RESISTANCE TO IODOFENPHOS AND MALATHION IN THE LESSER MEALWORM *ALPHITOBIUS DIAPERINUS*.

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ABSTRACT

Alphitobius diaperinus is a major pest in poultry houses due to the damage that it causes to insulation material and to its disease carrying capability. Recently, treatment failures for *Alphitobius diaperinus* have been reported and an investigation was undertaken to determine whether these populations were resistant to the insecticides approved by M.A.F.F. for fabric treatments. Using a method based upon the FAO recommended filter paper method for stored product beetles, tentative discriminating doses for iodofenphos and malathion were determined as 0.5% and 0.25% respectively. Using these discriminating doses it was found that almost half of the field populations tested were resistant to one or both insecticides. *These findings have serious* implications for both the feed milling and poultry industries.

INTRODUCTION

Alphitobius diaperinus is the major insect pest of poultry broiler houses. It's presence was first recorded from poultry units by Gould and Moses (1951), and since then it has been found infesting poultry houses in Europe, America, Asia, North and South Africa and Australasia. Their presence in caged-layer houses is thought to be beneficial in drying out manure, making conditions unfavourable for the development of fly larvae (Wallace *et al,* 1985), but if manure containing *A. diaperinus* is subsequently put on fields, the beetles may cause a public nuisance as reported by Jerrard and Wildey (1980). In deep litter broiler houses they are a major pest for two reasons; their ability to transmit diseases to the poultry, and also due to the damage they cause to insulation materials.

A. diaperinus was shown to be capable of transmitting Marek's disease (avian leukosis) (Eidson *et al,* 1966) and Gumboro disease (infectious bursitis) (Snedeker *et al,* 1967), but since the introduction of vaccines for the prevention of Marek's disease and Gumboro disease their role in disease transmission has greatly diminished. However, since 1987 a more virulent strain of Gumboro has appeared (Survashe, 1990) thus increasing the importance of *A. diaperinus* with regard to transmission of this disease. *A. diaperinus* are also known to carry serotypes of *Escherichia coli*, some of which are pathogenic to poultry, and *Salmonella* species (De Las Casas *et al*, 1968; Harein *et al*, 1970), and it is known that *Salmonella* can be carried through pupation to the adult beetle (Geissler and Kosters, 1978). Since the outbreak of *Salmonella* poisoning from infected eggs in the United Kingdom in 1988, eradication of all insect pests from poultry houses is desirable to prevent any possible routes of transmission to the flock and subsequently to humans.

Vaughan and Turner (1982) reported that the last instar larvae tunnel into insulation material to find a place for pupation and adult beetles then follow the larvae . The tunnelling by larvae severely damages the insulation material and it has been demonstrated that the insulative capacity of the material is reduced as a result of the damage (Vaughan *et al*,1984). This can severely increase costs to the poultry farmer, by increasing heating costs and eventually resulting in the need for premature replacement of the insulation.

In the United Kingdom, infestations of *A. diaperinus* in poultry houses have been controlled by using fabric treatments of either iodofenphos or fenitrothion. In 1983, a visit was made to a West Midlands turkey rearing unit , where the recommended fabric treatment of iodofenphos, used successfully for the previous eight years, was no longer effective. An investigation was carried out in conjunction with field trials, to determine whether this population had developed resistance to the insecticide. Several other field populations were also obtained and tested for possible resistance to both iodofenphos and malathion.

MATERIALS AND METHOD

A. diaperinus were bred in 3.18kg glass jars containing 750g poultry meal (BOCM 557 Farmgate Layers Meal), and a pad of cotton wool soaked in water, both to act as a water source and to encourage mould growth. Cultures were maintained at 25 deg. C , 70 % r.h. The life cycle of the beetles under these conditions took approximately 42 days.

Tests were set up based upon the FAO recommended filter paper method for stored product beetles (Anon,1974). Adults 3 to 6 weeks old were used for testing. Due to the larger size of this beetle, only ten insects were used in each of five replicates. A 24 hour exposure period was used. Tests were conducted at 25 deg. C, 70 % r.h.

Discriminating doses for malathion and iodofenphos were obtained using a susceptible strain from a farm in South Hampshire , where insecticides had not previously been used. The West Midland population and 41 other field populations and 1 laboratory population (Insectary) were tested for resistance to these insecticides using the determined discriminating doses.The Insectary population had been in culture in the laboratory for many years and had not been exposed to insecticides.

RESULTS AND DISCUSSION

All probit response lines are shown in Table I.

