Inheritance of phosphine resistance in *Sitophilus oryzae* (L.) (Coleoptera, Curculionidae)

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Abstract

Genetic crosses were carried out on a susceptible strain and a resistant strain of *Sitophilus oryzae* (L.). Time–mortality characteristics of susceptible and resistant parents and their F_1 hybrids, and response to time of the F_1 -backcross progeny and the reciprocal F_1 progeny were assessed in order to determine inheritance character. Results indicated that the response of the F_1 hybrid to phosphine was closer to its susceptible strain than to its resistant parent; there is no evidence of sex linkage between the RS and SR hybrids. χ^2 analysis of the response observed of the F_1-backcross progeny and the F_2 progeny rejected the null hypothesis of monogenic inheritance for resistance. Inheritance of phosphine resistance in *S. oryzae* is more complex and resistance appears to be controlled by more than one autosomal factor, but the major gene involved is incompletely recessive. The degree of dominance (D) to phosphine resistance for the species is –0.348.

Introduction

In recent years phosphine has become a popular fumigant for the disinfestation of stored grain and other commodities. In China this fumigant has been used from the early 1960s and almost total reliance has been progressively placed on phosphine because of its ease of use, wide availability and low residues. Currently, more than 80% of stored grain is dependent on phosphine fumigation for disinfestation. The continuous and widespread use of an insecticide can result in the rapid development of resistance. High resistance to phosphine has occurred where repeated fumigations are undertaken in poorly sealed storage (Taylor 1989).

Resistance to phosphine was first reported in a strain of *Sitophilus granarius* selected in the laboratory (Monro et al. 1972). Subsequently, the FAO global survey report (Champ and Dyte 1976) detailed the presence of low levels of phosphine resistance in several species of stored grain pests. Eight of 135 samples of *Sitophilus oryzae* tested from six countries were found to be resistant. The maximum resistance factor for *S. oryzae* was 2.5 times. More recently, a survey undertaken by Taylor (1989) revealed widespread resistance to phosphine in several major stored grain pests; 5 of the 8 samples of *S. oryzae* tested from South Asia and Brazil were resistant. It seems that phosphine resistance can be selected in most strains of all species of stored-grain pests (Taylor 1989; Winks 1986). Nevertheless, despite the importance and widespread occurrence of this phenomenon there is little information on the genetics of phosphine resistance in stored-grain pests (Ansell et al. 1990). This information is fundamental to an understanding of the evolution of resistance to phosphine and is a necessary basis for the development of procedures to manage this resistance.

In China, high-level resistance to phosphine was first detected in 1976 in a field strain of *S. oryzae* (Liang Quan 1976) from Mei county of Guangdong province, an area where phosphine had long been generated from calcium phosphide formulations. *S. oryzae* is regarded as the most important insect pest of stored grains in the southern provinces of China. In this report we present an analysis of the inheritance of phosphine resistance in adults of *S. oryzae*.

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Materials and Methods

Insect strains

The discriminating concentration of 0.04 mg phosphine/L for 20 hours as recommended by the FAO (Anon. 1975) was used to define resistance. The susceptible strain used in this study was collected originally in 1983 from Tibet and the resistant strain was supplied by Guangdong Institute of Cereal Science Research in 1976 from Mei county. The susceptible strain (T–16) was originally derived from 110 single pair crosses of an insect sample from Tibet. Newly emerged virgin adults were used to set up each single pair. These insects were allowed to oviposit on wheat for 2 weeks. The parents were then tested at 0.01 mg/L (20 hours) phosphine. Offspring of insects which failed to survive were pooled to form the susceptible strain. Before this investigation, the resistant strain (G–12) was selected for four generations with 1.6 mg/L and 3.6 mg/L (20 hours) and then 116 single pairs were tested at 3.6 mg/L. The progeny of pairs which survived this concentration were pooled to form the resistant strain.

Generating phosphine

Phosphine gas was generated by the action of acid on zinc phosphide dust as described in the FAO method (Anon. 1975). Phosphine concentration was determined using colorimetrically.

Phosphine susceptibility tests

In all cases, the responses to phosphine of parental strains and the F_1 and F_2 progenies were measured as mortality occurring at a range of exposure times at a constant concentration of 0.04 mg/L (Anon. 1975). This is the lowest concentration at a 20 hours exposure period that would give >99.99% mortality of homozygous susceptible individuals. Three batches of 40 adult insects were exposed to the fumigant at each of a range of time intervals. Insects were confined within 16 mL plastic bottles sealed with mesh. Fumigations were carried out in 1 L jars into which phosphine was injected through a rubber septum. After each exposure time the insects

were held in culture medium for 2 weeks at which time mortality was assessed.

