THE INHERITANCE OF PHOSPHINE RESISTANCE IN RHYZOPERTHA DOMINICA AND TRIBOLIUM CASTANEUM.

M.R ANSELL, C.E.DYTE and R.H.SMITH.

Dept. of Pure and Applied Zoology, University of Reading, PO BOX 228, Reading, RG6 2AJ, UK.

ABSTRACT

Preliminary results are presented from a study into the genetic mechanism responsible for resistance to phosphine in field caught strains of R.dominica and T.castaneum. In R.dominica the response of segregating populations to fumigation, at low dosages, can largely be explained in terms of a single gene. However resistance as displayed by the homozygous resistant strain appeared to require another gene(s). In T.castaneum the results appeared to show that full resistance was coded for by two genes; at high doses both genes were recessive. Linkage studies using visible markers in T.castaneum showed that resistance was linked to the mutant "pearl eye".

INTRODUCTION

In recent decades the number of fumigants available has decreased, now only phosphine and methyl bromide are in common usage. In many tropical developing countries almost total reliance is placed on phosphine because of ease of application, relative cheapness and wide availability. Any threat to its continued effectiveness has serious implications in these areas. The development of resistance represents such a threat.

Resistance to phosphine was first encountered in insect strains selected for resistance in the laboratory. Selection of Sitophilus granarius adults over 28 generations produced a three fold increase in tolerance to phosphine (Monro et al., 1972). Similarly selection of Tribolium castaneum adults over ten generations produced a twelve fold increase in tolerance (Kem, 1977).

Phosphine was included in the world wide survey of resistance sponsored by the United Nations Food and Agriculture Organisation (F.A.O.) in 1972-73. The F.A.O. survey report (Champ and Dyte, 1976) details the presence of resistance in field strains of a number of insect species including Rhyzopertha dominica and T.castaneum. Thus 22 of 92 strains of R.dominica tested contained resistant individuals, as did 15 of the 267 strains of T.castaneum. In the context of the F.A.O. survey a strain was defined as resistant if at least 1% survived a discriminating dose expected to produce 100% mortality in susceptible strains over a 20 hour exposure period; for R.dominica this dose was 0.03 mg/l (a concentration time product of 0.6 mg/l/hr), and for T.castaneum 0.04 mg/l (a C.T.P. of 0.8 mg/l/hr). Maximum resistance levels (12 fold for R.dominica and 6 fold for T.castaneum) did not

represent an immediate threat at that time but the authors did not exclude the possibility of control failure due to resistance in the future.

Since the F.A.O. survey both the geographic spread and the intensity of resistance have increased (Taylor and Halliday, 1986). At least one example of control failure partly attributable to resistance has been reported. In Dacca, Bangladesh, repeated whole store fumigations under conditions of poor gas-tightness have selected highly resistant strains of R.dominica, T.castaneum, Oryzaephilus surinamensis and Cryptolestes sp. (Tyler et al., 1983). The R.dominica strain was found to be 100 times as tolerant as susceptible insects of that species (Mills, 1983). The conditions that first fostered resistance, namely short exposure periods and poor gas-tightness, are prevalent in many areas. Without attention to these problems, phosphine may no longer offer complete control of insect pests particularly since resistance appears to extend to other lifestages e.g. eggs and pupae (Nakakita and Winks, 1981; Bell et al., 1977), where tolerances are naturally higher than in the adult.

One published study has involved highly resistant field strains. Selection of a field strain of T.castaneum from the Ivory Coast produced a rapid increase in resistance levels. Resistance remained high in the absence of selection. Crosses of resistant to susceptible stocks showed that resistance was semidominant. No significant differences could be identified between the progeny of reciprocal crosses (Bekon et al., 1988).

In the following paper some preliminary results from a current investigation into the mode of inheritance of phosphine resistance in adult R.dominica and T.castaneum are presented. The work has been funded by a grant from the Natural Resources Institute (N.R.I.) of the U.K. Overseas Development Administration.

MATERIALS AND METHODS

Insects

Resistant strains studied were field collected by N.R.I. as part of a study into the geographical spread of phosphine resistance. The R.dominica strain originated in Brazil and the T.castaneum strain in Pakistan. Susceptible strains of both species have been reared in the laboratory for many years and their origins are obscure.

