J Pest Sci
DOI 10.1007/s10340-017-0875-7

ORIGINAL PAPER

Relative tolerance and expression of resistance to phosphine in life stages of the rusty grain beetle, *Cryptolestes ferrugineus*

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Received: 29 January 2017 / Revised: 18 April 2017 / Accepted: 26 April 2017

Abstract *Cryptolestes ferrugineus* is a serious cosmopolitan pest of stored products. Frequent and indiscriminate usage of phosphine has caused the development of high levels of resistance to this fumigant. As there are few alternatives, it is imperative that resistance to phosphine is managed. Effective management requires knowledge of key factors driving the rate of selection. One of the most important factors is the response of each resistance genotype to phosphine, especially heterozygotes. Moreover, it is important to understand the expression of resistance in all life stages as all stages are subjected to selection during fumigation. We determined the relative tolerance and resistance levels to phosphine in all life stages of homozygous parental strains (susceptible and resistant) and their F_1 progeny (heterozygous) and estimated relative dominance of resistance within life stages over 48 h. In susceptible insects, relative tolerance was highest in eggs followed by pupae, then adults which had about the same tolerance as larvae. In homozygous resistant insects, the order of tolerance was adult = egg > pupae > larvae and in heterozygotes larvae > eggs > pupae > adults. All life stages expressed resistance with resistance ratios highest in adults > pupae > larvae > eggs. At LC_{50}, resistance was incompletely recessive in eggs, pupae and adults and incompletely dominant in larvae. Eggs and adults were also incompletely recessive at LC_{95}, but larvae were completely dominant and pupae were incompletely dominant. Our data showed that a proportion of heterozygotes in all life stages, the major carriers of resistance in the field, will survive at very high concentrations, particularly in the egg stage, forming a nucleus for reinfestation or dispersal of resistance.

Keywords Phosphine resistance · Immature stages · Dominance · Stored grain pests

Key message

- Resistance to phosphine in rusty grain beetle threatens control of this pest. Management is required as there are few viable alternatives.
- There is no information on the response to phosphine of immature life stages, particularly heterozygotes, for this species.
- The most tolerant life stages of homozygous parentals and heterozygous F_1 genotypes were adult = egg and larvae, respectively.
- Relative dominance increased with phosphine concentration, particularly in eggs, such that a proportion of heterozygotes, the carriers of resistance, may survive very high concentrations of phosphine.

Introduction

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), is an important secondary pest of stored grain and other dry commodities, mainly cereals, pulses and oilseeds and their

Communicated by C.G. Athanassiou.

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Published online: 10 May 2017

products (Hagstrum et al. 2013). It has a cosmopolitan distribution and is troublesome throughout the supply chain from farm storages to country silos, export terminals, processing plants and warehouses. Fumigation with phosphine (hydrogen phosphide, PH_3) is the preferred means of controlling this pest. However, the detection of very high levels of resistance to phosphine in some field populations of *C. ferrugineus* may jeopardise its role as an effective postharvest grain fumigant (Nayak et al. 2012). Loss of phosphine efficacy is of serious concern as there are few, if any, viable alternatives to phosphine for many commodities, particularly pulses and oilseeds (Bell 2000). Even for cereals, available alternatives consist of liquid contact insecticides (Daglish 2008) or the fumigant sulphuryl fluoride (Tsai, 2010), both of which have the potential to leave residues that may be unacceptable to markets.

Strong levels of resistance to phosphine have been detected in *C. ferrugineus* populations from India (Rajendran et al. 2004), China (Wang et al. 2006) and Australia (Nayak et al. 2012). In Australia, although strongly resistant phenotypes of this species have been reported from several locations, the frequency of resistance was relatively low (<15%) (Nayak et al. 2012), presenting an opportunity to develop strategies to manage the resistance or at least minimise its impact. Already, new phosphine protocols using higher concentrations and longer exposure periods have been developed to control homozygous strongly resistant insects (Kaur and Nayak 2014). These protocols, however, add to the cost of overall fumigation and are not achievable in many situations due to practical difficulties. In addition to destroying resistant insects, an effective management strategy requires tactics that will act to reduce the frequency of resistance alleles in the population and delay the appearance of individuals homozygous for the strongly resistant phenotype (Onstad 2008). Development of such tactics requires knowledge of the key factors that affect the rate at which resistance is selected in the field. One of the most important of these is the response of various resistant genotypes to the selecting agent, that is, their relative dominance (Onstad and Guse 2008). The response of heterozygotes to selection is particularly important because these will be the most frequent carriers of resistance during the early stages of its development (Roush and Daley 1990).

