12	86	72	52	100	90	6
13	100	100	52	100	96	10
CHECK	2	0	0	0	6	0

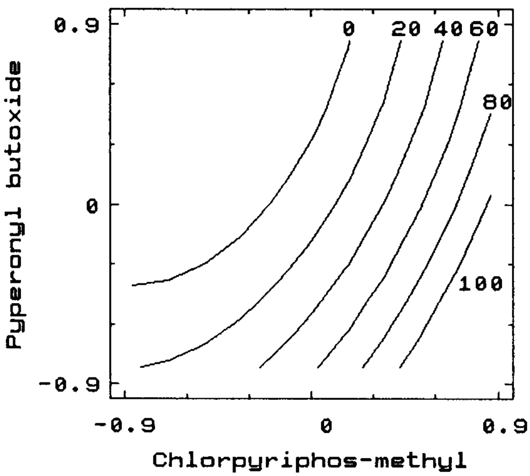

Figure 2: Activity on T.castaneum of Chlorpyriphos methyl and Piperonyl butoxide , without Deltamethrin

This negative interaction of Piperonyl butoxide is also found with Fenitrothion on *Tribolium castaneum* (Figure 3).

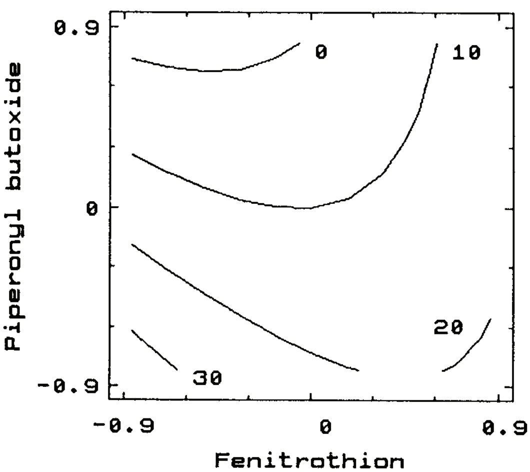

Figure 3: Activity on T.castaneum of Fenitrothion and Piperonyl butoxide without Deltamethrin

This antagonistic action is not found with all species. On *Sitophilus granarius* for instance, PB interact positively with Fenitrothion .

Generally speaking, it appears that antagonistic effect of Piperonyl butoxide is highest for Malathion and lowest for Fenitrothion, Chlorpyriphos-methyl activity being affected at an intermediate level.

On the other hand, antagonistic interaction of Piperonyl butoxide on Organophosphorous insecticides is strongest for *Tribolium castaneum*, intermediate for *Tribolium confusum*, *Sitophilus granarius* and *Sitophilus zeamais*, and lowest for *Rhyzopertha dominica* and *Oryzaephilus surinamensis*.

INTERACTION BETWEEN DELTAMETHRIN AND ORGANOPHOSPHOROUS COMPOUNDS

Insect susceptibility to chemical grain protectants depends both on chemical and on insect species. It is of obvious importance to take these differences into account when studying combinations of products. Synthetic pyrethroids are known to be especially active on *Rhyzopertha dominica*, and Organophosphorous coumpounds on *Sitophilus sp.*, but their spectrum of action is actually much wider. To optimize a combination of chemical one should consider these interactions of activity with the same interest than isolated ones.

By setting Piperonyl butoxide value at 0 ppm (-.816 in coded value) in efficacy equations, insect mortality is represented as a fonction of Deltamethrin and Organophosphorous insecticide concentration only. Activity of combined Deltamethrin and Fenitrothion on *Rhyzopertha dominica*, *Sitophilus granarius* , and *Tribolium castaneum*, is taken as an example. Differences in insect responses can be observed. For *Rhyzopertha dominica* (Fig.4), isomortality curves are almost parallel to Fenitrothion axes, which means that an increase in Fenitrothion concentration has basically no effect, Deltamethrin being the main and almost single factor of activity. For *Sitophilus granarius* the situation is quite different, as isomortality curves (Figure 5) indicate that Fenitrothion is the main factor, although an increase in Deltamethrin concentration results in a significant increase in *Sitophilus granarius* mortality.

