INSECTICIDE RESISTANCE IN POPULATIONS OF *ORYZAEPHILUS SURINAMENSIS* AND *CRYPTOLESTES FERRUGINEUS* FROM GRAIN STORES IN THE UNITED KINGDOM

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ABSTRACT

Discriminating doses have been set to enable the detection of resistance in *Oryzaephilus surinamensis* and *Cryptolestes ferrugineus* to all those organophosphorus insecticides currently available in the UK for use in association with stored grain. These doses were then used on strains of *O. surinamensis* and *C. ferrugineus* collected from farm and central grain stores in England and Wales, between 1987 and 1989. In *O. surinamensis* resistance to malathion, fenitrothion, pirimiphos-methyl, chlorpyrifos-methyl, etrimfos and methacrifos was found, and while some strains showed resistance to only one of these compounds, others were resistant to all six. Resistance in *C. ferrugineus* was limited to malathion, etrimfos, and fenitrothion, and in no strains was there resistance to more than one compound. An examination of the resistance spectra of the *O. surinamensis* strains suggests that several resistance mechanisms are involved. The extent of cross and multiple resistance is considered, in the light of these results, selection experiments and other work on the genetics of resistance in *O. surinamensis*, and with particular attention to chlorpyrifos-methyl resistance.

INTRODUCTION

In England and Wales, 90% of cereal grain is put initially into stores on the farms where it was grown. Subsequently some of the grain is moved into central stores, from where it will eventually go for processing or export. Once in store the grain is vulnerable to infestation by insect pests, the most important in the United Kingdom being the beetles, *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus* and *Sitophilus granarius*. The presence of pests and the need for long term storage, usually in floor stores, makes the use of insecticides, as grain protectants, particularly attractive. Four insecticides are currently available for use in the United Kingdom as grain protectants, these are pirimiphos-methyl, chlorpyrifos-methyl, etrimfos and methacrifos. Resistance to any of these insecticides in one of the major grain store beetle pests would make their control difficult under British conditions, and this would be especialy true if the resistance was coupled with cross-resistance to other organophosphorus insecticides.

Resistance in stored product beetles has been recognised since the early 1970s when a worldwide FAO survey investigated the resistance of eight species of stored product beetle to malathion and lindane (Champ & Dyte, 1976). In Great Britain, malathion resistance was found to be present in *Rhyzopertha dominica, Sitophilus granarius, S. oryzae, Tribolium castaneum* and *T. confusum*, but absent in *Oryzaephilus surinamensis, O. mercator* and *Sitophilus zeamais*. Lindane resistance was found in all eight species. Subsequently resistance in these beetles to organophosphorus insecticides appears to have increased worldwide (Champ, 1986). In Britain data for the years after the FAO survey (Muggleton, 1987) show that the frequency of malathion resistance increased in the decade following the early 1970s, but remained at, or about, the 1979-1982 level for the period 1983-1986. Data for 1984-1986 showed that for *O. surinamensis*, nearly 30% of the populations tested were resistant to pirimiphos-methyl and malathion , a small proportion (6%) were resistant to fenitrothion, but that nearly all (92%) were resistant to chlorpyrifos-methyl.

The disadvantages of these earlier data are that they are not a random sample, coming from premises with infestation problems and thus where resistance is more likely to be found, and that for most species it was possible to test for resistance to only one organophosphorus compound, malathion. The present paper gives the results from a random sample of farm grain stores in England, and for nearly all the central grain stores in England and Wales, and so should present a more accurate picture of the frequency of resistant populations in these stores. In addition, we have addressed the problem of a lack of resistance tests for compounds other than malathion, by developing new discriminating dose tests for both *O. surinamensis and C. ferrugineus*.

