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Detection and characterisation of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae)

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Abstract: As failure to control *Rhyzopertha dominica* (F.) with phosphine is a common problem in the grain-growing regions of Brazil, a study was undertaken to investigate the frequency, distribution and strength of phosphine resistance in *R. dominica* in Brazil. Nineteen samples of *R. dominica* were collected between 1991 and 2003 from central storages where phosphine fumigation had failed to control this species. Insects were cultured without selection until testing in 2005. Each sample was tested for resistance to phosphine on the basis of the response of adults to discriminating concentrations of phosphine (20 and 48 h exposures) and full dose–response assays (48 h exposure). Responses of the Brazilian *R. dominica* samples were compared with reference susceptible, weak-resistance and strong-resistance strains from Australia in parallel assays. All Brazilian population samples showed resistance to phosphine: five were diagnosed with weak resistance and 14 with strong resistance. Five samples showed levels of resistance similar to the reference strong-resistance strain. A representative highly resistant sample was characterised by exposing mixed-age cultures to a range of constant concentrations of phosphine for various exposure periods. Time to population extinction (TPE) and time to 99.9% suppression of population ($LT_{99.9}$) values of this sample were generally similar to those of the reference strong-resistance strain. For example, at 0.1, 0.5 and 1.0 mg L^{-1}, $LT_{99.9}$ values for BR33 and the reference strong-resistance strain were respectively 21, 6.4 and 3.7 days and 17, 6.2 and 3.8 days. With both strains, doubling phosphine concentrations to 2 mg L^{-1} resulted in increased $LT_{99.9}$ and TPE. High level and frequency of resistance in all population samples, some of which had been cultured without selection for up to 12 years, suggest little or no fitness deficit associated with phosphine resistance. The present research indicates that widespread phosphine resistance may be developing in Brazil. Fumigation practices should be monitored and resistance management plans implemented to alleviate further resistance development.

Keywords: phosphine; *Rhyzopertha dominica*; resistance; Brazil; fumigation

1 INTRODUCTION

Grain production in Brazil has doubled in the last 10 years to become a significant component of the economy. Concomitant with this increase in production has been a rapid increase in reliance by storage managers on fumigation with phosphine, primarily because of the high frequency of control failures with grain protectant insecticides. However, control failures with phosphine are now also common, possibly because most fumigations are undertaken in unsealed silos and in situations where sanitation is poor. As a consequence, many parcels of grain are repeatedly fumigated, and many storage managers are responding to control failures by applying very high doses of phosphine (Lorini I, unpublished). Resistance to phosphine in Brazilian insects was first detected in the 1980s[1] and confirmed in subsequent surveys.[2–4] Although establishing the presence of resistance, these studies did not attempt to characterise resistance in relation to dosage or control failure. In spite of the widespread and increasing occurrence of control failures with phosphine (Lorini I, unpublished), there is no recent information on the distribution, frequency or strength of resistance to this fumigant in Brazil. Previous studies and industry experience indicate that most control failures in Brazil have been caused by the lesser grain borer, *Rhyzopertha dominica* (F.).

Strong resistance in *R. dominica* has been reported from several regions internationally, requiring increases in either phosphine concentration or exposure period, or both, to overcome the resistance, and improvements in sealing to maintain these standards.[5–7] The aim of the present work was to investigate the frequency, distribution and strength of

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phosphine resistance in *R. dominica* in Brazil. This information will provide baseline data for the development of a strategy for the Brazilian grain industry to manage resistance to phosphine.

2 MATERIALS AND METHODS

Strains of *R. dominica* were collected from sites throughout the major grain-growing regions of Brazil and characterised for resistance to phosphine using the FAO method.[8] The strain with highest resistance was then further characterised by exposing mixed-age cultures to fixed concentrations of phosphine using a continuous flow method. Responses were compared with those from laboratory reference strains from Australia.