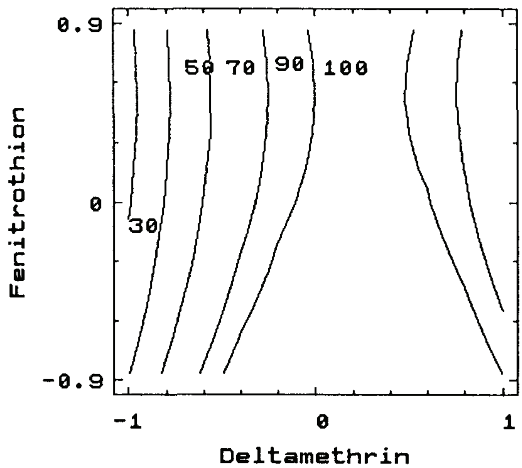

Figure 4: Activity on R.dominica of Deltamethrin and Fenitrothion without Piperonyl butoxide

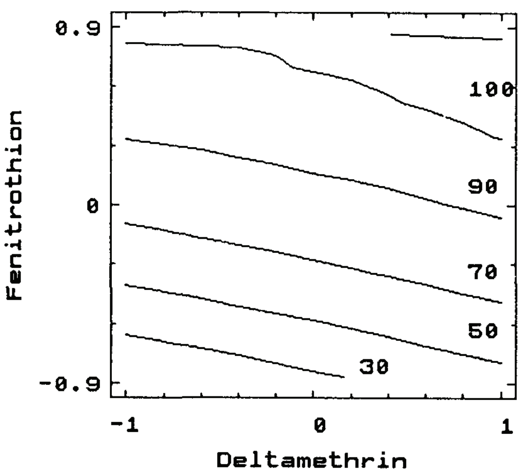

Figure 5: Activity on S.granarius of Deltamethrin and Fenitrothion without Piperonyl butoxide

Response of *Tribolium castaneum* (Figure 6) is different from previous ones, an reveals that this insect is almost equally susceptible to both active ingredients.

An increase of either Fenitrothion or Deltamethrin concentration resulting in an increase of mortality. This susceptibility however is quite low, the average level of activity being quite low on adults of *Tribolium castaneum*

(*Tribolium castaneum* practical control is mainly ensured at larval stages (COULON 1983)).

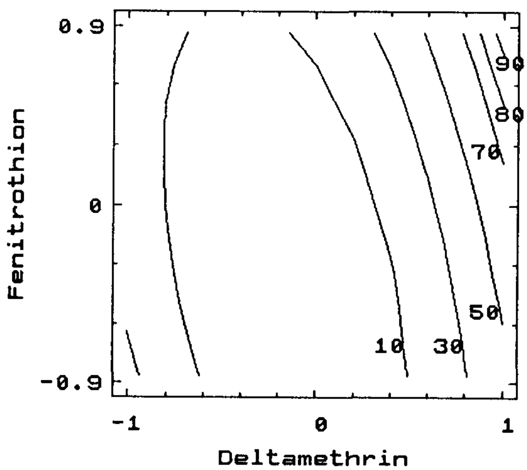

Figure 6: Activity on T.castaneum of Deltamethrin and Fenitrothion without Piperonyl butoxide

Similar observations could be made for other insects: Fenitrothion is the main factor for *Sitophilus zeamais*, with a positive interaction of Deltamethrin, Deltamethrin is the main factor for *Oryzaephilus surinamensis* with a positive interaction of Fenitrothion, and both compounds contribute to *Tribolium confusum* control.

Chlorpyriphos methyl shows a pattern of interaction with Deltamethrin qualitatively similar to Fenitrothion, with differences in rates to achieve the same efficacy, Chlorpyriphos methyl insecticidal activity being superior to that of Fenitrothion.

Isomortality curves indicate that interaction is not always constant and may depend on respective compound concentrations.

As these compounds have different degradation kinetics, proportion of chemicals in the grain are not constant during storage and interaction pattern is likely to change with time. This actually occurs, as is demonstrated by a comparison of Deltamethrin plus Fenitrothion activity against *Sitophilus granarius* after 6 months (Figure 5) and 9 months storage (Figure 7).

Although at 6 months, Fenitrothion is the main source of insecticidal activity, at 9 months and later, Deltamethrin becomes the main factor. This might be explained by the fact that Fenitrothion concentration in the grain dropped down below its threshold level of activity.

INTERACTION BETWEEN DELTAMETHRIN, ORGANOPHOSPHOROUS INSECTICIDES AND PIPERONYL BUTOXIDE

Activity of Deltamethrin and Organophosphorous insecticides combination has been studied "without Piperonyl butoxide". To change its "concentration" in the formula will allow to estimate its impact on the overall activity of the ternary combination.

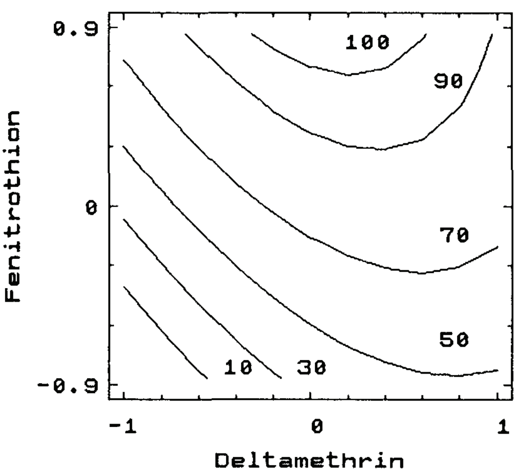

Figure 7: Activity on S.granarius of Deltamethrin and Fenitrothion without Piperonyl butoxide (9 months storage)

Three levels of Piperonyl butoxide concentration : 0, 2.5 and 5 ppm (-.816, 0, .816 in coded values) have been set in a combination of Deltamethrin plus Fenitrothion against *Sitophilus granarius* (Fig.5,8 & 9 respectively).

