PS4-6 – 6134

Resistance to Phosphine in *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) collected from wheat storages in Brazil

I. Lorini[1], P.J. Collins[2]

Abstract

Samples of *R. dominica* were collected from control failures occurring at central storages in three southern states of Brazil between 1991 and 2003. Resistance to phosphine was characterised in these samples using a range of tests including discriminating doses, full dose-response assays and mixed-age culture assays. Of the 19 samples tested, five were diagnosed with weak resistance and 14 with strong resistance. Resistance to phosphine in Brazil is widespread and strongly resistant strains are common. There is an urgent need to improve fumigation and pest management practices in Brazil to manage phosphine resistance.

Key words: Phosphine, *Rhyzopertha dominica*, Resistance, Brazil, Fumigation.

Introduction

The lesser grain borer, *Rhyzopertha dominica*, is the most severe pest of stored wheat in Brazil. Traditionally, grain protectants have been used to control this pest. However, because of widespread resistance and market reluctance to accept chemical residues, grain storage managers have been applying phosphine for the last 10-15 years. However, the industry has been experiencing many control failures with this fumigant. Because there is no practical alternative to phosphine, managers are responding to control failures by applying stronger and more frequent doses of phosphine. Fumigations are generally undertaken in unsealed silos and in many storages hygiene is very poor compounding the apparent resistance issue.

Although resistance to phosphine has previously been detected in *R. dominica* from Brazil (Taylor, 1989; Ansell et al., 1990; Pacheco et al., 1990; Sartori et al., 1990), these studies did not attempt to characterize resistance in terms of its strength or in relation to dosage. Our aim was to not only document the distribution of resistance but to also characterise its strength in relation to practical doses of phosphine used in grain storages and to use this information as a basis for developing a strategy to combat the development of resistance.

Materials and methods

Insects

Nineteen strains of *R. dominica* were collected

[1] Brazilian Agricultural Research Corporation (EMBRAPA), National Wheat Research Centre (Embrapa Wheat). Rodovia, BR 285, km 294, CEP 99001-970, Passo Fundo, RS, Brazil E-mail: ilorini@cnpt.embrapa.br

[2] Department of Primary Industries and Fisheries, 80 Meiers Road, Indooroopilly QLD 4068, Australia. E-mail: pat.collins@dpi.qld.gov.au,

from central storage sites throughout the major grain growing regions of southern and central Brazil over a period of 12 years. Most samples came from control failures at storages that had been used phosphine for many years. The response of these population samples to phosphine was compared with those of three reference laboratory strains from Australia: QRD14 (susceptible), QRD369 (weak resistance to phosphine) and QRD 569 (strong resistance to phosphine). The responses of these strains to phosphine and their resistance genotypes have been characterized previously (Collins et al., 2005; Schlipalius et al., 2002). QRD569 is homozygous for two major resistance genes coding for "strong resistance", while QRD369 is homozygous for one major resistance gene coding for "weak resistance".

Insects were maintained without further exposure to phosphine in the Stored Products Laboratory of the National Wheat Research Centre (Embrapa Wheat), Passo Fundo RS, Brazil until they were imported to Australia in 2004 (Australian Quarantine Inspection Service permit 200408925). The insects were then maintained in a quarantine facility at the Department of Primary Industries and Fisheries, Indooroopilly, Queensland, where all experiments were undertaken.

Characterising resistance to phosphine

The insect samples were subjected to a series of assays to characterise their resistance status. Initially, discriminating concentration tests were performed on adult insects to provide a diagnosis of the likely phosphine resistance phenotype of each strain. Each sample was then exposed to a range of concentrations of phosphine to further characterise resistance levels. Finally, all life stages of each strain were tested using a 'mixed-age culture' method that simulates field fumigations by exposing insect populations to a continuous flow of phosphine gas mixed with air. The advantage of the last method is that it relates resistance levels directly with realistic phosphine dosages.

Discriminating concentration tests

Adults were exposed to phosphine using a somewhat modified version of the standard FAO test for resistance to phosphine (FAO, 1975). Discriminating doses were 0.03 mg litre^{-1} for 20 h to separate susceptible from resistant insects, and to 0.25 mg litre^{-1} for 48 h to separate weak resistant from strong resistant (Collins et al., 2002). Phosphine was generated in the laboratory from a commercial formulation of aluminium phosphide and collected over acidified water and its concentration was determined by gas chromatography using a gas density balance. Two replicates of 50 beetles (1-3 weeks after eclosion) of each strain were tested at each discriminating concentration.

Dose-response lines

Each strain was exposed to a range of phosphine concentrations (0.016 to 2.0 mg litre^{-1}) for 48 h in desiccators using the FAO method (FAO, 1975) as described previously. Three replicates of 40 adult beetles (1-3 weeks after eclosion) were tested at each concentration level with 8-10 concentrations tested in each assay.