2.1 Insects

Twenty-two strains of *R. dominica* were used in these experiments. Nineteen population samples were collected over 12 years from central storages in southern and central Brazil (Fig. 1; Table 1). Most samples came from control failures at storages that had been using phosphine for many years. Repeated fumigations and increased application rates were typically employed in an attempt to eliminate these persistent infestations. Each sample was then maintained without further exposure to phosphine in the stored products laboratory of the National Wheat Research Centre (Embrapa Wheat), Passo Fundo RS, Brazil. The insects were imported to Australia in 2004 with quarantine permission (AQIS permit 200408925) and maintained in a quarantine facility at the Department of Primary Industries and Fisheries, Indooroopilly, Queensland.

The response of the Brazilian strains to phosphine was compared with three laboratory reference strains: one susceptible strain (QRD14), one weak-resistance strain (QRD369) and one strong-resistance strain (QRD 569). The responses of these strains to phosphine and their resistance genotypes have been characterised previously.[9,10] 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.

2.2 Discriminating concentration tests

Discriminating concentration tests were used to provide an initial diagnosis of the likely phosphine resistance phenotype of each strain. Adults were exposed to phosphine at 0.03 mg L^{-1} for 20 h and to 0.25 mg L^{-1} for 48 h using methods recommended by FAO[8] with some modification. The former dose was used to separate susceptible from resistant insects,[8] while the latter was used to separate weak-resistance from strong-resistance insects.[6] Phosphine was generated from a commercial formulation of aluminium phosphide and collected over acidified water. Its concentration was determined by gas chromatography using a gas density balance (Aerograph Model 90-P; Varian, Mount Waverley, Victoria, Australia) with dichlorofluoromethane (Refrigerant F22; Lovelock Luke, Mayne, Queensland, Australia) as a gas carrier. Two replicates of 50 beetles (1–3 weeks after eclosion) of each strain were confined within ventilated polystyrene vials with a small quantity of wheat grain inside gas-tight desiccators. Phosphine was injected through a septum in the lid of the desiccator to give the required concentration. Insects were exposed to phosphine at 25 °C and 70% RH. After exposure, the insects were maintained in a cabinet at 30 °C and 60% RH, and mortality was assessed after 7 days.

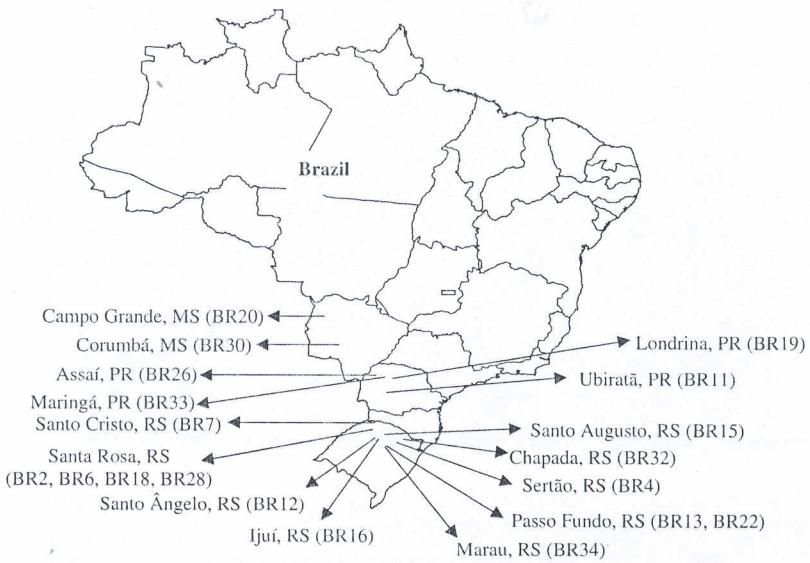

Figure 1. Distribution of *Rhyzopertha dominica* collection sites in southern to central Brazil. MS = Mato Grosso do Sul; PR = Paraná; RS = Rio Grande do Sul.

