A same-day test for detecting resistance to phosphine

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Abstract

A new test to identify resistance has been developed for three species of stored-product beetles, based on their mobility on paper cones during exposure to phosphine. Adults of the reference strains of *Cryptolestes ferrugineus* and *Tribolium castaneum* were all knocked down within 3 hours and those for *Sitophilus oryzae* within 4.5 hours in 0.31–0.42 mg/L phosphine. Homozygous resistant adults of all the species remained active for more than 20 hours with no overlap between susceptible and homozygous resistant populations.

When an F_1 was obtained by pooling the progeny of single pair crosses of homozygous resistant and susceptible individuals, a regression line intermediate between susceptible and resistant populations was obtained for each species, lying close to the susceptible population. There was considerable overlap between susceptible and heterozygous populations but it was still possible to set discriminatory exposure times, based on total knockdown of susceptibles, as follows: *C. ferrugineus*, 160 minutes at 0.36 ± 0.05 mg/L; *S. oryzae*, 272 minutes at 0.39 ± 0.03 mg/L; *T. castaneum*, 162 minutes at 0.38 ± 0.04 mg/L.

For one of the species, *T. castaneum*, 6 strains recently collected from provender mills in the U.K., which were identified as resistant by the standard FAO mortality test, were subsequently shown to be resistant by the new test method, with similar proportions of insects responding in both tests.

Introduction

Since the FAO survey of pesticide resistance in stored-product pests in the 1970s (Champ and Dyte 1976), the potential for problems arising from resistance to phosphine has been well known. Initially the levels of phosphine resistance detected were quite low, both in terms of incidence among different populations and in terms of the extent to which tolerance levels were increased among individual beetles. During the 1980s, however, very much higher tolerances were encountered among resistant populations, firstly in Bangladesh and India (Tyler et al. 1983; Taylor 1986), and later in parts of Africa, South America and Indonesia (Taylor 1989). Subsequent tests revealed that such populations also displayed resistance in the naturally tolerant immature stages and that there were serious implications for control (Price and Mills 1988; Mills et al. 1990). Furthermore, it has been established that high levels of resistance can result from selection with phosphine at any stage of the life cycle (Rajendran 1992).

In spite of growing resistance problems, phosphine is still effective in achieving control, provided that adequate gas concentrations can be maintained so that the exposure period can be extended. For this to be possible the storage structure needs to be sealed to pressure test standards (Banks and Annis 1980), or there needs to be some means of maintaining a constant gas concentration throughout a storage structure for the necessary exposure period (Winks 1990). The latter is possible with the continuous-flow systems developed in Australia and the U.K., which are based on a cylinder-based supply of phosphine in carbon dioxide.

With continuous-flow systems it is possible to extend an exposure period to any chosen duration, although this may increase costs sharply. It would be of considerable value if the resistance status of the pest population were known at the start of a treatment so that the need for any special measures could be ascertained. At present the discriminatory dose test for common beetle species relies on a 14-day mortality assessment following a 20-hour exposure to phosphine. A 15-day delay between discovery of an infestation and arranging a treatment is often unacceptable, and information on resistance is unlikely to be obtained in time to influence the control strategy. The possibility that resistance may be identified by the different activity levels of individuals under gas, a likely side effect of reduced uptake or active exclusion (Price 1984), has already been partly explored (Reichmuth 1992). In the current study a rapid diagnostic test has been developed for some common beetle pests by which the presence of resistance in populations can be identified within a few hours.

Methods

The insects used were from mixed-age populations. The reference insects were taken from laboratory stock cultures and the homozygous resistant insects from a population previously selected for phosphine resistance by the standard FAO mortality test. Heterozygotes were produced from single-pair crosses between virgin adults of the reference strain and the homozygous strain. Crosses were set up over several consecutive weeks and the progeny from all the crosses then combined. Every 3–4 weeks, depending on the life-cycle of the species, the heterozygotes were transferred to fresh food to prevent any of their progeny from emerging and contaminating the population with susceptible and homozygous resistant insects.

The insects were tested on paper cones (6.5 cm diameter, 3 cm high) in glass crystallising dishes (7 cm diameter, 4 cm deep). Molten paraffin wax was used to seal the base of the cones to the dishes to prevent the insects from getting under the edge of the paper. For glass-climbing insects, such as *Sitophilus oryzae*, the walls of the dishes were coated with a thin coating of fluon (an aqueous suspension of polytetrafluoroethylene) to prevent them from escaping. Tests were conducted in 6-litre glass desiccators, each fitted with a flat glass top, to enable clear observation of the insects, and a central dosing port fitted with a rubber septum.