In adults of *C. ferrugineus*, strong resistance to phosphine is conferred by two or more autosomal genes and both are expressed as incompletely recessive traits (Jagadeesan et al. 2016). Thus, strong resistance is fully expressed only in individuals that are homozygous for at least two of the major resistance genes. The pattern of relative tolerance and the expression of resistance to phosphine in different genotypes of other life stages in this species is unknown despite the fact that these stages are all targets of the fumigant, and thus, selection is also acting on these stages. Some limited research has indicated that dominance can vary with life stage in immatures of other insect pests of stored products. For example, under 6-day exposure to phosphine (144 h), resistance in the eggs and pupae of the rice weevil, *Sitophilus oryzae* (L.), was expressed as recessive and incompletely recessive, respectively, while it was semi-dominant and recessive in the eggs and pupae of *Tribolium castaneum* (Herbst), respectively (Collins et al. 1996). Furthermore, Kaur et al. (2012) showed that in the lesser grain borer, *Rhyzopertha dominica* (F.), eggs were semi-dominant and larvae and pupae were incompletely recessive in their response to phosphine. These results demonstrate the unpredictable nature of genetic dominance in the various life stages of these insects.

The study was carried out with two aims: (1) to determine the relative tolerance of all the life stages of *C. ferrugineus* to phosphine and (2) to characterise any changes in the expression of resistance (relative dominance) within life stages. In each life stage, three genotypes were used: homozygous for resistance (rr) and susceptibility (ss) and heterozygous for resistance (rs). The data obtained in this study will provide important information on factors influencing the rates of selection of resistance to phosphine in this species. This information is essential to the development of sustainable resistance management tactics. Strategies ignoring inheritance, relative tolerance and dominance may result in the development of ineffective fumigation practices and eventually to resistance outbreaks.

Materials and methods

Insect strains

Response to phosphine of *C. ferrugineus* was characterised in the eggs, larvae, pupae and adults of two parental strains: a phosphine-susceptible strain and a strongly phosphine-resistant strain, and their F_1 progeny.

The phosphine-susceptible reference strain QCF31, originating from a population sample collected from Cecil Plains in Queensland in 1998, has been maintained in the laboratory without exposure to insecticides and fumigants (Nayak et al. 2012). The strongly resistant parental strain QCF73 was collected from a central storage at Edgeroi in New South Wales in 2007 (Kaur and Nayak 2014). Before undertaking crosses, this strain was purified to homozygosity in the laboratory by in-breeding and selection with phosphine. The response to phosphine of the F_1 hybrid between these two strains was linear indicating homozygosity of the parental strains (Jagadeesan et al. 2016).

Insects were cultured in rolled oats (13% w/w moisture content) fortified with brewer's yeast (20:1 w/w) and maintained at 30°C and 60% r.h. under a 12:12-h light/dark photoperiod (Jagadeesan et al. 2013).

Preparation of individual life stages

To produce immature life stages from parental strains, groups of 50 unsexed adults were placed in jars containing 50 g rolled oats culture medium for 72 h. All adults were then removed from the jars, and the culture medium, containing unknown numbers of 1- to 3-day-old eggs, was fumigated with phosphine as described below. To obtain larvae and pupae, harvested eggs within the remaining jars were allowed to hatch and develop for 14 and 24 days to produce mid-instar larvae and pupae, respectively. These were then fumigated. Adult beetles (2 weeks post-eclosion) obtained from regular laboratory cultures were used for assays of the adult stage.

To produce F_1 progeny, larvae of parental strains were isolated in gelatine capsules containing 1–2 g finely ground rolled oats and allowed to mature into virgin adults. These were sexed based on a mandibular projection on males (Rilett 1949) and used to establish a series of reciprocal single-pair crosses, susceptible × resistant and resistant × susceptible. Bioassays of adults (Jagadeesan et al. 2016) as well as our preliminary work on other life stages confirmed that resistance was not sex linked; thus, responses of the reciprocal crosses were pooled for statistical analysis and interpretation.