Table I. Probit response lines for susceptible populations of *Alphitobius diaperinus* with a) iodofenphos and b) malathion.

a) Iodofenphos

Population	ED50	ED99.9	Slope	S.E.	H.F.
Insectary	0.046	0.17	5.42	0.62	1.0
	0.030	0.15	4.41	0.48	1.0
	0.042	0.24	4.11	0.42	1.0
	Combined line for all three lines				
	ED50 = 0.038		Slope = 4.29 +/- 0.50		
	ED99.9 = 0.20		H.F. = 3.8		
Hampshire (Initial)	0.17	0.64	5.31	0.56	1.0
	0.17	0.54	6.02	0.67	1.0
	0.15	0.47	6.41	0.85	1.0
	Combined line for all three lines				
	ED50 = 0.164		Slope = 5.79 +/- 0.38		
	ED99.9 = 0.56		H.F. = 1.0		
Hampshire (Recent)	0.056	0.58	3.05	0.35	1.0
	0.056	0.39	3.68	0.41	1.0
	0.058	0.39	3.75	0.42	1.0
	0.083	0.61	3.59	0.35	1.0
	Combined line for all four lines				
	ED50 = 0.067		Slope = 3.35 +/- 0.36		
	ED99.9 = 0.56		H.F. = 4.0		
Strain 29	0.032	0.39	2.86	0.33	1.0
Strain 34	0.14	0.42	6.94	0.83	1.0

b) Malathion.

Population	ED50	ED99.9	Slope	S.E.	H.F.
Hampshire	0.072	0.18	7.92	1.01	1.0
	0.067	0.17	7.84	0.99	1.0
	0.080	0.25	6.20	0.80	1.0
	0.030	0.28	3.26	0.38	1.0
	0.070	0.24	5.73	0.72	1.0

Combined line for all five lines

ED50 = 0.062 Slope = 4.18 +/- 0.67

ED99.9 = 0.34 H.F. = 11.0

Tentative discriminating doses for malathion and iodofenphos were found to be 0.25% and 0.5% respectively. The discriminating dose for iodofenphos was based on three probit mortality lines for the initial Hampshire population, which to our knowledge has never been exposed to insecticides. Further lines obtained for iodofenphos with this population at a later date (Hampshire recent) gave a discriminating dose of the same value, but the slope and ED50 values were reduced. The apparent change in this population may indicate that exposure to insecticides may have occurred in the past resulting in a low level of resistance to iodofenphos within the population. Three lines (Table 1a) were also obtained for the Insectary population with iodofenphos and these gave a combined ED99.9 value (discriminating dose) of only 0.2%, thus supporting the 0.5% discriminating dose. All lines obtained for the Hampshire and Insectary populations were shown to be parallel with a common slope of 4.25 +/- 0.14, but the common line for these lines gave a discriminating dose of 1.5% at the ED99.9 level, 0.7% at the ED99.0 level. Single lines for two other populations (Strains 29 and 34, Table 1a) have been obtained giving ED99.9 values of 0.39 and 0.42. The discriminating dose concentration for iodofenphos of 0.5% would appear effective as in all discriminating dose tests to date when the Hampshire population has been used as a standard, a concentration of 0.5% iodofenphos has resulted in 100% knockdown after 24 hours exposure. However, this concentration may have to be reviewed in the future.

Discriminating dose tests to date with malathion have been undertaken using a tentative discriminating dose of 0.25% based upon the 3 initial lines obtained using the (initial) Hampshire population. All discriminating dose tests using this population as a susceptible reference have resulted in 100% knockdown of this population after 24 hours. All probit-mortality lines obtained to date with this population (Table 1a) have proved to be parallel with a common slope of 5.3 +/- 0.28. However, when the combined line is generated a slightly higher discriminating dose of 0.34% at the ED 99.9% level is produced. The discriminating dose for malathion may therefore also have to be reviewed in the future dependant on further work.

Using the discriminating doses (Table II) it was found that 39% of the field populations tested were resistant to malathion and iodofenphos, 5% were resistant to malathion only, 0% were resistant to iodofenphos only and 56% were susceptible to both malathion and iodofenphos. Of the populations which were resistant to malathion 50% gave 0% response after 24 hours exposure to the discriminating dose.