For the initial tests to establish the discriminating times, 3 replicates of 20 insects were observed for each strain. The dishes were placed on a wire-mesh shelf inside the desiccator, approximately 6 cm from the top, and left to acclimatise in the test conditions of 25°C, 60% r.h., for at least 30 minutes before sealing with the glass top. The desiccators were then dosed with phosphine, using a gastight syringe through the central dosing port, and stirred with a magnetic stirrer for approximately 5 minutes. Timing was started from the introduction of fumigant. The insects were observed at intervals of 2–5

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minutes until all the reference insects were knocked down. Subsequently, a number of field strains of *Tribolium castaneum*, obtained from provender mills in the U.K., was tested for resistance using the FAO resistance test method. Six strains which were identified as resistant were then included in parallel tests. One test was observed every 2–5 minutes as before (exploratory test), and the other, which used 3 replicates of 50 insects, was left for a fixed period, based on the approximate discriminating knockdown times for each species, and then assessed for knockdown (discriminating test). In both cases each field strain was tested alongside the reference strain.

At the end of each test the concentration in the desiccators was analysed by gas chromatography, using a flame photometric detector, standardised with a cylinder formulation of phosphine in nitrogen.

Results

The probit regression lines for all the species showed a clear distinction between the reference and the homozygous resistant insects, with at least 12 hours between the last reference insect and the first resistant insect being knocked down. The regression lines for the heterozygotes all lay between those of the reference strain and those of the resistant strain, albeit very near to the reference lines (Figs. 1–3). Due to the closeness of the reference and heterozygote lines there was considerable overlap in their response times, although for *T. castaneum* and *Cryptolestes ferrugineus* there was always at least 1 hour between the last reference insect and the last heterozygote insect being knocked down. The results for *S. oryzae* (Fig. 3) showed less consistency among replicate tests and there was a greater degree of overlap between heterozygote and reference strain results than for the other two species.

The six field strains of *T. castaneum* identified as resistant by the FAO test were also shown to be resistant by the knockdown test and all but one regression line lay to the right of the mean reference line (Fig. 4). Generally, the level of activity in the knockdown tests was higher than the level of survival in the FAO tests. One strain (line 5 in Fig. 4), collected from Northfields, gave inconsistent results in both tests. Initially, one survivor was recorded in the FAO test and the strain was classified as resistant. However, a re-test showed no resistance. In the knockdown test no insects remained active in the exploratory test but one insect remained active at the end of the discriminating test (Table 1).

Table 1. A comparison between the results from the FAO test and those from the immobilisation test for *Tribolium castaneum*.

	Insects surviving/standing (%)			
Strain	FAO method		Knockdown method	
	1st test	Re-test	Exploratory	Discriminating
Calne	9.5	12.8	70.5	21.3
Chard	34.0	16.6	46.7	46.6
Melksham	9.5	18.2	63.2	17.3
Northfields	0.5*	susceptible	susceptible	0.7*
Wells	36.5	20.6	55.0	36.6
Wrexham	25.5	32.2	49.0	53.3

*Both these figures represent one survivor in each case.

Discussion

Previous attempts to identify resistance to phosphine, based on the ability of insects to remain active under gas, have been hampered by the difficulty of determining whether or not insects have become immobilised, and the complication of narcosis at high gas concentrations. Reichmuth (1992), for example, testing at concentration levels well above the 0.4 mg/L tested here, assessed resistance on the basis of insects not becoming 'motionless' or 'narcotised' within about half an hour, and presents results for *Rhyzopertha dominica* (F.). To date, narcotic concentration thresholds have not yet been identified for this species but it is known that narcotic thresholds for susceptible and resistant strains of *T. castaneum* differ widely, that for the susceptible strain lying near 0.5 mg/L, with much higher levels for resistant strains (Winks 1985).

The uncertainty associated with the narcotic responses of insects and the concentration levels at which they occur indicate that a resistance test based on such criteria is hard to interpret. For each species, consideration would have to be given to variation of narcotic response among strains and