Phosphine susceptibility tests

Phosphine was generated into a collection tube from commercial aluminium phosphide tablets (56%) that were immersed in an aqueous solution of sulphuric acid (5%) (FAO 1975). The source gas concentration was measured at the start of the experiment on a Clarus® 580 gas chromatograph (PerkinElmer, Waltham, MA) using a thermal conductivity detector with nitrogen as the standard (Kaur and Nayak 2014).

The response of insects to phosphine was measured at 25°C and 60% r.h., essentially following the recommended FAO method (FAO 1975) for testing resistance to phosphine, but with an exposure period of 48 h. We restricted the exposure period to 48 h to avoid eggs hatching into larvae during the fumigation. Jars containing the appropriate life stage with culture medium were placed inside airtight desiccators (4.0–6.0 L), and phosphine was injected into the desiccator through a septum in the lid using a gas-tight syringe. Phosphine was added at the concentration ranges of 0.05–20.0 mg/L for eggs and pupae, 0.003–12.0 mg/L for larvae and 0.003–16.0 mg/L for adults. Phosphine concentrations were monitored twice during the experiments, immediately (1 h) after the injection of the required volume of fumigant and at 30 h using a Clarus® 580 gas chromatograph (PerkinElmer) fitted with a flame photometric detector (Kaur and Nayak 2014). Any loss of gas was compensated by injecting the required amount of phosphine into the desiccator to maintain the desired concentration throughout the fumigation period (Kaur and Nayak 2014). After the exposure period, the experimental jars were removed from the desiccators and placed in a controlled environment at 30°C and 60% for up to 8 week to allow complete development of immature stages. The number of adults that emerged in the treated and untreated jars from each life stage was recorded from the fourth week and up to the eighth week. Adult mortality was recorded 7 day after completion of exposure period.

All experiments consisted of two full replications and each experiment consisted of two technical (sub) replicates. In describing the results of these experiments, the term 'tolerance' is used in comparisons among life stages within strains, or F_1 progeny, whereas the term 'resistance' is used when comparing results for the same life stage among strains. Tolerance (TR) and resistance (RR) ratios were calculated using the resistance ratio test described by Robertson et al. (2007). Degree of dominance (D) of resistance to phosphine was estimated using the formula described by Stone (1968) where $D = 1$ indicates complete dominance and $D = -1$ is complete recessivity. Scores $0 < D < 1$ indicate incomplete dominance, and scores $-1 < D < 0$ indicate incomplete recessivity.

Statistical analysis

Mortality response in eggs, larvae and pupae was estimated based on the procedure described previously by Jagadeesan et al. (2015) in which the number of live adults that emerged from control jars was compared with the number of live adults that emerged in treated jars for each of the respective life stages. These mortality values were corrected using Abbott's formula (Abbott 1925) and subjected to probit analysis (Finney 1979) under generalised linear regression models (McCullagh and Nelder 1989) using GenStat 16 statistical software (GenStat for Windows release 14.1). A significant deviance ratio should be expected for each life stage or strain if the test insects are responding strongly to the series of concentrations of the test insecticide. A heterogeneity value >1.0 indicates that mortality response of test cohorts was overdispersed and the existence of higher variability within the test cohort. Probit analysis using GenStat software accommodates these variations and adjusts the overall response in estimating the LC_{50} and LC_{95} values and their fiducial limits. These values were used to calculate tolerance or resistance

factors. For adults, the value of CM and ENT was obtained from the known number of dead insects and total number of insects that were used in the each treatment, respectively, followed by Abbott's correction (Abbott 1925).

Results

Relative tolerance of life stages

The results of probit analysis revealed that eggs of the susceptible strain had the highest LC_{50} value followed by pupae, with larvae and adults showing no significant difference (based on overlap of fiducial limits) (Table 1). The LC_{50} value of the eggs was about fivefold that of pupae and about 12-fold that of adults and larvae. This pattern of relative tolerance was also reflected in the LC_{95} values (Table 1). The high variance in response to phosphine shown by the eggs, as indicated by the relatively low slope and high heterogeneity factor (Table 1), resulted in a tolerance relative to adults of 50-fold at the LC_{95}. The slope of the response lines of the larvae and pupae was intermediate between the low slope of the egg and the very steep slope of the adult response to phosphine (Table 1; Fig. 1).