Mutant stocks of T.castaneum bearing the following autosomal markers were obtained from Phillip Harris Biological Ltd. (symbol and linkage group in parentheses): pearl (p,II), black (B,III), sooty (s,IV) and microcephalic (mc,V). A full description of all Tribolium mutants may be found in Sokoloff (1966).

A single mutant stock of R.dominica, black (b) (Champ and Genn, 1971), was acquired from the Ministry of Agriculture, Fisheries and Food, Slough Laboratory. The tolerance of the marker strains was assessed and all were found to be susceptible to phosphine.

R.dominica was cultured on whole wheat and T.castaneum on wholemeal flour enriched with 5% brewer's yeast by weight. Both species were held at 30^{0}C and 70% r.h..

At the start of the investigation a number of single pair lines of resistant insects were founded for both species. The parents of each successive generation were tested at the F.A.O. discriminating dose for that species. If the insects failed to survive, that line was discarded. After five generations the remaining lines were combined to form the resistant strains used in the study.

Phosphine

Phosphine was prepared by the action of water on aluminium phosphide pellets (Degesch), as described in F.A.O. Method no.16 (Anon., 1975). During the course of the project (after the 20 hour exposure tests) a phosphine

meter (Harris, 1986) was obtained from N.R.I.. This allowed the purity of the phosphine source to be measured accurately.

Toxicity Tests

The toxicity of phosphine was initially assessed over a 20 hour exposure period. Insects were starved overnight prior to testing. Insects were held on filter papers in 120 ml Beatson jars sealed with plastic mesh. Fumigations were carried out in 11 litre dessicators. The required volume of phosphine was injected into the dessicator by Hamilton gas-tight syringe via a rubber septum in the dessicator lid. All tests were carried out at 25°C. After testing the insects were removed from the dessicators, placed on the appropriate medium and held for 14 days prior to scoring at 25°C and 70% r.h.. Where longer exposure periods have been specified the insects were held on the appropriate food throughout the tests to reduce control mortality.

Toxicity data are often presented as fitted probit lines, which provide a useful description of the distribution of tolerances of a strain to the toxicant. However the probit transformation is at its weakest in pinpointing the two extremes of the distribution i.e. doses that produce almost 0 and 100% mortality, which are of great importance when attempting to separate individual genotypes. We concentrated on determining doses that discriminate between two genotypes i.e between susceptible (SS) and F1 or F1 and resistant (RR). Having determined discriminating doses, they were applied to segregating populations and the mortality compared with that expected on the basis of one or more genes segregating in a simple Mendelian fashion.

Mode of Inheritance

To determine the mode of inheritance reciprocal crosses were made between the RR and SS strains. The beetles were sexed as pupae and mated as single pairs. In the case of R.dominica this entailed changing the culturing medium to wheatbran since the pupae normally develop within individual grains. This greatly increased the generation time and larval mortality. Pupal mortality due to handling was also high. In later crosses, when it had been determined that no significant differences existed between the progeny of reciprocal crosses, wheat was used. Prior to the emergence of the offspring as adults, infested grains were isolated individually and newly emerged virgin adults from the required stocks were placed as single pairs in vials containing wheat. Although one half of the crosses would fail due to animals being of the same sex this proved to be less of a handicap than the time wasted using the former method.

F1 insects were intercrossed to produce segregating F2 generations or backcrossed to the susceptible or resistant stocks. These segregating populations were fumigated with the discrimating doses previously identified during the toxicity testing of SS, RR and F1 insects or with a variety of doses to produce a dose response curve.

Analysis of Linkage

In T.castaneum the mode of inheritance appeared to be two independently segregating genes. Thus we analysed for linkage between a mutant marker and one of a pair of complementary resistance genes. The expected segregations in the absence of linkage were determined on the premise of three independently assorting genes.

Initially all the test crosses were of the type ABcabC x ABcabC (where Aa and Bb are the two recessive loci coding for resistance and Cc the mutant marker gene) i.e. repulsion F2. Resistant insects were crossed to susceptible mutant insects (bearing one of the recessive markers p, mc, s or the dominant marker B). F1 progeny from both reciprocal crosses were intercrossed in single pairs. The parents were removed after three weeks. Three weeks after first emergence, all the adults present were removed, held for a further

week, and then dosed at the 20 hour discriminating dose for fully resistant insects. The insects were scored for resistance and mutant phenotype two weeks later. Data were analysed for deviations from the expected 45 : 15 : 3 : 1 ratio of phenotypes predicted by independent assortment.