The criterion of response was mortality, defined as the absence of movement during a two minute observation period. Results were corrected for control mortality using Abbott's formula (Abbott, 1925) and probit regressions (Finney, 1971) were fitted to the data using GenStat 6 software (GenStat, 2002), to obtain LC_{50} and $LC_{99.9}$ values, confidence limits and slopes. For comparison between response lines of strains, resistance factors were calculated dividing their LC_{50} by the LC_{50} of the reference susceptible strain.

Assays of mixed-age cultures

Mixed age culture assays were performed as described previously by Collins et al. (2005) to characterise the most resistant Brazilian strain by comparing it with responses obtained from the reference strong resistant and weak resistant

strains. Insect cultures were specially prepared so that they contained all life stages living in wheat. These were placed into stainless-steel chambers and phosphine and air were allowed to flow in and out in one direction controlled separately by mass flow controllers. The experiments were undertaken in rooms that maintained a constant temperature 30 ± 1 ºC. Moisture content of the grain was maintained at 12 % by passing the phosphine/air mixture through a water-bath set at an appropriate temperature.

Insects were removed from the fumigation after a pre-determined exposure period. All adults, live and dead, were extracted and counted. The cultures were then incubated for 8 weeks and again examined for the presence of live adult insects. In this way, the time taken, in days, required to completely control all life stages was determined at each test concentration.

Results

Response to discriminating concentrations

The three reference strains of *R. dominica* responded as expected: none of the susceptible strain (QRD14) survived either discriminating dose, while most weak resistant (QRD369) insects survived the lower discriminating dose but none survived the higher one, and a high proportion of individuals of the strong resistant strain (QRD569) survived the higher discriminating dose. Unexpectedly however, all strains of *R. dominica* from Brazil had survivors at one or both discriminating doses indicating that resistance was widespread and apparently at a high level. Of the 19 samples tested, five could be diagnosed with "weak" resistance and 14 with "strong" resistance.

Multiple dose assays

All four field samples diagnosed as possessing the weak resistance phenotype showed LC_{50} values significantly (based on non-overlap of 95 % CL) less than that of the reference weak resistant strain and this was reflected in the lower resistance factors. However, all but one of these strains had $LC_{99.9}$ values significantly (non-overlap of 95 % CL) higher than the reference weak resistant strain as a result of relatively shallow slopes displayed by these samples. Shallow slopes of response lines indicate high variance in response to phosphine in the samples. Resistance factors for "weak-resistance" strains ranged from 3 to 22.

Of the 14 population samples of *R. dominica* diagnosed with the strong resistance genotype, one, BR32 from Chapada, RS, had significantly (non overlap of 95 % CL) higher resistance than reference strong resistant strain. This difference was only two-fold at the LC_{50}, however. Four samples (BR13, BR19, BR26 and BR33) were not significantly different in their responses from this reference strain at both the LC_{50} and $LC_{99.9}$; all showing resistance factors > 200-fold. All other samples showed significantly lower LC_{50}s (non-overlap of 95% CL) than the reference strong resistant strain, with resistance factors ranging from 21 to 108, but similar $LC_{99.9}$ values. All "strong resistant" samples except one (BR11) had lower slopes than the reference strong resistant strain.

Time to population extinction assays

Four Brazilian population samples stood out as showing high LC_{50} and $LC_{99.9}$ values and, based on results of the discriminating dose assays, relatively high frequencies of the Strong resistance genotype. Of these, BR33, collected from a cooperative storage at Maringá in Paraná State in 2002, was chosen for further characterisation. This sample showed highest reproductive capacity and was therefore judged an appropriate candidate for assays testing variables associated with population growth.

For all strains tested, $LT_{99.9}$ values and times to population extinction (TPE) decreased as phosphine concentration increased from 0.05 to 1 mg/l. However, doubling the dose to 2 mg/l did not reduce $LT_{99.9}$ or TPE. In fact, TPEs were

longer at 2 mg/l than at 1 mg/l for both BR33 and the strong resistant strain and there was no significant difference in $LT_{99.9}$ values (measured as non-overlap of 95 % confidence limits).

Discussion

Results have revealed the seriousness of the phosphine resistance problem in Brazilian *R. dominica*. Not only is resistance at a very high frequency, but the majority of population samples also demonstrated the strong resistance genotype. In addition, these population samples contained significant numbers of resistant individuals despite having been cultured in the laboratory without selection for as long as 13 years (ca. 70 generations). This indicates that the resistance genes are quite stable in *R. dominica* populations and that any fitness deficit associated with resistance appears to be absent.