In contrast to the results for the susceptible strain, the adult stage of the highly phosphine-resistant strain showed the same level of tolerance to phosphine as the egg stage at LC_{50} (Table 1). These stages were about eightfold more tolerant than the larvae and about twofold more tolerant than pupae at the LC_{50}. There was again a large variance in the response of eggs and pupae to phosphine, as indicated by the relatively low slopes of the respective probit lines, resulting in very high LC_{95} values compared with their susceptible counterparts (Table 1).

Probit analysis of the F_1 progeny (S × R) assay results gave LC_{50} and LC_{95} values highest in larvae, followed by eggs, then pupae and lastly adults (Table 1). Larvae were about ninefold more tolerant to phosphine than adults, whereas eggs and pupae were about sixfold and threefold more tolerant, respectively. The slopes of the probit response lines of all life stages were relatively low indicating high variance in response to phosphine, closely resembling the response of the Strong-R parent. This variance was also reflected in high LC_{95} values shown by the eggs, larvae and pupae. The LC_{95} of F_1 larvae was equal to that shown by the larvae of Strong-R (Table 1).

Resistance and dominance

A comparison of the response of each life stage of the susceptible strain with those of the strongly resistant strain demonstrates that resistance is expressed in all life stages (Table 2). Within life stages, resistance ratios at the LC_{50} for the resistant strain were highest in adults (920×) followed by pupae (210×), larvae (100×) and eggs (80×) (Table 2; Fig. 1), with resistance ratios following the same order of ranking at the LC_{95}. Resistance ratios at the LC_{95} were around double those at the LC_{50}.

The response of the F_1 hybrids to phosphine determines the dominance of resistance in each life stage at heterozygous state relative to the homozygous parental genotypes (Table 2). At the LC_{50}, resistance to phosphine was expressed as incompletely recessive in eggs, pupae and adults, with respective degrees of dominance of −0.62, −0.38 and −0.56, whereas resistance was expressed in larvae as an incompletely dominant trait (0.54) (Fig. 1). Although the responses of heterozygotes (F_1) of all life stages were closer to the parental resistant strain (Table 2; Fig. 1) at LC_{95}, the degree of dominance for eggs (−0.14) and adults (−0.2) remained incompletely recessive, whereas the degree of dominance for larvae (0.98) and pupae (0.3) was changed from incompletely dominant to completely dominant (1.0) and incompletely recessive to incompletely dominant (0), respectively.

Discussion

Our results reveal that the response of the different life stages of *C. ferrugineus* to phosphine is complex, varying with life stage, resistance genotype and phosphine concentration. In insects homozygous for susceptibility to phosphine, the egg is clearly the most tolerant stage, with pupae and larvae showing a slightly higher tolerance than adults. This agrees with earlier limited indications that eggs are the most tolerant stage in this species (Barker 1969; Hole et al. 1976) and with data on other insect pests of stored products including moths (Bell 1976) and psocids (Ho and Winks 1995). In other beetle pests of stored products, pupae are usually more than or almost as tolerant as eggs (Hole et al. 1976; Howe 1973; Lindgren and Vincent 1966; Manivannan 2015), although these differences varied with fumigation exposure period (Hole et al. 1976; Manivannan 2015).

In contrast to susceptible insects, tolerance to phosphine in *C. ferrugineus* heterozygous for phosphine resistance (F_1 hybrids) is highest in larvae at median concentrations (LC_{50}). However, at high concentrations, i.e. around the LC_{95}, the three immature stages were not significantly different in relative tolerance and all were more tolerant than adults. There is very limited information for comparison on the response of immature stages heterozygous for resistance. Kaur et al. (2012) reported that the egg was the most tolerant stage of heterozygous phosphine-resistant *R. dominica* followed by pupae, then larvae and adults. The situation is different again in *C. ferrugineus* homozygous

Table 1 Relative tolerance (tolerance ratio) to phosphine of developmental stages of a phosphine-susceptible (S) and strongly phosphine-resistant (R) strains of *Cryptolestes ferrugineus* and their F_1 progeny