METHODS

Collection of beetles and data

In 1987 pre-harvest visits were made to 742 farms in England that grew cereal grain. Farms were stratified into three size groups by the area of cereal grain grown, and a further stratification was made into each of the five MAFF Regions, the aim being to visit 50 farms in each size category in each region. A random sample of farms in each category was supplied by the Agricultural Census Branch of MAFF. Data on storage practice was collected during the visits and baitbags and, where appropriate, other trapping methods were used to collect samples of beetles. Between October 1988 and March 1989 similar data were collected from central grain stores in England and Wales. This second exercise was limited to off-farm stores with over 1000 tonnes capacity, and excluded port sites. 182 sites with such stores were identified, and of these it proved possible to visit 171 sites.

Determination of discriminating doses

Dose-response data were obtained for each of eight strains of *O.*

surinamensis and two strains of *C. ferrugineus* known to be susceptible to malathion, and for a further two strains of *C. ferrugineus* known to be resistant to malathion but not to other organophosphorus insecticides. A minimum of five doses was used to produce each dose-response line and approximately 100 adult beetles were exposed at each dose. The method of exposing the beetles to the insecticide is described below. Two of the *O. surinamensis* strains had been cultured in the Laboratory since before the use of organophosphores insecticides. The remaining strains of both species were collected, from the field, between 1964 and 1983. The discriminating dose was set by taking the ED99.9 which typified those strains least tolerant of the insecticide (Muggleton (1987). Once a dose had been identified, suspected resistant strains were exposed to it, and the progeny of any survivors bred-up and tested at the putative dose. A significant increase in survivors among these progeny, compared to their parents, was taken as confirmation that the ability to survive the dose was inherited.

Bioassay technique

Adult beetles were exposed to doses of insecticide following the general procedures set out in FAO Method No. 15 (Anon., 1974), in which beetles are confined on insecticide impregnated filter papers by glass rings coated with 'Fluon' (an aqueous suspension of polytetrafluoroethylene). With the exception of methacrifos, the doses were applied to 'Whatman No. 1' filter papers in a mixture of 'Shell Risella' oil, petroleum ether and acetone in a ratio of 1:3:1 by volume. 0.5 ml of this mixture was applied to each filter paper which was then left to dry for 18 hours. Methacrifos was applied in a mixture of polyethylene glycol (molecular weight 300) and acetone in a ratio of 1:4 by volume. Technical grade insecticides were used throughout. The discriminating doses used to detect resistance are shown in Table I. All the tests were carried out in a constant temperature and humidity room maintained at 25 C and 70% r.h.; before testing the beetles had been reared for at least one generation in constant temperature and humidity rooms maintained at 25 C and 70% r.h. for O. surinamensis, and at 30 C and 70% r.h. for C. ferrugineus.

Selection experiments

Eight strains of *O. surinamensis* collected from farm grain stores were selected with chlorpyrifos-methyl for two generations. The selection was carried out by exposing adults for five hours on filter papers treated with half the discriminating dose of chlorpyrifos-methyl (i.e. 130mg/m). Sufficient adults were exposed to give 300 survivors which were then used to set up two cultures, using 150 survivors for each. As a control two cultures were set up using 150 unselected adults for each. Both selected and unselected parents were removed from the culture jars after three weeks. After nine weeks the progeny from the selected adults were again exposed to a dose of 130 mg/m of chlorpyrifos-methyl, and the survivors used to set up new cultures. At the same time new cultures from the unselected jars were also set up. Again the parents were removed at three weeks, and at nine to ten weeks the progeny from the jars containing both the selected and unselected strains were tested for resistance to each of the organophosphorus insecticides listed in Table I.

TABLE I The discriminating doses used to detect resistance. A five hour exposure period was used.