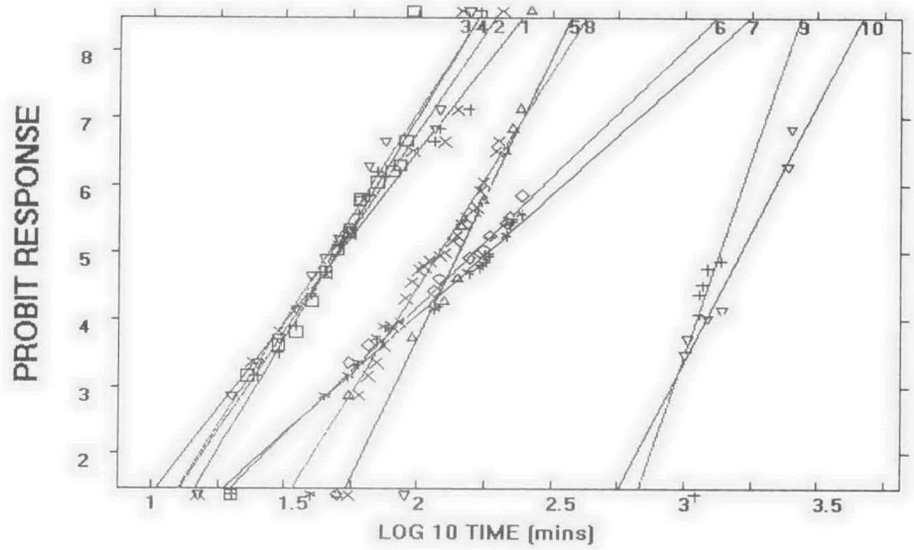

Fig. 1. Knockdown responses for *Tribolium castaneum* reference strain, homozygous resistant strain and heterozygotes which resulted from the crossing of these two, at concentrations of 0.4 + 0.03 mg/L. Lines 1–4 = reference strain, lines 5–8 = heterozygotes, lines 9–10 = homozygous resistant strain.

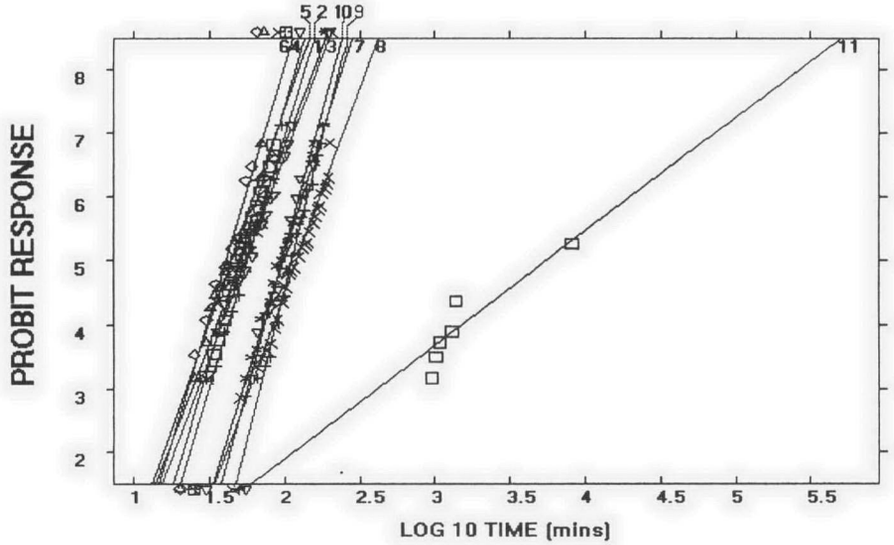

Fig. 2. Knockdown response for *Cryptolestes ferrugineus* reference strain, homozygous resistant strain and heterozygotes which resulted from the crossing of these two, at concentrations of 0.36 $^{+}$ 0.05 mg/l. Lines 1–6 = reference strain, lines 7–10 = heterozygotes, line 11 = homozygous resistant strain.