Although resistance to phosphine has been reported previously in Brazilian *R. dominica* populations, all but one of these studies (Sartori et al., 1990) were limited to testing population samples with a single discriminating dose, following the FAO method (FAO, 1975). A single discriminating dose indicates the presence of resistance but provides no information about its significance. Sartori et al. (1990) varied the dose and exposure period and found that some resistant adults could survive for 7 days at 0.3 mg litre^{-1}, but provided no information on other life stages which may be more tolerant to phosphine. Our study differs from earlier work in two important aspects. Firstly, we used two discriminating doses on adults to diagnose the likely resistance phenotype in each sample with reference to representative laboratory strains, followed by full dose-response assays of adults. Secondly, we were about to estimate the significance of the resistance to real world insect pest management by investigating the responses to phosphine of mixed-age cultures living in wheat.

Although the population samples studied here were biased in that they came from control failures and were not strictly random samples, they do demonstrate clearly the extent of the problem in the cooperative storage system. So how has this situation developed? We believe there are four major contributing reasons. Firstly, insect pest populations are high because of the favourable climate and little or no insect management is practiced on farms. Secondly, phosphine is used extensively by storage managers. Thirdly, storages are generally not sealed before fumigation so that under-dosing is routine and concentrations are not monitored (Lorini, 2003). Under-dosing has allowed the survival of insects heterozygous for resistance genes and re-fumigation, because of initial failures, has resulted in selection of populations with high frequencies of insects homozygous for resistance genes. Finally, there is very little knowledge of the correct application of phosphine in the industry. Control failures are now common and the typical response of storage managers is to re-fumigate and to apply a higher dose of aluminium phosphide. There is obviously an urgent need to change fumigation and pest management practices in Brazil to manage phosphine resistance. Silos used for fumigation must be sealed to a standard that retains gas long enough and at high enough concentration to ensure complete control of resistant insects. In addition, a national approach, including research and extension institutions in partnership with industry, needs to be taken so that strategies to manage resistance to phosphine to protect Brazil's domestic and international grain markets can be developed and implemented.

Acknowledgements

The senior author thanks the Queensland Department of Primary Industries and Fisheries (Australia) for providing laboratory facilities for this study. We thank the Australian Quarantine and Inspection Service (AQIS) for permitting importation of the Brazilian insects. The senior author thanks the Brazilian Agricultural Research Corporation (Embrapa) and the National Council

for Scientific and Technological Development (CNPq) – Brazil, for the postdoctoral agreement and scholarship, respectively.

References

Abbott W.S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economical Entomology 18, 265-267.

Ansell M.R., Dyte C.E., Smith R.H., 1990. The inheritance of phosphine resistance in *Rhyzopertha dominica* and *Tribolium castaneum*. In: Proc. Fifth International Working Conference on Stored-product Protection, ed by Fleurat-Lessard F, Ducom P, Bordeaux, France, 9-14 September 1990. Institut National de la Recherche Agronomique, Paris, France, pp. 961-970.

Collins P.J., Daglish G.J., Bengston M., Lambkin T.M., Pavic H., 2002. Genetics of resistance to phosphine in *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Journal of Economical Entomology 95, 862-869.

Collins, P.J., Daglish, G.J., Pavic, H., Kopittke, K.A., 2005. Response of mixed-age cultures of phosphine-resistant and susceptible strains of the lesser grain borer, *Rhyzopertha dominica*, to phosphine at a range of concentrations and exposure periods. Journal of Stored Products Research 41, 373-385.

FAO, 1975. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative method for adults of some major pest species of stored cereals, with methyl bromide and phosphine. FAO Method Nº 16. FAO Plant Prot Bull 23, 12-25.

Finney D.J., 1971. Probit Analysis, Third ed. Cambridge University Press, London.

GenStat, 6 Committee, 2002. GenStat for Windows, Sixth. ed. Numerical Algorithms Group, Oxford, UK.

Lorini I., 2003. Manual técnico para o manejo integrado de pragas de grãos de cereais armazenados. Embrapa Trigo. Passo Fundo, RS. 80 pp.

Pacheco I.A., Sartori M.R., Taylor R.W.D., 1990. Levantamento de resistência de insetos-pragas de grãos armazenados à fosfina no Estado de São Paulo. Coletânia ITAL 20, 144-154.

Sartori M.R., Pacheco I.A., Vilar R.M.G., 1990. Resistance to phosphine in stored grain insects in Brazil. In: Proc. Fifth International Working Conference on Stored-product Protection. Bordeaux, France, 9-14 September 1990. Institut National de la Recherche Agronomique, Paris, France, pp. 1041-1050.

Schlipalius D.I., Cheng Q., Reilly P.E.B., Collins P.J., Ebert P.R., 2002. Genetic linkage analysis of the lesser grain borer *Rhyzopertha dominica* identifies two loci that confer high-level resistance to the fumigant phosphine. Genetics 161, 773-782.

Taylor R.W., 1989. Phosphine-a major grain fumigant at risk. Int Pest Control 31, 10-14.