Insecticide	*O. surinamensis*		*C. ferrugineus*	
	% concn in oil	deposit (mg/m)	% concn in oil	deposit (mg/m)
Malathion (mal)	0.3	78	1.0	260
Fenitrothion (fen)	0.5	130	0.2	52
Pirimiphos-methyl (p-m)	0.6	156	0.5	130
Chlorpyrifos-methyl (cp-m)	1.0	260	0.5	130
Methacrifos (meth)	0.4*	104	-	-
Etrimfos (etr)	0.2	52	0.15	39

* in polyethylene glycol

RESULTS

a) Assessment of resistance

Trapping for beetles was carried out at all 742 farms included in the exercise and at 157 of the central grain store sites, where 283 individual storage structures were sampled. *O. surinamensis* from 30 farm stores and 28 central stores, at 20 sites, were bred-up and tested for resistance to six organophosphorus insecticides. *C. ferrugineus* from 22 farm stores and 21 central stores, each at a separate site, were bred-up and tested for resistance to five organophosphorus insecticides. The results of the tests of the populations of *O. surinamensis* and *C. ferrugineus* from each store are shown in Tables II - V, together with the overall percentage of populations resistant to each insecticide.

b) Selection with chlorpyrifos-methyl

The results of two generations of laboratory selection with chlorpyrifos-methyl on the response, to six organophosphorus insecticides, of strains of *O. surinamensis* from eight farm grain stores are shown in Table VII. Following selection with chlorpyrifos-methyl there was a significant increase in the proportion of individuals resistant to both chlorpyrifos-methyl and etrimfos in five strains, to chlorpyrifos-methyl, methacrifos and etrimfos in one strain, to chlorpyrifos-methyl only in one strain and to etrimfos only in one strain. There were no significant differences in the frequency of resistance to any of the other insecticides.

DISCUSSION

The results show that in the UK resistance to organophosphorus insecticides is limited in *C. ferrugineus*, but is widespread in *O. surinamensis*. For farm stores, the frequency of resistance in *O. surinamensis* to pirimiphos-methyl, chlorpyrifos-methyl and fenitrothion is similar to that found in inland premises between 1984 and 1986 (Muggleton, 1987). Resistance to malathion appears, however, to be less frequent (13% compared to 29.5%), but the difference is not significant (X = 3.119). In contrast there are some striking differences between the resistance found in *O. surinamensis* from in farm and central stores. In general resistance was detected more frequently in the central store *O. surinamensis* populations than in those from the farm stores. The notable exception to this is methacrifos resistance which was much less frequent in the central stores than in the farm stores. The high frequency of detection of resistance in the central stores compared with the farm stores probably has two causes, the more extensive use of insecticides in central stores and the concentration in central stores of grain from many different sources. The lower detection rate of methacrifos resistance in central stores is puzzling. It would seem to suggest that methacrifos resistance can be inherited separately from resistances to other organophosphorus compounds.

Table II The results of discriminating dose tests on strains of *O. surinamensis* collected from farm grain stores. The figures are percentage knockdown.

Strain no.	mal	fen	p-m	cp-m	meth	etr
24	100	100	97	28	97	88
84	100	100	94	49	75	86
134	100	100	99	77	94	90
227	100	100	100	71	98	100
2239	100	100	100	87	95	100
301	100	100	100	68	51	98
339	100	100	100	71	100	94
302	100	100	100	30	92	100
408	100	100	100	93	100	100
606	100	100	98	95	100	83
465	100	100	100	71	100	100
768	100	100	100	83	91	92
2653	100	100	100	81	100	100
2703	100	100	100	96	89	100
2706	99	100	100	91	100	100
2817	100	100	100	51	75	100
647	92	100	98	86	69	91
691	95	100	97	32	57	90
743	100	100	100	75	88	100
816	100	100	89	58	73	44
3033	100	100	100	33	92	96
883	100	100	100	92	100	96
891	100	100	100	80	93	95
903	100	100	100	61	62	92
968	100	100	100	94	95	100
1002	100	100	100	78	78	100
945	100	100	100	95	84	98
1000	100	100	100	80	69	97
1022	100	100	100	74	12	62
1040	80	98	96	61	94	90
% Resistant	13	3	27	100	60	77

For *O. surinamensis* it is possible to tabulate the various combinations of resistance found in each of the populations, and this tabulation is shown in Table VI. It is immediately evident that no strains are susceptible to all six insecticides, and that all but three strains show multiple resistance to organophosphorus compounds. Of particular interest is the large number of strains resistant to chlorprifos-methyl, etrimfos and methacrifos in spite of the fact that only 4.7% of farms and 26.7% of central stores used any one of these compounds, compared to 39.6% of farms and 69.4% of central stores which used pirimiphos-methyl. This widespread resistance is difficult

Table III Results of discriminating dose tests on strains of *C. ferrugineus* collected from farm grain stores. Figures are percentage knockdown.