Table II.The frequency of resistant *Alphitobius diaperinus* populations in various animal units.[1]

Origin	Populations tested	Number of resistant populations found: Iodofenphos	Malathion
Turkey unit	6	6	5 [2]
Chicken unit	30	10	10 [3]
Pig unit	2	0	0
On animal feed	4	1	1
Unknown origin	2	0	0

[1] Three populations gave responses of greater than 95% to either malathion or iodofenphos and these were considered susceptible for the purposes of the table. All other populations gave a response less than 95%.
[2] One population not yet tested with malathion
[3] Two populations not yet tested with malathion

The West Midland population was shown to survive 5% malathion for three days, 1% malathion for 6 days, and 2% iodofenphos gave a 66% knockdown response after 24 hours. Dose response lines for the West Midland population were not obtained probably due to loss of resistance by the population whilst in culture. Mortalities between 8 and 100% at the discriminating dose have been observed for this population with iodofenphos, and up to 16% at the discriminating dose with malathion. To increase the level of resistance in the West Midland population, insects were bred on culture media containing iodofenphos. To date insects have survived and bred at a concentration of 100mg/kg iodofenphos.

As can be seen from Table 1 there has been an increased response to iodofenphos at the ED50 level from the initial Hampshire population to the recent population. After the population had been in culture for approximately three years, all but the lowest of the five serial doses used for the dose-mortality lines gave 100% knockdown after 24 hours exposure. Thus lines were obtained using a lower dose range (Table 1a Hampshire recent) and gave an ED99.9 equal to that originally determined but the slope of the line had moved from 5.79 to 3.35 (Table 1a). The reason for this is not known, but the problems encountered whilst producing dose-mortality lines with *A. diaperinus* may be in part due to the larger size of this beetle compared with most stored product insects for which this test method was devised. Currently, topical application of the insecticide is being used, to assess whether this method will produce less variation in the probit response lines.

CONCLUSIONS

A high incidence of resistant populations have been detected and this has serious implications for both the feed milling and poultry industries. Currently, only fenitrothion and iodofenphos both approved by M.A.F.F., are used for the fabric treatment of poultry houses against *A. diaperinus*. In recent field trials (Cogan *et al* unpublished results) M.A.F.F. recommended field doses of fenitrothion failed to control iodofenphos resistant populations indicating cross-resistance to fenitrothion. It would appear from our findings that the use of these insecticides on many poultry farms at recommended levels would have very little, if any, effect on population numbers, and may result in the ineffective use of insecticides within the poultry industry. Due to the damage that they cause, especially with high population numbers, and their disease carrying capability, the presence of highly resistant populations is a cause for concern. Recently, sensitivity to *A. diaperinus* in research workers has been reported (Schroeckenstein *et al*, 1988) and it is possible that allergic reactions may occur amongst poultry workers if continually exposed to this beetle.

Feed mills receive bran from outside the U.K. and this may often be contaminated with *A. diaperinus*. One of the resistant populations of *A. diaperinus* reported here was imported in rice bran from India. It is therefore possible that *A. diaperinus* contaminated feed may be sent to poultry farms and the resistant populations may be spread. Some large poultry producers have their own feed mills which may supply a large number of poultry farms in one area. *A. diaperinus* infestations may therefore be passed from feed mills to poultry units and vice versa. The rapid spread of resistance within a large poultry group may be seen from our results where 9 out of 10 *A. diaperinus* populations from poultry units in one area in the West Midlands were resistant to iodofenphos, and all 7 of the populations tested with malathion were resistant.

Potentially pathogenic microorganisms carried by *A. diaperinus* must be of concern to the feed milling industry, as these may be passed on to the poultry via the feed and subsequently to humans.

The presence of resistant populations has serious implications for both feed milling and poultry industries and there is therefore an urgent need for alternative treatment methods to eradicate *A. diaperinus*.

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RESISTANCE A L' IODOFENPHOS ET AU MALATHION DU TENEBRION DES POULAILLERS, *ALPHITOBIUS DIAPERINUS* PANZER

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RESUME

Des études en laboratoire basées sur la méthode de la mesure de la résistance des coléoptères des denrées stockées, (recommandées par les normes de la FAO), sur papier filtre ont été réalisées afin de déterminer les réponses dose/mortalité à l'iodofenphos et au malathion chez les populations sensibles d'*Alphitobius diaperinus* (Ténébrion des poulaillers).

Des doses discriminantes ont été utilisées pour étudier les populations de terrain au Royaume-Uni, un certain nombre d'entre elles ont présenté une résistance à la fois à l'iodofenphos et au malathion.

Les implications de la résistance à ces produits chimiques sont discutées en tenant compte de la dissémination des populations résistantes d'*A. diaperinus* dans l'industrie agro-alimentaire et dans l'élevage des volailles.