The presence of linkage between pearl and resistance was suggested, and therefore F2 crosses in coupling (ABCabc x ABCabc), and coupling backcrosses (ABCabc x abcabc) were produced and fumigated. Resistant pearl insects were crossed to susceptible, wildtype individuals in single pairs. The resulting F1 were intercrossed (both reciprocal crosses used) or backcrossed to the resistant pearl stock as single pairs. The adults were removed after three weeks. Each cross was then checked at three day intervals for the presence of pupae. Pupae were removed and sexed, held until the emerged adults were two weeks old and dosed. After a further two weeks the offspring were scored for resistance and pearl phenotype. F2 coupling data were subjected to Chi Square analysis to test for deviations from the segregation given above, whilst the backcross data were tested against the expected 3 : 1 : 3 : 1 phenotypic ratio. Where linkage was evident the crossover % was found using the Product Method (Immer, 1930).

RESULTS

Toxicity tests and Mode of Inheritance : R.DOMINICA

In all the following figures the dashed vertical lines represent the outer limits of a range of doses that discriminate between two phenotypes over the stated exposure period.

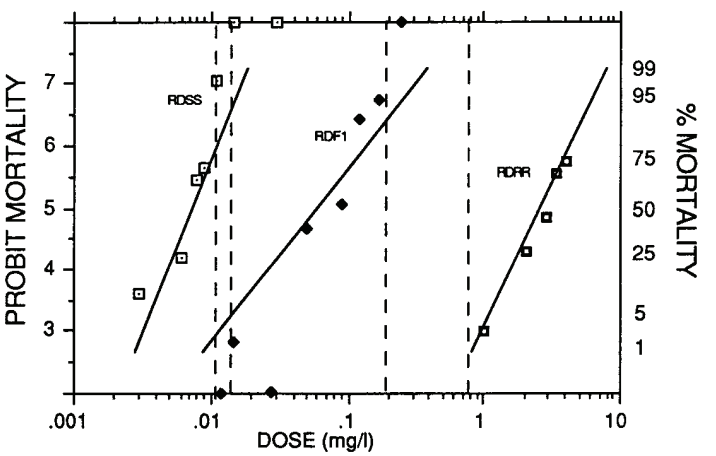

Fig.1 DOSE RESPONSE CURVES OF SS,F1 AND RR PHENOTYPES OF R.DOMINICA 20 HOUR EXPOSURE

The data (in Fig 1) were heterogeneous, this may have been because the resistant strain was not homozygous or because of inaccurate dosing (tested prior to use of phosphine meter). There was no evidence of significant differences between sexes or the progeny from reciprocal crosses in their response to phosphine, thus the data were combined. Resistance is semidominant and autosomal. The separation of susceptible and F1 populations does not appear complete when gauged by comparison of their respective probit lines. However, in practice, doses of between 0.012-0.015 mg/l discriminate accurately between the two genotypes. There is better separation of F1 and resistant insects; doses of between 0.2-0.8 mg/l give 100% mortality when applied to F1 insects and 0% when applied to resistant. F2 progenies were fumigated using these discriminating doses (Table I). Given a single gene one expects 25% mortality at the lower dose and 75% at the higher.

TABLE I R.DOMINICA F2 (20 HOUR EXPOSURE)

No.of families	Dose	No. of insects	% Mortality Observed	% Mortality Expected	Chi sq dev. from expected
6	0.012	452	24.4	25	1.5
12	0.015	1170	24.6	25	0.8
8	0.2	791	84.8	75	50.9**
13	0.25	1037	85.3	75	59.6**
4	0.3	379	84.4	75	18.0*

* indicates significant deviation from expectation, $p < 0.05$

The significance of the data is complicated by heterogeneity within some of the dose classes. However, there is a good fit between the single gene expectation and the 0.012-0.015 mg/l results, but less agreement between the expectation and the 0.2-0.3 mg/l data. This is confirmed by the response of an F2 population dosed to produce a response curve (Fig 2).