Cross procedure

To determine the mode of inheritance reciprocal mass crosses were made between susceptible and resistant strains to obtain F_1, F_2 and backcross progeny (F_1–BC) following the plan outlined by Collins (1986). Virginity was assured by isolating each sex one day after emergence of adults (Halstead 1963) and the appropriate cross was made.

Reciprocal F_1 crosses were made to test for dominance and whether resistance was autosomal or sex linked. The single gene hypothesis was tested by exposing the F_2 and the F_1–BC progeny to a full range of time–mortality plots at the fixed concentration of 0.04 mg/L. If a single gene is responsible for resistance then plateaus will occur in the F_2 regression line about at 25 and 75% mortality and in the F_1–BC progeny regression line about at 50% mortality.

Statistical analyses

The results of phosphine susceptibility tests of the parental strains and their reciprocal F_1 and F_2 progenies were fitted to log time–probit mortality curves by method of Finney (1952). Goodness-of-fit was tested using χ^2(P=0.05) and LT_{50} values were estimated. The resistance factors were obtained as the simple ratio of the LT_{50} of the resistant strain against the susceptible strain. Degree of dominance (D) is based on the following calculation (Stone 1968).

$$D = \frac{2LT_{50}(RS) - LT_{50}(RR) - LT_{50}(SS)}{LT_{50}(RR) - LT_{50}(SS)}$$

Relative potency analysis (Finney 1952) was used to test for differences between the reciprocal crosses of the F_1 (i.e., between progeny of R × S and S × R) and of their F_2 progeny (i.e., between progeny of RS × RS and SR × SR). For assays of the F_2 and F_1–BC progenies, differences between observed and expected responses to phosphine were tested using the χ^2 analysis described by Finney (1952). To test the hypothesis that a single gene controlled resistance the expected proportion responding at each time plot was calculated as following (Georghiou 1969, Collins 1986):

(a) for F_2 progeny: $X_y = W_{(SS)}0.25 + W_{(SR)}\,0.5 + W_{(RR)}\,0.25$ where X = the expected response at a given time *y*; and W = the observed response of SS, SR and RR genotypes at time *y*, obtained directly from respective regression lines.

(b) for F_1–BC to RR parent: $X_y = W_{(SR)}0.5 + W_{(RR)}0.5$

Results

Results of reciprocal crosses of the resistant (G–12) and susceptible (T–16) strains indicated the response of the F_1 hybrids to phosphine was closer to its susceptible parent than to the resistant strain at the LT_{50} (Table 1, Fig. 1).

Relative potency analysis showed that there was no evidence of sex linkage between the progeny of R × S and S × R (relative potency [95% limits] = 1.03 [0.97 – 1.09]) and maternal effects were absent. Resistance, therefore, was autosomally inherited and degree of dominance (*D*) was –0.348 for *S. oryzae* to phosphine resistance.

The observed response of the F_1 – BC progeny showed a lack of a plateau at 50% mortality (Fig. 1) and χ^2 analysis (χ^2 = 80.48; df = 7; p<0.005) rejected the null hypothesis of single gene control of resistance. Inheritance of phosphine resistance in *S. oryzae* appears to be controlled by more than one autosomal factor but the major gene involved is incompletely recessive. The shape of the F_2 regression line (Fig. 2) indicated that there was no plateau at 25 and 75% mortality and X^2 analysis showed that the null hypothesis of monogenic inheritance was rejected (X^2 = 290.78; df = 7; p 0.005). Reciprocal F_2 progenies were not significantly different in their response to phosphine (relative potency [95% limits] = 1.02 [0.96–1.08]).

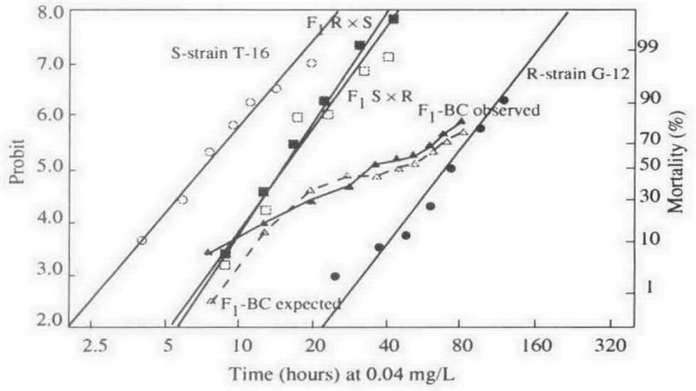

Fig.1. Comparison of the observed response of *S. oryzae* to phosphine and the expected response of the F_1–BC progeny for resistance controlled by a single gene and calculated from the response of SS, F_1 and RR phenotypes. ○ = SS (T–16), □ = F_1 (S × R), ■ = F_1 (R × S), ▲ = F_1–BC to RR progeny observed, Δ = expected response of F–BC progeny, ● = RR (G-12).