Table 1. Corrected mortality response of field and reference strains of *Rhyzopertha dominica* to discriminating concentrations (DC) of phosphine and multiple dose assays. 100 adult insects were tested at each DC. Control mortality ranged from 0.0 to 11.0%. The exposure period of dose–response assays was 48 h, and LC values are in mg L^{-1} of phosphine

Strains and population samples		Discriminating concentrations (DC)		Multiple dose assays					
Identifier	Origin (year collected)	0.03 mg L^{-1} for 20 h	0.25 mg L^{-1} for 48 h	Deviance	df	Slope (±SE)	LC_{50} (95% CL)	$LC_{99.9}$ (95% CL)	RF[a]
Laboratory reference strains									
Susceptible	Oakey (1971)	100	100	56.74	4	2.5 (±0.67)	0.0016 (0.00080–0.0025)	0.027 (0.010–0.57)	–
Weak-R	Condamine (1990)	3.1	100	19.89	6	5.0 (±0.69)	0.047 (0.041–0.054)	0.20 (0.14–0.34)	29
Strong-R	Millmerran (1997)	4.0	16.7	6.746	6	2.4 (±0.15)	0.34 (0.30–0.39)	6.8 (4.8–10)	214
Brazilian field population samples diagnosed weak-resistant									
BR6	Santa Rosa (1993)	69.7	100	2.884	6	1.2 (±0.15)	0.0047 (0.0021–0.0079)	1.9 (0.89–6.9)	3
BR7	Santo Cristo (1994)	42.4	100	8.937	6	1.5 (±0.14)	0.013 (0.0088–0.017)	1.6 (0.89–3.8)	8
BR15	Santo Augusto (1999)	18.0	100	0.7380	4	3.8 (±0.42)	0.022(0.01–0.025)	0.14 (0.10–0.24)	14
BR18	Santa Rosa (1999)	12.5	100	2.431	6	2.4 (±0.17)	0.034 (0.030–0.039)	0.68 (0.48–1.0)	22
BR30	Corumbá (2001)	15.1	100	3.394	6	2.2 (±0.17)	0.028 (0.024–0.032)	0.72 (0.49–1.2)	18
Brazilian field population samples diagnosed strong-resistant									
BR2	Santa Rosa (1991)	15.8	84.6	11.87	6	1.6 (±0.10)	0.043 (0.036–0.051)	3.9 (2.5–7.1)	27
BR4	Sertão (1994)	2.1	62.2	20.46	5	1.8 (±0.22)	0.17 (0.13–0.23)	9.6 (4.1–37)	108
BR11	Ubiratã (1998)	1.0	72.0	2.119	4	3.3 (±0.22)	0.11 (0.10–0.12)	0.96 (0.74–1.3)	71
BR12	Santo Ângelo (1998)	7.1	78.0	8.335	6	1.9 (±0.11)	0.072 (0.063–0.082)	2.9 (2.0–4.7)	45
BR13	Passo Fundo (1998)	5.0	35.3	66.78	6	2.2 (±0.41)	0.35 (0.24–0.54)	8.5 (3.4–57)	220
BR16	Ijuí (1999)	5.1	61.0	55.21	6	1.7 (±0.29)	0.10 (0.064–0.15)	6.2 (2.2–43)	63
BR19	Londrina (1999)	3.0	8.0	69.31	6	2.2 (±0.42)	0.35 (0.23–0.55)	9.1 (3.4–70)	222
BR20	Campo Grande (1999)	9.9	62.8	14.04	6	1.9 (±0.15)	0.14 (0.11–0.17)	6.2 (3.6–13)	86
BR22	Passo Fundo (2000)	30.6	97.0	10.26	6	2.0 (±0.14)	0.033 (0.028–0.038)	1.1 (0.77–1.9)	21
BR26	Assaí (2000)	1.0	30.3	20.85	4	1.9 (±0.32)	0.44 (0.32–0.67)	18 (6.3–130)	277
BR28	Santa Rosa (2001)	6.0	74.7	24.15	6	2.0 (±0.23)	0.067 (0.051–0.087)	2.5 (1.3–7.1)	42
BR32	Chapada (2002)	9.3	7.5	80.74	6	2.2 (±0.57)	0.88 (0.56–1.7)	21 (6.3–720)	549
BR33	Maringá (2002)	0.0	29.3	24.44	6	2.4 (±0.28)	0.42 (0.33–0.53)	8.0 (4.4–20)	261
BR34	Marau (2003)	18.7	53.3	19.78	6	1.3 (±0.16)	0.059 (0.041–0.082)	12 (4.8–54)	37

[a] RF = resistance factor.

