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Genetic characterization of field-evolved resistance to phosphine in the rusty grain beetle, *Cryptolestes ferrugineus* (Laemophloeidae: Coleoptera)

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ABSTRACT

Inheritance of resistance to phosphine fumigant was investigated in three field-collected strains of rusty grain beetle, *Cryptolestes ferrugineus*, Susceptible (S-strain), Weakly Resistant (Weak-R) and Strongly Resistant (Strong-R). The strains were purified for susceptibility, weak resistance and strong resistance to phosphine, respectively, to ensure homozygosity of resistance genotype. Crosses were established between S-strain × Weak-R, S-strain × Strong-R and Weak-R × Strong-R, and the dose mortality responses to phosphine of these strains and their F_1, F_2 and F_1-backcross progeny were obtained. The fumigations were undertaken at 25 °C and 55% RH for 72 h.

Weak-R and Strong-R showed resistance factors of 6.3× and 505× compared with S-strain at the LC_{50}. Both weak and strong resistances were expressed as incompletely recessive with degrees of dominance of −0.48 and −0.43 at the LC_{50}, respectively. Responses of F_2 and F_1-backcross progeny indicated the existence of one major gene in Weak-R, and at least two major genes in Strong-R, one of which was allelic with the major factor in Weak-R. Phenotypic variance analyses also estimated that the number of independently segregating genes conferring weak resistance was 1 ($nE = 0.89$) whereas there were two genes controlling strong resistance ($nE = 1.2$). The second gene, unique to Strong-R, interacted synergistically with the first gene to confer a very high level of resistance (~80×). Neither of the two major resistance genes was sex linked. Despite the similarity of the genetics of resistance to that previously observed in other pest species, a significant proportion (~15 to 30%) of F_1 individuals survived at phosphine concentrations higher than predicted. Thus it is likely that additional dominant heritable factors, present in some individuals in the population, also influenced the resistance phenotype.

Our results will help in understanding the process of selection for phosphine resistance in the field which will inform resistance management strategies. In addition, this information will provide a basis for the identification of the resistance genes.

1. Introduction

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) is a cosmopolitan insect pest that infests a wide range of stored cereals and processed commodities [1,2]. Until recently, phosphine (PH_3) fumigation has been effective in controlling this species in Australia, however, strongly resistant populations of *C. ferrugineus* have now been detected that threaten market access of infested commodities [3]. Resistant populations detected in Australia are capable of developing very high levels of resistance to phosphine, up to 1300×, significantly higher than levels reported in other grain insect pests [4].

The development of resistance is an evolutionary process in which a heritable change occurs within an insect population as an intrinsic response to selection imposed by humans [5]. An understanding of the genetics of resistance in *C. ferrugineus* may help us identify the factors driving the development of strong resistance to phosphine in this species. This information is crucial to the development of rational and sustainable resistance management. Strong resistance to phosphine in the lesser grain borer, *Rhyzopertha dominica* (Fabricius) [6], the red flour beetle, *Tribolium castaneum* (Herbst) [7], and the rice weevil, *Sitophilus oryzae* (Linnaeus) [8], is mediated by two major autosomal recessive genes, *rph1* and *rph2*. In homozygous isolation, each of these genes confers only weak resistance, ~4–30×, however, when they occur together in one individual, they interact synergistically and provide a very high level of resistance, up to ~600× [6–8]. Genes *rph1* and *rph2* are expressed as incompletely recessive in all the three species, irrespective of the phenotypes, and are not sex linked [6–9]. This genetic information together with well-established efficacy data against *R. dominica* [10], *T. castaneum* [11] and *S. oryzae* [12–14] facilitated the development and implementation of a resistance management strategy

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that has been effective in containing the resistance problem [15] or at least delaying further evolution of high level of resistance in these pest species in Australia [16].

No prior information is available on the genetics of phosphine resistance in *C. ferrugineus* despite the extraordinary levels of resistance detected in this species [4,17]. To support the development of a rational, effective resistance management strategy, our aim was to determine the inheritance of resistance to phosphine in this species. We investigated key determinants in the selection of resistance including the number of resistance genes, their mode of inheritance, their relative dominance and gene interactions, if any, in two field-evolved resistant strains.

2. Materials and methods

2.1. Insect strains

Three field-derived strains of *C. ferrugineus* were used in this study; phosphine-susceptible, QCF31, weakly resistant, QCF37 and strongly resistant, QCF73 [4]. Throughout this report we refer to these strains as S-strain, Weak-R and Strong-R, respectively. Before the commencement of genetic crosses, both Weak-R and Strong-R were fumigated at 0.04 mg L^{-1} over 48 h and 1.0 mg L^{-1} over 144 h, respectively, for three successive generations to promote homozygosity within the strains. All insects were cultured on a standard dietary medium of rolled oats + cracked sorghum + yeast (75:20:5%) at 30 °C and 65% RH [18].

2.2. Inheritance of phosphine resistance

2.2.1. Genetic crosses

2.2.1.1. Multiply mated intercrosses (MIC). To determine the mode of inheritance of phosphine resistance in *C. ferrugineus* three multiply mated (mass) intercrosses (MIC) were set up: S-strain × Weak-R; S-strain × Strong-R and Weak-R × Strong-R. Each cross consisted of 100 virgin adult males (♂) of one parent and 100 virgin adult females (♀) of the other parent. The resulting F_1 hybrids were used to produce segregating F_2 intercross and backcross progeny (F_1-BC). F_2 insects were obtained by allowing F_1 progeny to randomly mate with each other for two weeks. In the case of backcross, two F_1-BC populations were obtained from each parental cross; S-strain × Weak-R; S-strain × Strong-R and Weak-R × Strong-R, by crossing virgin F_1 females from each cross, back to virgins males of each parental strain [(F_1 ♀ × Weak-R ♂ and F_1 ♀ × S ♂); (F_1 ♀ × Strong-R ♂ and F_1 ♀ × S ♂); (F_1 ♀ × Strong-R ♂ and F_1 ♀ × Weak-R ♂)]. In F_1, F_2 and F_1-BC, reciprocals (F_1', F_2' and F_1-BC′) were also established as a replicate to the original cross and their dose mortality response was compared and pooled with originals for analysis, if the responses were not significantly different.