Strain no.	mal	fen	p-m	cp-m	etr
123	100	100	100	100	100
84	100	100	100	100	100
147	100	100	100	100	100
227	100	100	100	100	100
264	100	100	100	100	100
278	100	100	100	100	100
327	100	100	100	100	100
339	100	100	100	100	100
384	100	100	100	100	100
302	100	100	100	100	100
313	100	100	100	100	100
606	100	100	100	100	100
462	94	100	100	100	100
2714	100	100	100	100	100
673	100	100	100	100	100
725	71	100	100	100	100
819	100	100	100	100	97
889	100	100	100	100	100
1021	100	100	100	100	100
3004	100	100	100	100	100
1000	100	100	100	100	100
1023	100	100	100	100	100
% Resistant	9	0	0	0	5

Table IV Results of discriminating dose tests on strains of *C. ferrugineus* collected from central grain stores. Figures are percentage knockdown.

Strain no.	mal	fen	p-m	cp-m	etr
3021/3	100	100	100	100	100
4003/1	100	100	100	100	100
4008/1	100	100	100	100	100
4009/2	100	100	100	100	100
4011/1	100	83.9	100	100	100
4012/1	100	100	100	100	100
4016/2	100	100	100	100	100
5006/1	100	100	100	100	100
5009/1	100	100	100	100	100
5012/2	100	100	100	100	100
5050/2	100	100	100	100	100
5055/1	100	100	100	100	97.8
5072/2	100	100	100	100	100
5075/1	100	100	100	100	100
6005/1	100	100	100	100	100
6012/1	72.0	100	100	100	100
6013/3	100	100	100	100	100
6017/1	74.0	100	100	100	100
7005/1	85.6	100	100	100	100
7012/1	100	100	100	100	100
8002/1	100	100	100	100	96.5
% Resistant	14	5	0	0	10

Table V Results of discriminating dose tests on strains of *O. surinamensis* collected from central grain stores. The figures are percentage knockdown.

Strain no.	mal	fen	p-m	cp-m	etr	meth
3002/1	100	100	100	67.6	59.4	100
3002/2	88.7	100	100	80.7	70.9	100
3015/1	100	100	92	60.6	53.5	100
4003/1	100	100	97.9	78.2	73.2	100
4008/1	100	100	94.9	65.1	54.5	98.2
4009/1	100	100	97.4	91.7	40.0	100
4009/2	100	100	96.8	92.3	60.3	100
4009/3	100	100	98.0	93.7	67.0	100
4012/1	100	100	100	63.2	75.7	96.9
4016/2	100	100	92.2	41.9	79.0	100
5009/1	52.3	67.7	73.6	88.7	21.7	100
5019/1	100	100	92.3	86.6	74.5	100
5065/2	100	100	100	67.0	68.0	100
6010/1	100	100	78.6	73.5	61.8	100
6010/2	100	100	98.4	85.2	95.7	100
6010/3	100	100	100	92.2	98.5	100
6013/3	100	100	91.1	41.5	79.8	100
6017/1	100	100	94.9	75.5	53.9	94.4
6018/1	22.7	51.1	55.6	83.8	10.4	100
7002/1	4.5	14.4	7.2	65.3	1.9	100
7005/1	100	100	94.8	93.1	59.0	100
7005/2	100	100	92.1	97.8	62.6	100
7009/1	92.4	100	91.0	85.3	38.4	100
7012/1	11.8	32.6	6.0	22.6	0	8.3
7014/1	83.1	98.9	90.1	89.5	67.4	95.7
7022/1	100	100	97.2	55.1	83.5	92.9
7022/3	100	100	92.1	63.7	78.1	93.6
7022/4	100	100	91.4	72.4	82.5	98.0
% Resistant	25	18	82	100	100	29

to explain, unless it is as a result of cross-resistance to compounds used frequently in the past, such as malathion and fenitrothion, or to the most frequently used at present, pirimiphos-methyl.