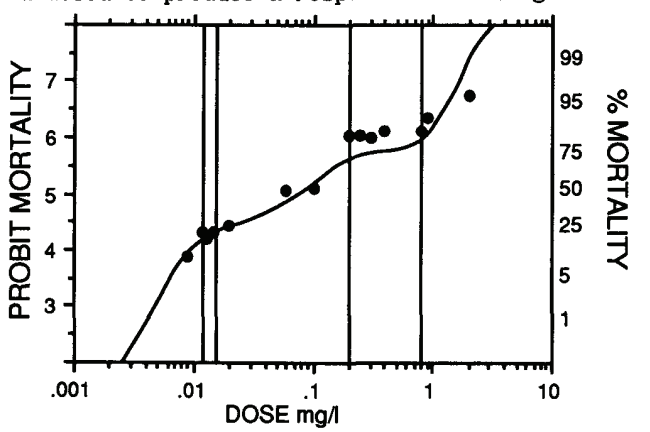

Fig.2 THE RESPONSE TO DOSE OF R.DOMINICA F2 20 HOUR EXPOSURE

The curves fitted to this and the following figures are based on a theoretical single gene segregation and not the observed data.

Since there is the possibility that the tolerances of susceptible and F1 insects overlap when dosed over 20 hours we investigated the dose response relationships of SS, RR and F1 populations using a 72 hour exposure period (see Fig 3).

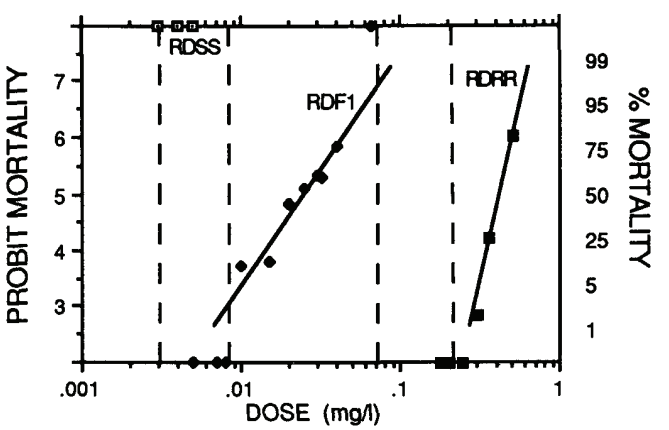

Fig.3 DOSE RESPONSE CURVES OF SS, F1 AND RR PHENOTYPES OF R.DOMINICA 72 HOUR EXPOSURE

Extremely low doses of phosphine produce complete mortality of susceptible insects; given the methods of dosing employed it was impractical to produce a response curve for this genotype. However there was good separation between the SS and F1 phenotypes making it possible to set discriminating doses of 0.003 to 0.008 mg/l for SS and F1 insects and 0.07 to 0.2 mg/l for F1 and RR populations. Data from F2 and backcross (F1 x SS) progenies dosed over 72 hrs are shown in Fig 4 and 5.