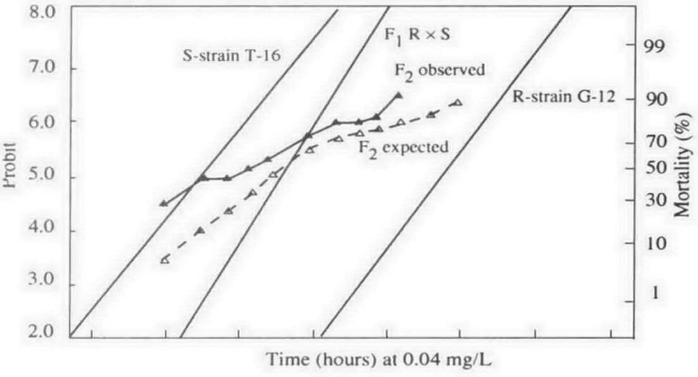

Fig. 2. Comparison of the observed response of *S. oryzae* to phosphine and the expected response of the F_2 progeny for resistance controlled by a single gene and calculated from the response of SS, F_1 and RR phenotypes. ▲ = F_2 progeny observed, Δ = expected response of F_2 progeny.

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References

Anon. 1975. Recommended methods for detection and measurement of resistance of agricultural pests to pesticide. Tentative method of adults of some major pest species of stored cereals, with methyl bromide and phosphine. FAO Method No. 16. FAO Plant Protection Bulletin, 23, 12–26.

Ansell, M.R., Dyte, C.E. and Smith, R.H. 1990. Inheritance of phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica*. In: Fleurat-Lessard, F. and Ducom, P., ed., Proceedings

Table 1. Response to phosphine of T–16, G–12 and their reciprocal F_1 progenies of *Sitophilus oryzae*.

Strains	n	LT_{50} (95% FL) Time (hours) at 0.04 mg/L	Slope (SE)	Resistance factor
T–16 (S)	840	7.6 (7.0–8.1)	5.31 (0.17)	–
G–12 (R)	840	69.1 (65.4–73.1)	5.99 (0.69)	9
F_1 (SXR)	840	15.8 (14.8–16.7)	6.67 (0.50)	2
F_1 (RXS)	840	15.6 (14.7–16.5)	6.96 (0.79)	2

of Fifth International Working Conference on Stored-product Protection, Bordeaux, France, September 1990, 961–970.

Champ, B.R. and Dyte, C.E. 1976. Report of the FAO global survey of pesticide susceptibility of stored grain pests. Rome, Food and Agriculture Organization.

Collins, P.F. 1986. Genetic analysis of fenitrothion resistance in the sawtoothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Cucujidae). Journal of Economic Entomology, 79, 1196–1199.

Finney, D.J. 1952. Probit analysis 2nd ed. London, Cambridge University Press.

Georghiou, G.P. 1969. Genetics of resistance to insecticides in houseflies and mosquitoes. Experimental Parasitology, 26, 224–255.

Halstead, D.G.H. 1963. External sex differences in stored-products of Coleoptera. Bulletin of Entomological Research, 54, 119–134.

Liang Quan 1976. Resistance to phosphine in four major species of stored grain pests in Guangdong, China. Sichuan Liangyou Keji. 1976, 4, 1–11 (in Chinese).

Monro, H.A.U., Upitis, E. and Bond, E.J. 1972. Resistance of a laboratory strain of *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) to phosphine. Journal of Stored Products Research, 8, 199–207.

Stone, B.F. 1968. A formula for determining degree of dominance in cases of monofactoral inheritance of resistance to chemicals. Bulletin of the World Health Organization, 38, 325–326.

Taylor, R.W.D. 1989. Phosphine—a major grain fumigant at risk. International Pest Control, 31, 10–14.

Winks, R.G. 1986. The effect of phosphine on resistant insects. In: Proceedings of GASGA Seminar on Fumigation Technology in Developing Countries. London, Tropical Development and Research Institute.