Strain/cross	Developmental stage	n^a	Slope ± SE	LC_{50} (95% FL[b]) (mg L^{-1})	LC_{95} (mg L^{-1})	HF[c]	df	Deviance ratio	TR[d] at LC_{50} (CI[e])	TR[d] at LC_{95} (CI[e])
S	Egg	13,077	2.2 ± 0.185	0.093 (0.080–0.11)	0.52	16.1	34	366.16	12 (10–14)	53 (38–72)
	Larvae	13,752	3.64 ± 0.06	0.0085 (0.0075–0.0096)	0.024	44.9	34	188.65	1.1 (1.0–1.2)	2.4 (2.1–2.8)
	Pupae	16,875	2.98 ± 0.19	0.018 (0.017–0.019)	0.064	10.2	70	1112.1	2.4 (2.1–2.7)	6.5 (5.3–8.0)
	Adult	2700	14.31 ± 2.8	0.0076 (0.0069–0.0083)	0.012	15.5	16	136.83	1.0	1.0
R	Egg	8413	1.58 ± 0.20	7.41 (5.71–9.26)	81.1	25.4	29	69.35	8.5 (6.4–11)	19 (9.8–38)
	Larvae	10,350	2.41 ± 0.24	0.867 (0.71–1.05)	4.18	29.2	34	262.9	1.0	1.0
	Pupae	24,291	1.59 ± 0.13	3.77 (3.05–4.52)	40.9	29.2	70	203.65	4.3 (3.3–5.7)	9.8 (6.0–16)
	Adult	2700	4.32 ± 0.59	7.01 (5.95–8.06)	16.9	16.5	16	88.20	8.1 (6.4–10)	4.0 (2.6–6.1)
F_1 (S × R)	Egg	14,985	1.2 ± 0.08	0.212 (0.166–0.266)	4.99	19.1	34	357.00	6.2 (4.5–8.5)	23 (13–42)
	Larvae	20,745	1.46 ± 0.07	0.304 (0.25–0.37)	4.06	25	34	443.8	8.9 (6.7–12)	19 (11–33)
	Pupae	26,505	0.98 ± 0.05	0.095 (0.073–0.12)	4.58	17.6	34	446.22	2.8 (2.0–3.8)	21 (12–37)
	Adult	3141	2.05 ± 0.24	0.034 (0.0266–0.0428)	0.216	9.51	19	154.65	1.0	1.0

[a] Number of insects subjected to phosphine bioassay, excluding control

[b] *FL* fiducial limits

[c] Heterogeneity factor (HF) = total deviance − regression deviance/(N − 1) df, where N is total number of treatments

[d] Tolerance ratio (TR) = LC_{50} (or LC_{95}) of life stage/LC_{50} (or LC_{95}) of least tolerant life stage, within each strain