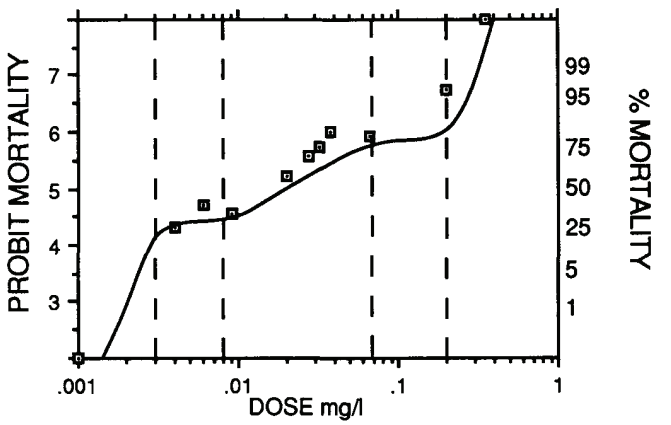

Fig.4 THE RESPONSE TO DOSE OF R.DOMINICA F2 72 HOUR EXPOSURE

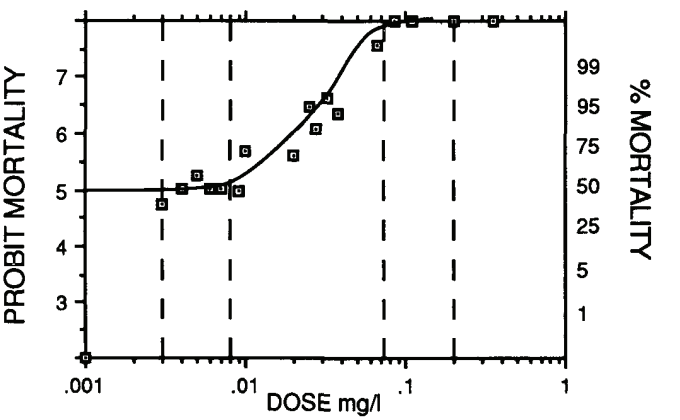

Fig.5 THE RESPONSE TO DOSE OF R.DOMINICA F1 X SS BACKCROSS 72 HOURS EXPOSURE

Analysis of linkage

Resistance in R.dominica is not linked to the black mutant.

Toxicity Tests and Mode of Inheritance : T.castaneum

With a 20 hours exposure period, the dose response data (Fig.6) for T.castaneum phenotypes were heterogeneous. There was no evidence of differences between the sexes or between the offspring of reciprocal crosses. Resistance is semidominant and autosomal. There was overlap between the distributions of tolerance of SS and F1 populations, it was impossible to set a discriminating dose to separate the two phenotypes. Doses of between 0.25 - 0.9 mg/l discriminate between F1 and RR phenotypes.

F2 and backcross (to the RR stock) progenies were bred and dosed at concentrations within this discriminating range. Individual progenies were combined prior to testing. The results are given in Table II

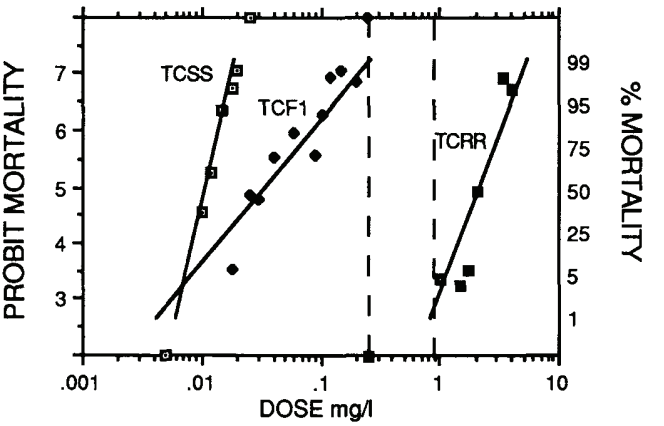

Fig.6 THE RESPONSE CURVES OF SS, F1 AND RR PHENOTYPES OF T.CASTANEUM 20 HOUR EXPOSURE

Table II T.castaneum F2 and Backcross (20 hour exposure)

Dose (mg/l)	F2 No. tested	F2 % Resistant expected	F2 % Resistant observed	Backcross No. tested	Backcross % Resistant expected	Backcross % Resistant observed
0.25	752	25	9.4	282	50	37.6
0.275	348	25	7.5	301	50	35.1
0.395	441	25	6.8	220	50	25.9

Expectancies calculated on the basis of a single recessive gene.

There was a significant shortfall in the proportion of resistant insects segregating in both crosses given the single gene hypothesis. One possible reason for the shortfall was that the RR strain was not homozygous for resistance. Therefore the original resistant strain (TcRRo) was further selected at doses (0.25, 1.0 or 2.0. mg/l) that were sure to remove all heterozygote contamination, given a 20 hour exposure.

Toxicity testing of the new resistant strains (TcRR0.25, TcRR1.0 and TCRR2.0) showed no significant alteration in the response to dose. Further F2 and backcross (BC) progenies were produced using the new RR strains as the source of resistance and fumigated at doses within the discriminating range as individual families (Table III).