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2.3 Dose–response lines

Assays were also carried out to determine the dose–response curve of each *R. dominica* strain to phosphine further to characterise resistance. Each strain was exposed to a range of phosphine concentrations (0.016–2.0 mg L^{-1}) for 48 h in desiccators using the FAO method[8] as described in Section 2.2. 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 2 min observation period. Results were corrected for control mortality using Abbott's formula,[11] and probit regressions[12] were fitted to the data using GenStat 6 software[13] to obtain LC_{50} and $LC_{99.9}$ values, confidence limits and slopes. For comparison between response lines of strains, resistance factors were calculated by dividing the LC_{50} by the LC_{50} of the reference susceptible strain.

2.4 Time to end-point mortality

An experiment was undertaken to validate assessing adult mortality 7 days after exposure instead of 14 days after exposure as recommended by the FAO method.[8] Time to end-point mortality was determined after exposure of adults to phosphine using the FAO method. Adults of the Brazilian, phosphine-resistant field sample BR33 were exposed to phosphine in desiccators as described in Section 2.2. The experiment consisted of two test concentrations, 0.3 and 1.0 mg L^{-1}, both for a 48 h exposure period, and 11 mortality assessment times, from 1 h to 10 days. It was expected that these doses would produce mortalities of about 50 and 85% respectively. Mortalities at each exposure period were derived from separate assays. Each assay consisted of three replicates of 50 adults (1–3 weeks after eclosion). A randomised block design was used, and the results were analysed using ANOVA and *F*-test.

2.5 Assays of mixed-age cultures

Mixed-age culture assays were performed as described previously by Collins *et al.*[6] to characterise the most resistant Brazilian strain by comparing it with responses obtained from the reference strong-resistance and weak-resistance strains. In this technique, cultures of insects living in whole grain and containing all life stages are exposed to fixed concentrations of phosphine using a continuous flow application of fumigant mixed with air. The advantage of this method is that it simulates exposure of insect populations to phosphine in the field. Briefly, each mixed-age culture is set up by placing 50 *R. dominica* adults into 160 g of wheat (12% moisture content, wet weight basis) maintained in glass jars at a constant temperature of 30 °C and 60% RH for 8 weeks. Each mixed-age culture was then placed into a cylindrical cage made from 50 mm sections of PVC pipe (92 mm inner diameter). Each end of the pipe was sealed with fine stainless steel mesh allowing movement of gases but preventing escape of the insects. The cages were constructed with a 15 mm flange at the bottom which fitted tightly into the top of the cage below. Untreated controls were prepared in parallel to every batch of test insects and incubated under the same conditions, except that they were not exposed to phosphine. Mass flow controllers (Brooks, Fisher Rosemount, Melbourne or Sierra, Procon, Brisbane, Australia) and a series of flow monitors governed the flow of gases in one direction from the cylinders through the tubing and fumigation chambers and vented out to the atmosphere through a fume hood. The experiments were undertaken in laboratories that were maintained at a constant temperature of 25 ± 1 °C. The phosphine concentration was monitored daily to ensure that it remained constant. Gas samples were drawn from sample points above and below each fumigation chamber, and the phosphine concentration was measured using a pulsed-flame photometric detector mounted in a Varian Star 3600CX gas chromatograph. Each sample was injected using a gas-tight syringe and carried by nitrogen through a 25 m megabore column (0.53 mm ID) packed with GSQ. Injector, oven and detector temperatures were 165, 100 and 250 °C respectively.

Phosphine concentrations ranging from 0.05 to 2.0 mg L^{-1} were tested for exposure periods of up to 14 days. Mixed-age cultures were removed from the fumigation after a predetermined exposure period, and all adults, live and dead, were sieved from the cultures and counted. The cultures were then incubated at 30 °C and 60% RH for 8 weeks and again examined for the presence of live adults representing eggs, larvae or pupae that had survived the fumigation and completed development.