Interaction of Piperonyl butoxide is clearly negative as an increase in its concentration results in a sharp reduction of insecticidal activity of the combination.

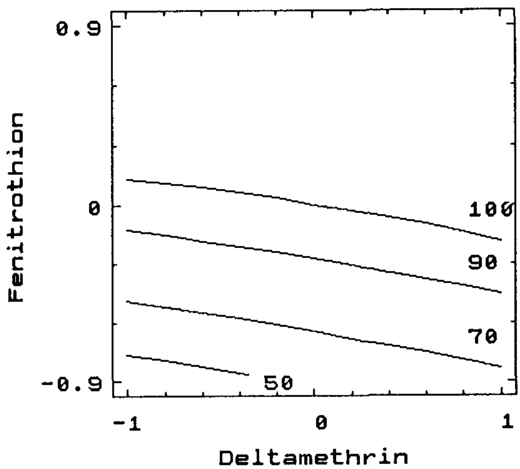

Figure 8: Activity on S.granarius of Deltamethrin and Fenitrothion with 2.5 ppm Piperonyl butoxide

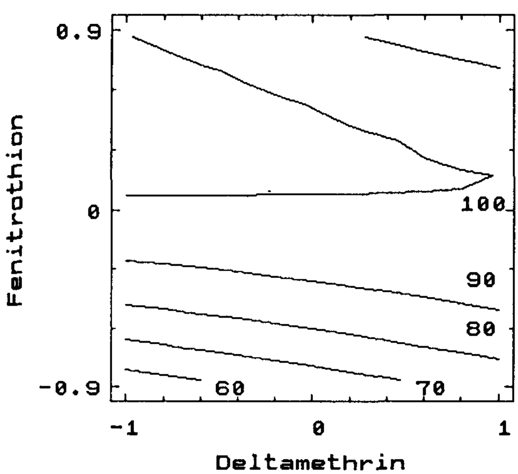

Figure 9: Activity on S.granarius of Deltamethrin and Fenitrothion with 5 ppm Piperonyl butoxide

The same phenomenon occurs with other pests, and has been found too with Chlorpyriphos-methyl.

DIFFERENCES AMONG ORGANOPHOSPHOROUS INSECTICIDES

Differences in insecticidal potency and physico chemical stability among Organophosphorous insecticide compounds can be globally quantified, by comparing their relative potencies after various storage periods (Relative potency is defined as the ratio of concentrations of two compounds giving the same activity).

This relative potency depends on many parameters, such as insecticide nature, grain species, insect (SAMSON and PARKER 1989), as well as on humidity (SAMSON, PARKER and JONES 1988).

As an exemple, in Figure 5 and Figure 10 Fenitrothion and Chlorpyriphos-methyl are compared against *Sitophilus granarius* after a 6 months storage (Piperonyl butoxide concentration = 0 ppm). A visual observation of isomortality curves shows very similar paterns of activity, allowing global comparison .

The intercept of 100 % isomortality curve with Organophosphorous insecticides axis is at a level of 6.5 ppm for Fenitrothion and 3.2 ppm for Chlorpyriphos-methyl, which leads to a Relative potency close to 2, meaning that about twice as much Fenitrothion as Chlorpyriphos-methyl is necessary to have the same activity at six months on *Sitophilus granarius*.

For storage period of 3, 6, 9 or 12 months, Relative potency between Chlorpyriphos-methyl and Fenitrothion against *Sitophilus granarius* varies, with respective values of 1, 2, 12 and > 15. This rapid increase of relative potency should obviously be interpreted as a consequence of a shorter half life of Fenitrothion compared to Chlorpyriphos-methyl under our laboratory storage condition.