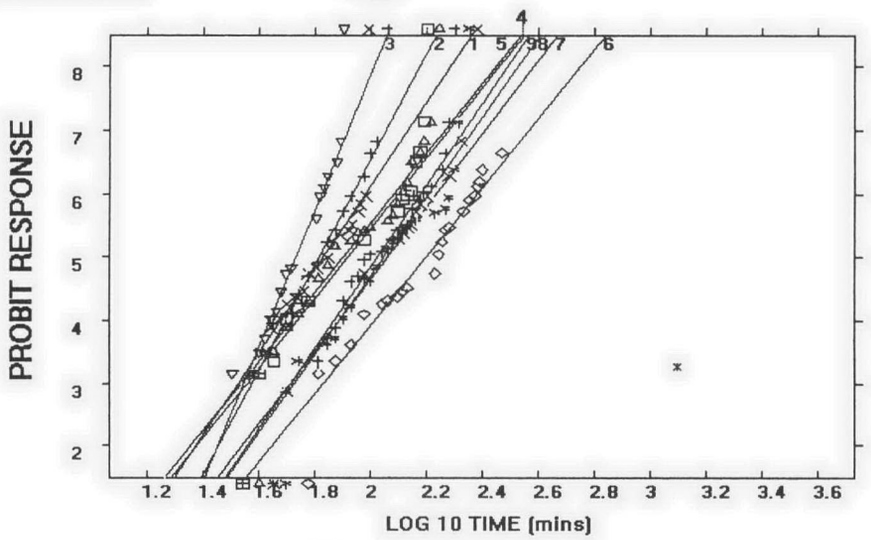

Fig. 3. Knockdown responses for *Sitophilus oryzae* reference strain, homozygous resistant strain and heterozygotes which resulted from the crossing of these two, at concentrations of 0.39 $^{+}$ 0.03 mg/l. Lines 1–5 = reference strain, lines 6–9 = heterozygotes, * = first homozygous resistant individual knocked down.

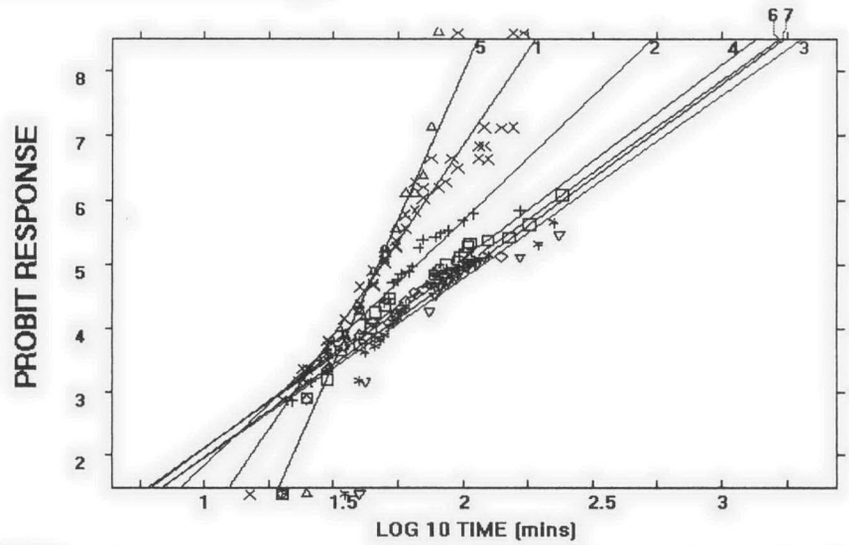

Fig. 4. Knockdown responses for six field strains of *T. castaneum* compared with the mean response line for the reference strain at concentrations of 0.38 $^{+}$ 0.04 mg/l. Line 1 = mean response line for the reference strain, lines 2–7 = field strains identified as resistant by the FAO mortality test.

among genotypes within a strain. For these reasons the current study is based on a knockdown response at concentrations below that which initiate narcosis in *T. castaneum*. Knockdown is more clearly defined from the normal movements of an insect, and the use of paper cones renders the assessment of knockdown much easier. Knocked-down individuals are defined as those unable to retain their grip on the sloping side of the cone and which fall to the bottom and are unable to stand. The number of test insects was initially only 20 per replicate for the experimental observations which provided the regressions of knockdown against time. This was done to aid accurate observation in obtaining the provisional discriminatory knockdown times, though no limit need be placed on the number of individuals in a discriminatory dose test . A larger sample size would enable the detection of low incidences of resistant individuals.

For *T. castaneum*, good agreement was obtained between the results of the current test method and those of the FAO discriminatory dose test. The correlation extended to the point that a strain showing a marginal result in the FAO test also gave a marginal result in the new knockdown test. Thus, these preliminary results indicate that the new test method is likely to be as efficient as the FAO test in identifying resistance . It has the considerable advantage of being able to give a result within a working day so that the findings can be used to formulate a fumigation strategy. It has also made it possible to differentiate heterozygous insects from susceptible ones, provided that a sufficient number of heterozygotes is present in the population sample at the time of testing. This gives the added advantage of being able to detect the presence of resistance in a population at a relatively early stage in the selection process, before homozygous genotypes become established in response to inadequate phosphine fumigations. In order to make full use of the advantages presented by this test more information is required on the mortality responses of all life stages of heterozygous resistant insects.

At present only three species have been tested using the new method and further tests are required on these with a larger number of strains, both resistant and susceptible according to the FAO method, to establish a more reliable discriminating time between resistance and susceptibility. It is clearly desirable also that the test method is investigated against other species for which an FAO-based discriminatory dose test is available.

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