A comparison of the results of the discriminating dose tests (Tables II and V) shows no evidence of correlations between knockdown frequencies for any of the compounds. This does not mean to say that cross-resistance is not involved. In some earlier work, two strains of *O. surinamensis*, one homozygous for malathion resistance, the other homozygous for malathion susceptibility, were selected from a single multiple organophosphorus resistant field strain collected in England. When the dose response data for these two strains was compared it was evident that the loss of the malathion resistance gene was accompanied by a reduction in resistance to pirimiphos-methyl, etrimfos and fenitrothion, as well as to malathion. There was no similar loss of resistance to chlorpyrifos-methyl or methacrifos. This result suggests that in this strain there is cross-resistance between malathion, pirimiphos-methyl, etrimfos and fenitrothion, but that resistance to chlorpyrifos-methyl and methacrifos is inherited separately. Wallbank and Rose (1986) found that in an Australian strain of *O. surinamensis* the relationship they observed between mixed function oxidase activity and fenitrothion resistance did not extend to chlorpyrifos-methyl, again suggesting the existence of a separate resitance mechanism for this compound.

Table VI The combinations of insecticide resistance found in populations from farm and central stores. (R = resistant, S = susceptible)

Chlorpyrifos-methyl	R	R	R	R	R	R	R	R	R	R	R	R	S
Methacrifos	R	R	R	R	S	S	R	S	S	S	S	S	S
Etrimfos	R	R	R	R	R	R	S	S	S	R	R	R	S
Pirimiphos-methyl	R	R	R	S	R	S	S	S	S	R	S	R	S
Malathion	R	R	S	S	S	S	S	R	S	R	R	R	S
Fenitrothion	R	S	S	S	S	S	S	S	S	R	S	S	S
no of strains with each combination	3	2	9	9	13	5	8	1	3	3	1	1	0

Table VII The percentage of each strain resistant to each of six organophosphorus insecticides following two generations with (sel.), or without (unsel.) selection with chlorpyrifos-methyl.

Strain		Insecticide					
		cp-m	meth	etr	p-m	fen	mal
M84	unsel.	7.4	0	12.1	0	0	0
	sel.	30.0	0.4	29.3	0	0	0
	X	8.5	-	22.8	-	-	-
M339	unsel.	12.5	0	23.1	0	0	0
	sel.	51.9	0	45.7	1.5	0	0
	X	72.6	-	18.8	-	-	-
L408	unsel.	11.7	0	5.4	1.2	0	0
	sel.	65.4	0	10.2	1.7	0	0
	X	157.9	-	4.0	-	-	-
M465	unsel.	10.6	0	2.6	0	0	0
	sel.	29.6	0	9.6	3.0	0	0
	X	21.3	-	9.5	-	-	-
M691	unsel.	43.0	0	4.6	0	0	0
	sel.	50.0	0.8	18.0	0.8	0	0
	X	1.8	-	12.2	-	-	-
M1002	unsel.	12.4	0	3.7	0	0	0
	sel.	22.1	0	6.6	1.8	0	1.2
	X	8.5	-	2.2	-	-	-
L1040	unsel.	20.6	0.7	5.0	0	2.6	4.8
	sel.	40.4	2.2	19.6	0.4	0	0
	X	25.2	-	25.0	-	-	-
S2706	unsel.	17.8	1.1	1.6	0	0	0
	sel.	31.7	9.3	7.4	2.2	0	0
	X	14.0	18.2	10.4	-	-	-