Table III T.castaneum F2 and Backcross (20 hour exposure)

Cross	Dose (mg/l)	Source of Resistance	No. of Progenies	No. of Insects	Mean % Resistant
F2	0.25	TCRR0.25	3	142	13.4
F2	0.25	TCRR1.0	4	214	9.8
F2	0.25	TCRR2.0	6	333	15.6
F2	0.5	TCRR0.25	2	130	6.2
F2	0.5	TCRR1.0	2	92	12.0
F2	0.5	TCRR2.0	3	243	7.0
F2	1.0	TCRR0.25	3	147	4.8
F2	1.0	TCRR1.0	3	128	7.0
F2	1.0	TCRR2.0	5	174	6.3
BC	0.25	TCRR0.25	2	78	29.5
BC	0.25	TCRR1.0	3	109	33.0
BC	0.5	TCRR1.0	4	176	35.2
BC	1.0	TCRR0.25	2	110	21.0
BC	1.0	TCRR1.0	6	256	23.8

The results are comparable to those derived using the original resistant strain. The shortfall is not due to heterozygosity of the resistant strain. We also tested whether resistant insects had significantly longer developmental times and thus had not all emerged as adults at the time when the offspring were removed for testing. There was no evidence to suggest that this was the case.

The data suggest that at doses of 0.8-1.0 mg/l resistance was due to the action of two fully recessive genes. Thus F2 and backcross to resistance progenies would contain 6.25% and 25% resistant insects. The observed data do not differ significantly from these expectancies which were used in the analysis of linkage data.

We have attempted to separate SS and F1 using a longer exposure period. However toxicity testing at 72 and 120 hours has shown that the ranges of tolerance of the two phenotypes overlap although to a lesser extent than that seen at 20 hours.

Analysis of Linkage

Linkage analysis using Group I markers was not performed since resistance is not sex linked. The results of linkage tests involving markers from linkage groups II-V are given in Table IV (F2 R = F2 repulsion, F2 C = F2 coupling and BC C = backcross coupling).

Table IV T.castaneum (20 hour exposure 0.8mg/l)

Cross	Marker	No.of Families	Number of Progeny Without-Marker Susc	Without-Marker Res	With-Marker Susc	With-Marker Res	Chi squ Linkage	Cross-Over %
F2 R	mc	14	599	35	126	8	0.003	-
F2 R	s	12	385	16	98	5	0.153	-
F2 R	p	12	601	55	233	9	6.64*	34.8
F2 R	B	8	87	8	265	10	3.49	-
F2 C	p	8	608	23	102	9	4.3*	35.1
BC C	p	17	674	125	523	234	137.0**	34
BC C	female	17	331	62	282	114	17.8*	36
BC C	male	17	334	63	241	120	32.4*	32

DISCUSSION

When attempting to analyse data from families segregating for resistance genes, the phenotype expressed is dependent on the dose of pesticide applied. Thus a gene that appears dominant at one dose may be recessive at a higher dose and non-protective at a dose higher still.

In the case of R.dominica, F2 and backcross progeny appeared to show the segregation of a single dominant gene when fumigated with the lower discriminating dose (SS from F1 and RR). However when dosed at the higher discriminating dose F2 progeny showed a significant shortage of the resistant phenotype, in comparison to the single gene expectation. It appears probable that a second recessive gene is necessary for resistance to this higher dose. To confirm the existence of a second gene we are presently backcrossing repeatedly to the susceptible strain. This should separate the two genes and allow identification of lines carrying one or both.

Analysis of the T.castaneum data is more problematic since we do not have a dose to discriminate between SS and F1(and RR) insects. Mortality in F2 and backcross progenies increased as the concentration increased within the range of discriminating doses that separate F1 and RR insects. It appears that phenotypes with tolerances intermediate between F1 and RR were produced by the segregations. When dosed at the upper extreme of the discriminating

range approximately 6.25% were resistant in an F2 and 25% in a backcross. Such segregations are expected from two recessive genes.

The markers used in linkage work were chosen because of their availability to us, and their reported good penetrance and viability. There are recognised markers for 9 of the 10 linkage groups in T.castaneum, thus further study may identify a second linkage relationship as confirmatory evidence of a two gene system. We are presently attempting to gain further mutants located in linkage group II which may be located closer to the resistance gene. These markers should allow us to position the resistance gene relative to other known genes in linkage group II and may be of use in separating the resistance genes from each other. The very fact that linkage is so clearly demonstrable suggests that resistance is not the expression of a truly polygenic system. T.castaneum is the only coleopteran pest for which there are extensive data on the genetics, population dynamics and ecology. As such it is the ideal candidate for studies into possible methods of manipulating populations to limit the spread of resistance. In order to accomplish this task it is first necessary to understand the mode of inheritance of resistance and the degree of resistance produced.