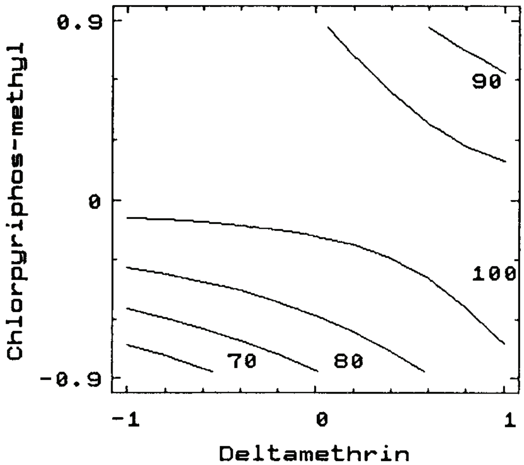

Figure 10: Activity on S.granarius of Deltamethrin and Chlorpyriphos methyl without Piperonyl butoxide

CONCLUSION

In the complex study of associations between pyrethroids and organophophorous insecticides for stored grain treatment, powerful experimental methodology has greatly facilitated a global apprehension of phenomenoms, although it does not explain them. To study a combination of three products, Doehlert uniform network only 13 experimental points were needed to evaluate products action and interactions. In the specific case of Deltamethrine as pyrethroid, Fenitrothion, Chlorpyriphos methyl or Malathion as organophosphorous compounds, and piperonyl butoxide, main conclusions from this study are:

- Piperonyl butoxide antagonizes Organophosphorous action, with some exceptions and is not desirable in the association.
- Single action Deltamethrin and Organophosphorous compounds is very complementary, and their interaction is always positive.

This justifies fully the concept of their association. Interactions intensity depends on respective concentration ratio. Different half lives of products and expected lenght of protection will therefore dictate treatment rates. The following are simplified recommendations, to be adapted according to local situations :

For 6 to 9 months protection, in tropical conditions:		
Deltamethrin:	+ Chlorpyrifos methyl:	5 ppm
0.5 ppm	+ Fenitrothion :	>5 ppm
	+ Malathion :	10 ppm
in temperate conditions:		
Deltamethrin:	+ Chlorpyrifos methyl:	1,25 ppm
0.125 ppm	+ Fenitrothion :	5 ppm
	+ Malathion :	7.5 ppm

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VALIDATION EXPERIMENTALE ET MODELISATION EN TANT QU'OUTILS DE DEVELOPPEMENT DES COMBINAISONS ENTRE LA DELTAMETHRINE, LE CHLORPYRIPHOS-METHYL ET LE PYPERONYL BUTOXIDE POUR OPTIMISER LES FORMULES DES INSECTICIDES DE PROTECTION DES GRAINS

J. NICOLAS (1), P. VINCENT (1), H. TRALONGO (2), P. LANTERI (3), et R. LONGERAY (3)

(1) ROUSSEL UCLAF, CEBAT, BP 1, 13367 Marseille Cedex 11
(2) ROUSSEL UCLAF, 31 quai A. Barbes, BP 123, 69583 Neuville/Saône Cedex
(3) ESCIL, Université Lyon 1, 69622 Villeurbanne Cedex

RESUME

Les composés pyréthroïdes et organophosphorés ont des spectres d'activité différents mais complémentaires vis-à-vis des ravageurs du grain. Il parait intéressant de les combiner pour la protection des grains. La concentration optimale des matières actives dépend de plusieurs facteurs, par ex. : les conditions de stockage, la durée de la protection, le coût, etc. Lorsqu'on associe plusieurs insecticides, il faut considérer leurs interactions (positives ou négatives). Afin d'étudier simultanément plusieurs des plus importants de ces facteurs en laboratoire, tout en maintenant le volume de travail dans des limites raisonnables, on a expérimenté un modèle basé sur le réseau uniforme de Doehlert pour appliquer la méthodologie surfaces-réponse. On a choisi, pour cette étude, deux insecticides de contact persistants largement utilisés, la deltaméthrine et le chlorpyriphos-méthyl ainsi que le pyréronyl butoxyde, un synergiste des pyréthroïdes. Du blé traité a été stocké en conditions tropicales (32° C, grain infesté par *R. dominica*, *S. zeamais* et *T. castaneum*) ou en conditions tempérées (20° C, grain infesté par *O. surinamensis*, *S. granarius* et *T. confusum*). L'efficacité du traitement a été contrôlée toutes les quatre semaines pendant un an. Les doses d'application ont été calculées à l'intérieur d'un cadre expérimental bien défini : concentration de deltaméthrine de 0 à 5 ppm, concentration du chlorpyriphos-méthyl de 0 à 7,5 ppm, et du pypéronyl butoxyde de 0 à 5 ppm. Les modèles empiriques résultant de ces traitements montrent clairement que la deltaméthrine et le chlorpyriphos-méthyl sont complémentaires. Les interactions positives diminuent l'intérêt du synergiste. En raison des différences de leurs demi-vies dans le grain, leur combinaison optimale lors du traitement dépend à la fois de la période de protection désirée et des conditions de stockage. Bien que ces expériences aient été faites en laboratoire, elles permettent de mieux comprendre les phénomènes étudiés. Elle sont sans valeur vis-à-vis de recherches futures sur le terrain où les facteurs environnementaux sont incontrôlables et ne permettent pas de bien saisir l'importance des paramètres et leurs interactions.