2.2.1.2. Single pair intercrosses (SIC). Three single pair intercrosses (SIC) (one ♀ + one ♂) were also established with the virgin parents (S-strain × Weak-R; S-strain × Strong-R and Weak-R × Strong-R) to investigate the expression of resistance at higher concentrations of phosphine in the F_1 generation. The mortality response data of three F_1 hybrid populations obtained from single pair intercrossing and multiply mated intercrossing were compared to control for skewness or errors associated with sexing, multiple mating or other strain based genetic background variation unrelated to phosphine resistance.

2.3. Phosphine susceptibility tests

Phosphine was generated from aluminium phosphide and its concentration measured using a gas chromatograph (Perkin-Elmer Clarus 580) according to a procedure described previously by Daglish et al. [14]. Phosphine susceptibility in adults of the parental strains and the crosses (MIC and SIC) was assessed using the FAO recommended bioassay method at a range of phosphine concentrations (0.005 to 8.0 mg L^{-1}) in gas-tight desiccators (4 to 6 L) for 72 h at 25 °C and 55% RH [4]. Mortality was assessed 7 days after the completion of the exposure period. The entire experiment was replicated twice with each test concentration for each replicate consisting of three batches of 50 adult beetles.

The parents and F_1 hybrids from each of the three MIC were also fumigated at exposure periods of 48 and 144 h, in addition to the standard 72 h exposure, to detect any variation in the expression of resistance (degree of dominance) associated with length of exposure period, especially at the higher concentrations of phosphine. The estimated LC_{50} values under the respective exposure periods were fitted to the standard equation $C^n t = k$, where k is constant and n is the toxicity index that decides the effect of exposure period over concentrations [13]. A value of n less than 1.0 is expected in the case that the expression of resistance factors varies significantly with exposure period on these strains/hybrids [14].

2.4. Data analysis

2.4.1. Resistance factor, dominance and sex linkage

The response of parental strains and reciprocal F_1 progeny in each cross were analysed and fitted to log dose-probit mortality response curves [19] using Genstat software version 16.0 [20]. Resistance factors were calculated as the ratio between LC_{50} value of resistant parental strains or F_1 hybrids with LC_{50} value of S-strain or Weak-R [21], depending on the crossing scheme. Sex linkage (influence of maternal factors) [19] was tested by overlap of 95% fiducial limits at LC_{50} and relative potency analysis in the response of reciprocal F_1 hybrids. The degree of dominance was estimated from the response of parental strains and reciprocal F_1 hybrids in each cross, based on LC_{50} and $LC_{99.9}$, according to the method of Stone [22] to reveal variations (if any) in the expression of the phenotype in F_1 hybrids against concentration. A high number of test insects ($n = 600$) were used for each concentration in the probit analysis for estimating $LC_{99.9}$, so that the estimates were reliable and close representative of the observed data.

2.4.2. Number of genes conferring resistance

Three methods were used to determine the number of genes conferring resistance to phosphine in *C. ferrugineus*. First, the observed response of the F_2 and F_1-BC progeny to phosphine was compared visually to an expected response assuming monogenic inheritance. According to Tsukumoto et al. [23], if resistance is conferred by a single recessive gene, then a plateau or point of inflection should occur in the log dose-probit response line of the F_2 at around 75% and in the F_1-BC response line at around 50%. Second, the null hypothesis of monogenic inheritance of resistance was tested on the basis of goodness-of-fit [24] between observed mortality and the theoretical expectations of F_2 and F_1-BC curves according to Georgiou et al. [25] using a modified chi-square [26], that incorporates the heterogeneity factor of the parental strains. Since, the observed response of F_1 hybrids in the three crosses differed significantly at high concentrations from the expected response for incompletely recessive inheritance, a theoretical F_1 curve was established based on incomplete recessive inheritance model and used in predicting the expectations for monogenic F_2 and F_1-BC response curves. The third test was based on the procedure outlined by Lande [27] to approximate the number of freely segregating genetic factors (nE) associated with a heritable phenotype. The nE was estimated by comparing the genotypic and phenotypic variances that contribute quantitative trait difference between two populations. Thus, $nE = (\mu_{P2} - \mu_{P1})^2 / 8\,\sigma^2_S \leq n$, where μ_{P1} and μ_{P2} are the $\log_{10}$ of the LC_{50} values of the strongly resistant and susceptible or weakly resistant strains, respectively, depending on the crossing scheme, and n was the actual number of genes. The additional genetic variance ($\sigma^2_{S\text{-}Var}$) associated with the F_1-BC generation was estimated as:

$\sigma^2_{S\text{-}Var} = \sigma^2_{B1} + \sigma^2_{B2} + [\sigma^2_{F1} + 0.5^*(\sigma^2_{P1}) + 0.5^*(\sigma^2_{P2})]$, where, σ^2_{B1}, σ^2_{B2}, σ^2_{F1}, σ^2_{P1} and σ^2_{P2} refer to the phenotypic variances of the