Statistical analyses were performed on the total number of adults recovered at the end of the exposure and after 8 weeks incubation. For each phosphine concentration, times to 99.9% suppression of the population ($LT_{99.9}$) were calculated using probit analysis without transformed doses, Wadley's method,[12] using GenStat 6 software.[13] Wadley's method is used when the numbers of subjects exposed to a dose is unknown but an estimate can be obtained from the control. The authors tested logit and complementary log–log in addition to probits but found that the probit model, without transformed doses, was most suitable. Times to population extinction (TPE), defined as the earliest exposure period from which there was no emergence provided that this was also true in samples from longer exposure periods, were also recorded.[14]

3 RESULTS

3.1 Response to discriminating concentrations

The three reference strains of *R. dominica* responded as expected. A 100% mortality response was scored against the susceptible strain at both discriminating

doses, most weak-resistance insects survived the lower discriminating dose (0.03 mg L^{-1} for 20 h), but none survived the higher one (0.25 mg L^{-1} for 48 h), and a high proportion of individuals of the strong-resistance strain survived the higher discriminating dose (Table 1). 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. Furthermore, two Brazilian strains had more survivors than the reference strong-resistance strain at the higher discriminating dose.

3.2 Multiple dose assays

All four field samples of *R. dominica* 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-resistance strain, and this was reflected in the lower resistance factors (Table 1). However, all but one of these strains had $LC_{99.9}$ values significantly (non-overlap of 95% CL) higher than the reference weak-resistance strain as a result of the 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, had significantly (non-overlap of 95% CL) higher resistance than the reference strong-resistance strain (Table 1). This difference was only twofold at the LC_{50}, however. Four samples (BR13, BR19, BR26 and BR33) were not significantly different from this reference strain in their responses at both the LC_{50} and $LC_{99.9}$, all showing resistance factors >200-fold. All other samples showed significantly lower LC_{50} values (non-overlap of 95% CL) than the reference strong-resistance strain, with resistance factors ranging from 21 to 108, but similar $LC_{99.9}$ values. All strong-resistance samples except one (BR11) had lower slopes than the reference strong-resistance strain.

3.3 End point mortality assessment

Mortality of adults of the BR33 sample was assessed at 1 h and on each day between 1 and 10 days after test insects were removed from exposure to the fumigant. At both concentrations there were no significant differences between mortalities recorded at any of the assessment times (at 0.3 mg L^{-1}, $F = 1.376$, df = 10 and $P < 0.005$; at 1.0 mg L^{-1}, $F = 1.788$, df = 10 and $P < 0.005$). Mean mortality was 51.5% at 0.3 mg L^{-1} and 86.7% at 1 mg L^{-1}.

3.4 Response of mixed-age cultures

Four population samples of *R. dominica*, BR19, BR26, BR32 and BR33, 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 (Table 1). 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.

Mixed-age assay control numbers for BR33, including insects counted immediately after the exposure period and at 8 weeks, had a mean (±SE) of 2268 (±1003). Control means for the two reference strains were 1294 (±362) for the reference strong-resistance strain and 2590 (±507) for the susceptible strain.

For all strains of *R. dominica* tested, $LT_{99.9}$ values and times to population extinction (TPE) decreased as phosphine concentration increased from 0.05 to 1 mg L^{-1} (Table 2). However, doubling the dose to 2 mg L^{-1} did not reduce $LT_{99.9}$ or TPE. In fact, TPEs were longer at 2 mg L^{-1} than at 1 mg L^{-1} for both BR33 and the strong-resistance strain, and there was no significant difference in $LT_{99.9}$ values (measured as non-overlap of 95% confidence limits). Although probit analysis of BR33 data produced higher $LT_{99.9}$ values than for the strong-resistance strain at 0.05 and 0.1 mg L^{-1}, these were not significantly different based on non-overlap of confidence limits. Both $LT_{99.9}$ values and TPEs at 0.5–2 mg L^{-1} also indicated very similar responses in these two strains. Only data for assays at 0.5 and 2 mg L^{-1} were adequate for probit analysis of the responses of the S-strain. $LT_{99.9}$ values were significantly lower than those for the resistant strains at 0.5 mg L^{-1}, but not at 2 mg L^{-1}. There was no significant decrease in $LT_{99.9}$ at 2 mg L^{-1} compared with the response at 0.5 mg L^{-1}, and, as observed with BR33 and the strong-resistance reference strain, TPE was similar at these two concentrations.