We have examined the relationship between chlorpyrifos-methyl resistance and that for the other compounds by selecting eight of the strains from farm grain stores with chlorpyrifos-methyl. Following selection with chlorpyrifos-methyl the proportion of the population resistant to chlorpyrifos-methyl should increase as should the proportion resistant to any other compounds showing cross-resistance to chlorpyrifos-methyl. As can be seen from Table VII the results show clear evidence of cross-resistance between chlorpyrifos-methyl and etrimfos in seven out of the eight strains; in one of these strains the cross-resistance extends to methacrifos. It has been suggested that, in view of the limited use of the insecticide, the resistance to chlorpyrifos-methyl maybe the result of a natural tolerance to that compound (Muggleton, 1987). This suggestion appears to be supported by the finding of chlorpyrifos-methyl resistant strains of *O. surinamensis* in grain stores in Minnesota in the absence of chlorpyrifos-methyl usage (Subramanyan et al, 1989). The results presented here suggest that this natural tolerance also extends to etrimfos and hence the unexpectedly high occurrence of etrimfos resistance we have found. The wide range of knockdown frequencies observed also suggest that this natural tolerance is present as a polymorphism in many, but not all, populations of *O. surinamensis*.

ACKNOWLEDGEMENTS

Part of this work was supported by a research grant from the Home-Grown Cereals Authority. We should also like to thank Cheminova, Ciba-Geigy, Dow, ICI, Sandoz and Sumitomo for their gifts of technical grade insecticides; Mrs J. Eade, Miss L.M. Farmer, Mrs M.J. O'Donnell, Miss L.A. Sharrock and Dr J. Waller for their work developing the discriminating doses, Miss S. Blades, Miss L. Nair, Mr J. C. Starzewski and Miss I.T. Taylor for applying them and recording the results and to Miss S. Smith, Miss Taylor and Mrs A. Bedi for their work on the selection experiments.

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RESISTANCE AUX INSECTICIDES DES POPULATIONS D'*ORYZEAPHILUS SURINAMENSIS* ET *CRYPTOLESTES FERRUGINEUS* DES STOCKS DE GRAINS AU ROYAUME-UNI

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RESUME

On a procédé à l'établissement de doses discriminantes afin de détecter la résistance d'*Oryzeaphilus surinamensis* et de *Cryptolestes ferrugineus* à tous les insecticides organophosphorés d'usage courant sur les grains stockés au R.U. Le test de la dose discriminante a été utilisé en suivant le protocole FAO et consiste à exposer un adulte à une feuille de papier buvard imprégnée d'un insecticide. Ces tests ont alors été utilisés sur des souches de *O. surinamensis* et de *C. ferrugineus* récoltées au hasard sur des échantillons de grains stockés dans des fermes ou en vrac, en dehors des fermes, en Angleterre ou au Pays de Galles, entre 1987 et 1989. Chez *O. surinamensis* on a découvert une résistance au malathion, au fénitrothion, au pirimiphos-méthyl, au chlorpiryphos-méthyl, à l'estrimfos et au méthacrifos. Alors que certaines souches ont présenté une résistance à un seul de ces composés, d'autres étaient résistantes aux six substances. Toutes les souches de *O. surinamensis* se sont avérées résistantes au chlorpyrifos-méthyl. La résistance de *C. ferrugineus* est limitée au malathion, à l'étrimfos et au fénitrothion. Ce phénomène de résistance à plus d'un composé n'est apparu que chez une seule souche. Comparée à des travaux antérieurs sur *O. surinamensis*, la résistance à tous les composés, excepté le malathion, semble augmenter. Un examen de l'étendue de la résistance de *O. surinamensis* suggère que plusieurs mécanismes sont mis en jeu. Le niveau élevé des résistances croisées ou multiples doit être considéré à la lumière de ces résultats ainsi que de divers autres travaux sur la génétique de la résistance de *O. surinamensis*. Il semble probable que la résistance généralisée au chlorpyriphos-méthyl constatée chez *O. surinamensis* soit, en partie, le résultat d'une tolérance naturelle à cet insecticide présente sous forme de polymorphisme chez de nombreuses populations et pouvant s'étendre au méthacrifos.