[e] *CI* confidence interval

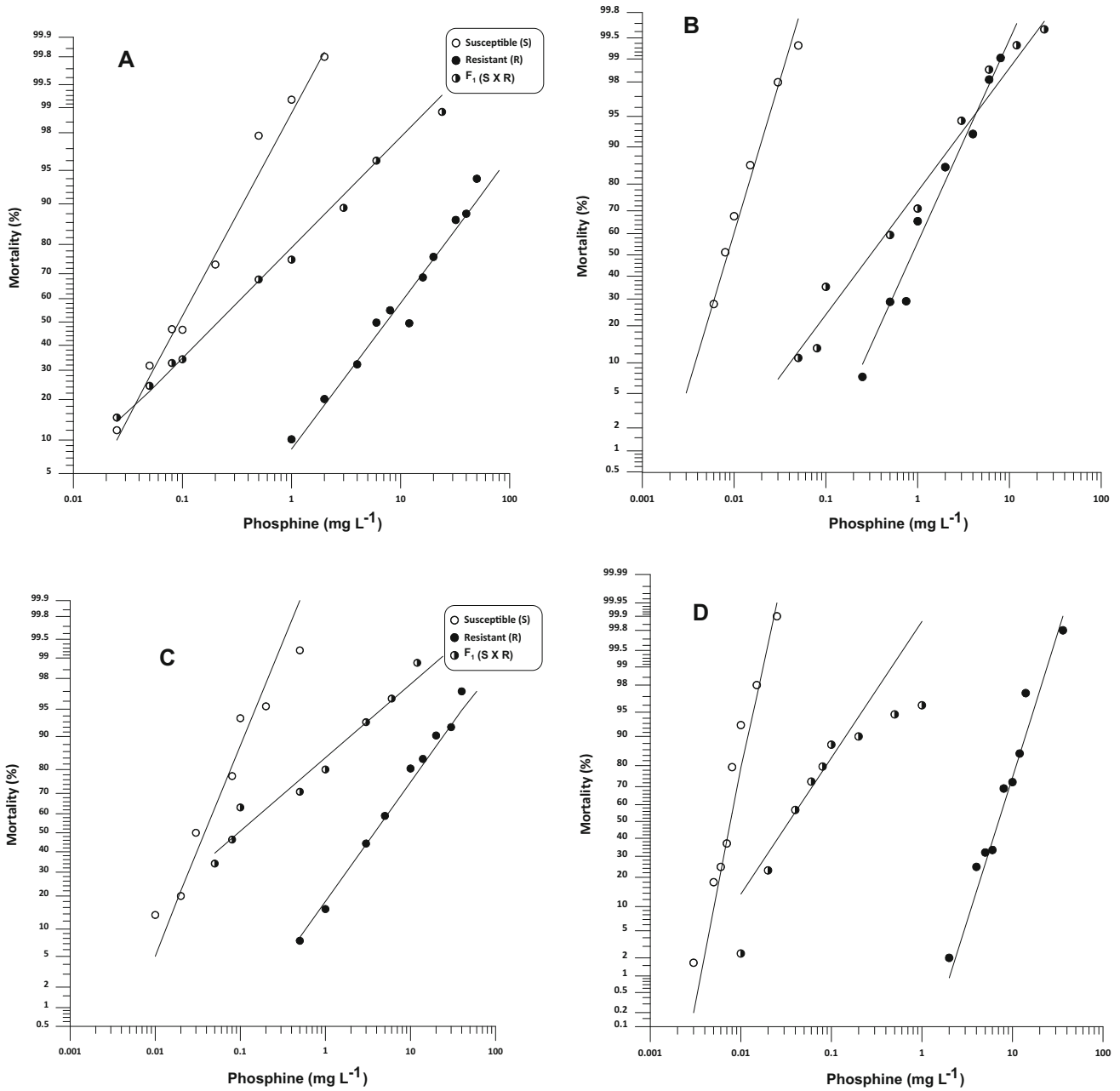

Fig. 1 Response of eggs (**a**), larvae (**b**), pupae (**c**) and adults (**d**) of *Cryptolestes ferrugineus* strains: phosphine susceptible (S), strongly phosphine resistant (R) and their F_1 progeny to phosphine at a range of concentrations for an exposure period of 48 h

for resistance (represented by the responses of the strongly resistant strain). With this genotype, tolerance to phosphine in the adult was about equal to that in the egg stage at median concentrations and higher than that of the pupae. At high concentrations, pupae were more tolerant than adults, but both were less tolerant than eggs. This contrasts with *R. dominica* where homozygous resistant adults were more tolerant to phosphine than both eggs and pupae (Kaur et al. 2012).

Resistance to phosphine in *C. ferrugineus* is conferred by two major genes (Jagadeesan et al. 2016); therefore, there are nine possible genotypes that could be expected in a population that segregates for resistance. In these experiments, we measured responses in the three of the nine possible genotypes, that is, homozygous resistant (r_1r_1; r_2r_2), F1 hybrid (r_1s_1; r_2s_2) and homozygous susceptible (s_1s_1; s_2s_2). Other genotypes are difficult to identify using phenotypic screening.