REFERENCES

Anon. (1975) Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. F.A.O. Pl. Prot. Bull. 23, 12-35.

Bell C.H., Hole B.D. and Evans P.H. (1977) The occurrence of resistance to phosphine in adult and egg stages of strains of Rhyzopertha dominica (F.) (Coleoptera : Bostrichidae). J. stored Prod Res. 13, 91-94.

Bekon K.A., Le Torc'h J.M., Fleurat-Lessard F. (1988) Tolerance a la phosphine chez une race geographique de Tribolium castaneum (Herbst) (Col. : Tenebrionidae). L'Agronomie Tropicale 1988, 43-47.

Champ B.R. and Dyte C.E. (1976) Report of the F.A.O. Global Survey of Pesticide Susceptibility of Stored Grain Pests. F.A.O. Plant Production and Protection Series No. 5.

Champ B.R. and Genn B.G. (1971) Some mutants of stored product Coleoptera. J. stored Prod. Res. 7, 293-298.

Harris A.H. (1986). A conductimetric method for the determining of the concentration of phosphine during fumigation. Proc GASGA Seminar on Fumigation Technology in Developing Countries, T.D.R.I., Slough, 18th-21st March 1986, pp56-63.

Immer F.R.(1930) Formulae and tables for calculating linkage intensities Genetics 15, 81-98.

Kem T.R. (1977) Selection of a strain of Tribolium castaneum (Herbst) resistant to phosphine. J. ent Res. 1, 213-217.

Mills K. A. (1983) Resistance to the fumigant hydrogen phosphide in some stored product species associated with repeated inadequate treatments. Mitt. dtsch. Ges. allg. angew. Ent. 4, 98-101.

Monro H.A.U., Upitis E. and Bond E.J. (1972). Resistance of a laboratory strain of Sitophilus granarius (L.) (Coleoptera : Curculionidae) to phosphine. J. stored Prod. Res. 8, 199-207.

Nakakita H. and Winks R.G. (1981) Phosphine resistance in immature stages of a laboratory strain of Tribolium castaneum (Herbst) (Coleoptera : Tenebrionidae). J. stored Prod Res. 17, 43-52.

Sokoloff A. (1966) The genetics of Tribolium and related species. Academic Press, New York.

Taylor R.W.D. and Halliday D. (1986) The geographical spread of resistance to phosphine by coleopterous pests of stored products. Proc. 1986 Brit. Crop Prot. Conf. - Pests and Diseases. 2, 607-613.

Tyler P.S., Taylor R.W.and Rees D.P. (1983) Insect resistance to phosphine fumigation in food warehouses in Bangladesh. International Pest Control 25 (1), 10-13.

HERITABILITE DE LA RESISTANCE A LA PHOSPHINE CHEZ *TRIBOLIUM CASTANEUM* ET *RHYZOPERTHA DOMINICA*

M. ANSELL, C.E. DYTE et R.H. SMITH

University of Reading
Dept. of Pure & Applied Zoology, P.O. Box 228
Reading RG6 2JA, U.K.

RESUME

Des études génétiques ont été entreprises sur des souches de laboratoire sensibles des deux espèces et sur des souches de terrain définies résistantes selon les critères de la FAO. Les souches de terrain ont subi des pressions de sélection afin de concentrer les gènes homozygotes responsables de la résistance. Les paramètres dose/mortalité des parents résistants et sensibles ainsi que de leur descendance F1 ont été mesurés chez les adultes afin de déterminer les doses discriminantes.

Ces doses discriminantes ont été utilisées pour séparer les descendants F2 et descendants de croisements en retour. Les proportions relatives d'individus ayant survécu à la fumigation ont été comparées aux proportions attendues d'une transmission monogénique bi et polygénique. Jusqu'à présent, les résultats indiquent qu'il y a héritabilité monogénique chez *Rhyzopertha dominica*. L'héritabilité de *Tribolium castaneum* est plus complexe mais apparaît mettre en jeu deux gènes principaux avec, peut-être, des gènes modificateurs. Rien n'indique qu'il y ait linkage sexuel chez ces espèces, mais un linkage a été trouvé au moins sur un gène marqueur de *Tribolium castaneum*.