4 DISCUSSION

The present results demonstrate clearly that strong resistance to phosphine in *R. dominica* is widespread in the important grain-growing regions of Brazil. Of the 19 samples collected between 1991 and 2003, 100% were resistant to phosphine and 74% were diagnosed with the strong-resistance genotype. The earliest sample showing strong resistance was collected in 1991, indicating that this genotype has been present in Brazil since at least that time. An important observation is that 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 there is no fitness deficit associated with resistance. In several cases, resistance levels in the Brazilian field samples showed LC_{50} and $LC_{99.9}$ values similar to

Table 2. Response to phosphine of mixed-age cultures of *Rhyzopertha dominica*. A phosphine-resistant Brazilian strain (BR33) is compared with the reference susceptible S-strain and the reference strong-resistance StR-strain. $LT_{99.9}$ values are times (days) to population extinction compared with untreated controls

Strain	Phosphine concentration ($mg\,L^{-1}$)	Probit analysis				Times to population extinction (days)
		Deviance	df	$LT_{99.9}$	95% confidence limits	
BR33	0.05	657.1	3	27	[a]	>14
	0.1	975.9	4	21	19–26	>14
	0.25	496.3	4	18	[a]	14
	0.5	518.6	4	6.4	5.5–9.3	5
	1	10.16	4	3.7	3.5–4.0	3
	2	462.7	4	4.9	3.8–15	>6
Strong-resistance	0.05	142.7	3	18	16–32	>14
	0.1	240.8	4	17	14–32	>14
	0.25[b]					<9
	0.5	133.7	4	6.2	5.5–7.6	6
	1	9.088	2	3.8	3.5–4.1	4
	2	10.02	3	3.9	3.6–4.2	5
Susceptible	0.05[b]					<10
	0.1[b]					<9
	0.25[b]					<9
	0.5	128.1	4	2.5	2.0–4.0	6
	1[b]					2
	2	4.578	3	3.1	2.4–5.3	5

[a] Confidence limits not calculated as a slope are not significantly different from zero.
[b] Data not adequate to perform probit analysis.

those of the reference strong-resistance strain from Australia, indicating that resistance had been selected to homozygosity in these populations.

Resistance to phosphine has been reported previously in Brazilian *R. dominica* populations.[1–4] All but one of these studies (Sartori *et al.*[4]) were limited to testing population samples with a single discriminating dose, following FAO method 16.[8] A single discriminating dose indicates the presence of resistance but provides no information about its significance. Sartori *et al.*[4] varied the dose and exposure period and found that some resistant adults could survive for 7 days at $0.3\,mg\,L^{-1}$, but provided no information on other life stages that may be more tolerant to phosphine. The present study differs from earlier studies by investigating the responses of adults to phosphine as well as the responses of mixed-age populations. Use was made of 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. Finally, the response of a representative strain (BR33) in simulated fumigations of mixed-age populations was characterised. This information is crucial to the future development of a strategy to manage resistance to phosphine in Brazil.

In spite of the fact that resistance to phosphine is very high in *R. dominica* from Brazil, it has been demonstrated that resistant populations can be controlled at reasonable concentrations of phosphine provided that the insects are exposed for long enough. The present results demonstrate that all life stages of BR33 were eliminated in less than 4 days at $1.0\,mg\,L^{-1}$. However, doubling the concentration to $2.0\,mg\,L^{-1}$ increased the time required to achieve population extinction (Table 2). This phenomenon was repeated in all test strains (Table 2) and has been reported previously.[6] It is clear that increasing doses much beyond $1\,mg\,L^{-1}$ does not result in a decrease in fumigation time and, in fact, may increase the time required for complete control.

A major disadvantage of FAO method 16 is that, although the exposure period is short, 20 h, the assay is not assessed for another 14 days.[8] The 14 days is required to allow the assay to reach end-point mortality, i.e. time for the insects to respond fully to the toxicant – to either die or recover.[15] However, the 14 day delay makes this assay of little practical use for the grain industry, where rapid diagnoses of resistance are required so that informed decisions can be made about pest or resistance management options. Results from the present assays with the phosphine-resistant strain BR33 suggest that, at least for this species, end-point mortality is reached in less than an hour after exposure to phosphine and may be occurring during the exposure period. This rapid time to end-point provides the potential for the FAO test[8] to be used for practical resistance management. Further work is required to confirm that all resistance strains and genotypes behave in the same manner as BR33, and to determine if the FAO assay[8] can be shortened for other species.