The relatively higher tolerance of eggs in susceptible insects has been attributed to the impermeable proteinaceous egg shell and embryonic membrane protecting the actively growing embryo (Price and Bell 1981). In

Table 2 Resistance ratio and degree of dominance expressed in response to phosphine of developmental stages of *Cryptolestes ferrugineus* strains: phosphine susceptible (S), strongly phosphine resistant (R) and their F_1 progeny

Developmental stage	Strain	LC_{50}	RR[a] (CI[b])	DD[c]	LC_{95}	RR (CI[b])	DD[c]
Eggs	S	0.093	1.0		0.52	1.0	
	R	7.41	80 (61–104)		81.1	150 (81–300)	
	F_1 (S × R)	0.212	2.3 (1.7–3.0)	−0.62	4.99	9.5 (5.8–16)	−0.14
Larvae	S	0.0085	1.0		0.024	1.0	
	R	0.867	100 (86–130)		4.18	180 (120–250)	
	F_1 (S × R)	0.304	36 (30–73)	0.54	4.06	170 (120–250)	0.98
Pupae	S	0.018	1.0		0.064	1.0	
	R	3.77	210 (170–260)		40.9	640 (430–940)	
	F_1 (S × R)	0.095	5.2 (4.1–6.8)	−0.38	4.58	71 (48–100)	0.3
Adult	S	0.0076	1.0		0.012	1.0	
	R	7.01	920 (790–1100)		16.9	1700 (1300–2300)	
	F_1 (S × R)	0.034	4.5 (3.6–5.6)	−0.56	0.216	22 (14–34)	−0.2

[a] RR = resistance ratio: within life stage, LC_{50} or LC_{95} of R or F1 hybrid/LC_{50} or LC_{95} of S

[b] *CI* confidence interval

[c] Degree of dominance (DD) = (2log LCRS-Log LCR-Log LCS)/(Log LCR-Log LCS)

addition, this stage, as well as the pupae, tends to show reduced metabolic activity and lower respiration rates (Emekci et al. 2001, Price and Bell 1981) both of which may slow the toxic activity of phosphine. Resistance to phosphine is also expressed strongly in adults as well as in eggs in *C. ferrugineus* and in *R. dominica* (Kaur et al. 2012). The presence of resistance genes imparts a relatively large change in response (resistance factor) in adults compared with eggs. This indicates that the high tolerance of eggs to phosphine in resistant insects is due to an interaction of the innate physiological properties of the eggs with the action of resistance mechanisms, whereas the response of resistant adults is due primarily to the action of resistance mechanisms. Thus, resistance, measured as resistance ratio, is generally expressed most strongly in adults.

Higher than expected survival of insects at high concentrations of phosphine may also in part be attributed to a narcotic effect. Narcosis is an enhanced tolerance to phosphine that occurs at high concentrations and short exposure periods, that is, 5–10 h (Winks and Waterford 1986) and is more pronounced in resistant insects. Although the exposure period used in these experiments was 48 h, the very high concentrations used may have induced narcosis in the response of heterozygotes and resistant homozygotes. A possible phosphine-induced narcotic effect was also observed in the response of resistant *R. dominica* populations exposed to concentrations greater than 1 mg/L (Collins et al. 2002).

The expression of resistance to phosphine also changes with life stage and phosphine concentration. We observed incomplete recessivity of phosphine resistance genes in *C. ferrugineus*. This is typical of adult insects of stored products and has been observed in *C. ferrugineus* (Jagadeesan et al. 2016), *R. dominica* (Collins et al. 2002), *T. castaneum* (Bengston et al. 1999; Jagadeesan et al. 2012) and *S. oryzae* (Daglish et al. 2014; Nguyen et al. 2015). The expression of resistance in immature stages of insect pests of stored products is poorly understood, however. We found in *C. ferrugineus* that at median concentrations resistance was incompletely recessive in eggs, pupae and adults but incompletely dominant in larvae. At high concentrations, we found that resistance was expressed as incompletely recessive in eggs, but larvae were close to fully dominant, and in pupae, resistance was expressed as incompletely dominant. The greater than expected survival of heterozygotes (F_1 hybrids) at higher concentrations of phosphine may have been the result of the presence of additional dominant factors present in a proportion of the population (Jagadeesan et al. 2016). At 48-h exposure period, as used in these experiments, *R. dominica* resistance is semi-dominant in eggs and incompletely recessive in larvae and pupae (Kaur et al. 2012). At longer exposure periods, *S. oryzae* resistance to phosphine has been reported to be recessive in eggs and incompletely recessive in pupae, while in the eggs and pupae of *T. castaneum* resistance to phosphine was semi-dominant and recessive in expression, respectively (Collins et al. 1996). In almost all known cases of phosphine resistance in insect pests of stored products, dominance is incomplete and can range from incomplete recessivity to semi-dominant, with the

former being the most common mode of inheritance of resistance (Opit et al. 2012).