The present results have revealed the seriousness of the phosphine resistance problem in Brazilian

R. dominica. Not only is resistance at a very high frequency in these samples, but the majority of population samples also demonstrated the strong-resistance genotype. 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? The authors believe there are several contributing reasons. Firstly, insect pest populations are high because of the favourable climate, and little or no insect management is practised on farms. Secondly, phosphine is the treatment of choice by most storage managers because of problems of widespread resistance to grain protectants. Thirdly, storages are generally not sealed before fumigation, so that underdosing is routine and concentrations are not monitored.[16] Underdosing has allowed the survival of insects heterozygous for resistance genes, and refumigation, 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 refumigate and to apply a higher dose of aluminium phosphide. Wheat, for example, is typically fumigated 3–4 times while in storage (Lorini I, unpublished). 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 a 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.

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REFERENCES

1 Taylor RW, Phosphine – a major grain fumigant at risk. *Internat Pest Control* **31**:10–14 (1989).
2 Ansell MR, Dyte CE and Smith RH, The inheritance of phosphine resistance in *Rhyzopertha dominica* and *Tribolium castaneum*, in *Proc Fifth International Working Conference on Stored-product Protection, Bordeaux, France, 9–14 September 1990*, ed. by Fleurat-Lessard F and Ducom P. Institut National de la Recherche Agronomique, Paris, France, pp. 961–970 (1990).
3 Pacheco IA, Sartori MR and Taylor RWD, Levantamento de resistência de insetos-pragas de grãos armazenados à fosfina no Estado de São Paulo. *Coletânia ITAL* **20**:144–154 (1990).
4 Sartori MR, Pacheco IA and Vilar RMG, 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*, ed. by Fleurat-Lessard F and Ducom P. Institut National de la Recherche Agronomique, Paris, France, pp. 1041–1050 (1990).
5 Acda MA, Bengston M and Daglish GJ, Response to phosphine of susceptible and resistant strains of *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrychidae) from the Philippines. *Asia Life Sciences* **9**:103–113 (2000).
6 Collins PJ, Daglish GJ, Bengston M, Lambkin TM and Pavic H, Genetics of resistance to phosphine in *Rhyzopertha dominica* (Coleoptera: Bostrichidae). *J Econ Entomol* **95**:862–869 (2002).
7 Wang D, Collins PJ and Gao X, Optimising indoor phosphine fumigation of paddy rice bag-stacks under sheeting for control of resistant insects. *J Stored Prod Res* **42**:207–217 (2006).
8 FAO, 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 (1975).
9 Collins PJ, Daglish GJ, Pavic H and Kopittke RA, Response of mixed-age cultures of phosphine-resistant and susceptible strains of lesser grain borer, *Rhyzopertha dominica*, to phosphine at a range of concentrations and exposure periods. *J Stored Prod Res* **41**:373–385 (2005).
10 Schlipalius DI, Cheng Q, Reilly PEB, Collins PJ and Ebert PR, 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 (2002).
11 Abbott WS, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).
12 Finney DJ, *Probit Analysis*, 3rd edition. Cambridge University Press, London (1971).
13 *GenStat, 6 Committee, GenStat for Windows*, 6th edition. Numerical Algorithms Group, Oxford, UK (2002).
14 Winks RG and Hyne EA, Measurement of resistance to grain fumigants with particular reference to phosphine, in *Proc Sixth International Working Conference on Stored-product Protection, Canberra, Australia*, ed. by Higley E, Wright EJ, Banks HJ and Champ BR. CAB International, Oxford, UK, pp. 244–249 (1994).
15 Winks RG, The toxicity of phosphine to adults of *Tribolium castaneum* (Herbst): time as a response factor. *J Stored Prod Res* **18**:159–169 (1982).
16 Lorini I, Manual técnico para o manejo integrado de pragas de grãos de cereais armazenados. Embrapa Trigo, Passo Fundo, RS, 80 pp. (2003).