The dominant expression of resistance in larvae heterozygous for resistance was an unexpected result, especially when adults, the other active stage, were incompletely recessive. In addition, larvae of *C. ferrugineus* are highly active compared to larvae of *R. dominica* and *S. oryzae*, in which resistance to phosphine was expressed as incompletely recessive. Thus, it appears that expression of resistance in insects can be plastic particularly in the immature stages where expression is influenced by other background genetic factors (Collins et al. 1996; Kaur et al. 2012; Jagadeesan et al. 2016). It is also possible that a proportion of the larval test cohort in our bioassays, could have transformed into the quiescent pre-pupal stage (Rilett 1949) just before or during the fumigation. This may have increased the apparent overall tolerance of the larval stage. This effect, in contrast, was not observed in larvae of homozygous resistant parent. This could be due to inherent developmental differences between the larvae of the heterozygous (F_1) and homozygous resistant genotypes in *C. ferrugineus*. For example, in *R. dominica,* insect life stages homozygous for phosphine resistance showed significant delay in development than their susceptible and heterozygote counterparts (Kaur et al. 2012).

It is important to understand the response of immature stages as well as adults to phosphine, as all life stages of these pests infest the commodity and are subject to fumigation and therefore selection for resistance. In particular, understanding the response of heterozygotes (F_1) is central as these will be the most common resistance genotype in the population. In practice, for several of reasons, a wide range of dosages can occur during a fumigation and between fumigations (Collins et al. 1996; Reed 1997). Our data show that a proportion of F_1 hybrids (heterozygotes) may survive even at very high concentrations, particularly eggs, but also adults, larvae and pupae, forming a nucleus for reinfestation of the commodity and resulting in very rapid selection of a strongly resistant population. This is a key finding that stresses the importance of achieving and maintaining the required concentration (c) and time (t) regime in field fumigations. In Australia, aluminium phosphide fumigations in large, well-sealed steel silos (greater than 375 m^3) maintain an average concentration of about 1.5 g/m^3 ($\sim$1000 ppm) for 20 days. In addition, cylinder-based application dosages range from 30 to 700 ppm and 3- to 25-day exposure period, depending on temperature (APVMA 2017). Research is revealing that these application rates are marginal for complete control of strongly resistant *C. ferrugineus* (Kaur and Nayak 2014). Thus, for resistant *C. ferrugineus*, fumigations performed below the benchmark will in fact screen for strongly resistant heterozygotes and homozygotes, which in turn mate and reproduce, resulting in a higher proportion of strongly resistant homozygotes in succeeding generations that will ultimately eliminate all susceptible alleles in the local population, leading to rapid development and spread of resistance (McKenzie 1996). Additional factors that can influence the efficacy of phosphine, such as commodity temperature and sorption, and operational factors, such as number of fumigations of the same insect population, will also influence the rate of selection. Survival of heterozygous resistant adults is also important as these insects provide a source for dispersal of resistance to other grain storages and the flow of resistance genes into the broader population. As this study involved the response of individual life stages of *C. ferrugineus* to phosphine at a fixed exposure period of 48 h, future research should be directed towards establishing their responses under longer fumigation periods, so that we can better understand the dynamics of selection for resistance under field conditions.

Author contributions

PC, MV, MN and RJ conceived and designed the research. MV and RJ conducted the experiments, analysed the results and drafted the manuscript. PC, MN, CS and MS modified the manuscript significantly. All authors read and approved the manuscript.

Acknowledgements The senior author wishes to thank Prof Gimme Walter for supporting his training fellowship at the University of Queensland and Queensland Department of Agriculture and Fisheries. We acknowledge the support of the Australian Government's Australia-India Strategic Research Funds (GCF010006 and AISRF48516), under which the research, training and analysis were completed. We thank Virgine T Singarayan, Hervoika Pavic and Linda Bond for excellent technical assistance. We thank Dr Greg Daglish for his constructive comments on the manuscript.

Funding This study was in part funded by the Australian Government's Australia-India Strategic Research Funds (Grant Numbers GCF010006 and AISRF48516).

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

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