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Effect of diet on phosphine toxicity, rate of development and reproduction of the rice weevil *Sitophilus oryzae* (Linnaeus)

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ABSTRACT

One of the loci responsible for strong phosphine resistance encodes dihydrolipoamide dehydrogenase (DLD). The strong co-incidence of enzyme complexes that contain DLD, and enzymes that require thiamine as a cofactor, motivated us to test whether the thiamine deficiency of polished white rice could influence the efficacy of phosphine fumigation against insect pests of stored grain. Three strains of *Sitophilus oryzae* (susceptible, weak and strong resistance) were cultured on white rice (thiamine deficient), brown rice or whole wheat. As thiamine is an essential nutrient, we firstly evaluated the effect of white rice on developmental rate and fecundity and found that both were detrimentally affected by this diet. The mean time to reach adult stage for the three strains ranged from 40 to 43 days on brown rice and 50–52 days on white rice. The mean number of offspring for the three strains ranged from 7.7 to 10.3 per female over a three day period on brown rice and 2.1 to 2.6 on white rice. Growth and reproduction on wheat was similar to that on brown rice except that the strongly resistant strain showed a tendency toward reduced fecundity on wheat. The susceptible strain exhibited a modest increase in tolerance to phosphine on white rice as expected if thiamine deficiency could mimic the effect of the *dld* resistance mutation at the *rph2* locus. The strongly resistant strain did not respond to thiamine deficiency, but this was expected as these insects are already strongly resistant. We failed, however, to observe the expected synergistic increase in resistance due to combining thiamine deficiency with the weakly resistant strain. The lack of interaction between thiamine content of the diet and the resistance genotype in determining the phosphine resistance phenotype suggests that the mode of inhibition of the complexes is a critical determinant of resistance.

1. Introduction

The fumigant phosphine is the major disinfestant used worldwide for the control of the rice weevil, *Sitophilus oryzae*, and other insect pests of stored grain. This fumigant is extremely important in world trade and food security as no practical substitute is available for general use. Strong resistance to phosphine is now widespread, with strong resistance in *S. oryzae* reported in China (337x) (Zeng, 1998), India (425x) (Rajendran, 1998), Brazil (73x) (Athié et al., 1998), all determined at 20 h exposure and in Australia (52x) after a 48 h exposure (Nguyen et al., 2015). Weak phosphine resistance in *S. oryzae* is monogenic, whereas strong resistance is the product of at least two major genes. In both cases, resistance is incompletely recessive and autosomally encoded (Li and Li, 1994; Daglish et al., 2014; Nguyen et al., 2015). The gene responsible for weak resistance, *So_rph1*, in combination with resistance alleles at an additional locus, *So_rph2* results in the strong resistance phenotype (Nguyen et al., 2015).

The *So_rph2* gene encodes the enzyme dihydrolipoamide dehydrogenase (DLD) (Nguyen et al., 2016). The orthologue of the *So_rph2* gene is also responsible for the strong phosphine resistance phenotype in three other insect pests of stored grain: *Rhyzopertha dominica*, *Tribolium castaneum* (Schlipalius et al., 2012) and *S. oryzae* (Nguyen et al., 2016). DLD is a subunit of three different mitochondrial α-ketoacid dehydrogenase enzyme complexes, each of which depends on thiamine pyrophosphate (TPP) as

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an essential cofactor (Mathews and Vanholde, 1996). TPP is a cofactor of only three other enzymes in most animals, including insects. The degree of overlap between the utilisation of DLD and TPP in cellular biochemistry suggests the possibility that dietary thiamine might influence resistance.

A recent study showed that suppression of the *dld-1* gene in wild type individuals of the nematode, *Caenorhabditis elegans*, results in resistance to phosphine (Schlipalius et al., 2012). We hypothesize that thiamine deficiency could likewise inhibit the activity of the α-ketoacid dehydrogenase complexes, thereby inducing resistance to phosphine, producing a phenocopy of genetic resistance due to mutation of the *dld* gene. As animals are unable to synthesize thiamine, they must take up thiamine from their diet (Pauling, 1970). Thiamine is found primarily in the aleurone layer and germ of whole grain, 85% of which is lost from white rice as a result of the milling process (Lebiedzińska and Szefer, 2006). Therefore, insects cultured on white rice will be exposed to a diet with a decrease in thiamine content. From our understanding of genotypic resistance, we infer that thiamine deficiency would result in weak resistance in a normally sensitive strain, but would synergistically enhance the phenotype of a genotypically weakly resistant (*rph1*) strain, resulting in a strong resistance phenotype.

The aim of this study was to test the hypothesis that a thiamine deficient diet will suppress the activity of the α-ketoacid dehydrogenase complexes, thereby inducing resistance to phosphine. Furthermore, we wished to determine whether thiamine deficiency would interact with the known resistance factors as expected of a phenocopy of a *dld* gene mutation. As thiamine is an important factor in energy metabolism, we monitored the effect of the dietary deficiency through the rate of development and fecundity of *S. oryzae*.

2. Materials and methods

2.1. Insect strains

This study used three *S. oryzae* strains collected in Australia that have been previously characterized according to their phosphine resistance genotypes (Daglish et al., 2014; Nguyen et al., 2015). A susceptible strain, the S-strain (LS2), collected in 1965 from Brisbane, Queensland; a weakly resistant strain, called the WR-strain (QSO335), also collected in Queensland from a central storage in Millmerran in 1990; and a strongly resistant strain, the SR-strain (NNSO7525), which was sampled from Widgelli, New South Wales in 2009. Strains were maintained on whole, certified organic wheat (12% moisture content − m.c) at 25 °C and 55% relative humidity (r.h) from the time of collection to the start of the experiment.

2.2. Culturing on tested diets

This experiment measured fecundity, development and response to phosphine of insect strains cultured on three different diets: white rice, brown rice and whole wheat. All grains are organic to ensure that they are free of insecticide residues. The white rice is that had been subjected to milling to remove the embryo and bran (pericarp plus aleurone), while the brown rice and whole wheat retain the bran and embryo.

As our insects cultures are routinely maintained exclusively on whole wheat, insects cultured on whole wheat are used throughout this study as a control. For rice diets, 100 adults of each strain were cultured separately on either 150 g white rice or 150 g brown rice (both at 14% m.c). The cultures were incubated at 25 °C and 55% r.h. After three weeks of oviposition, adults were removed and the rice was incubated until the next generation had emerged. Two week old offspring (approximately 100 individuals) were then transferred to fresh grain of the respective type of rice and incubated for three weeks to produce the next generation. This process on rice was carried out over five generations before starting the experiment.

2.3. Rate of development and fecundity

For each strain, two week old adults (30 individuals) were placed in small glass jars containing either 50 g of brown rice, white rice or whole wheat at 25 °C and 55% r.h. These insects were removed after three days of egg laying and their sex was determined according to Halstead (1963) to count the number of females. Adult emergence was recorded every day for four weeks after setting up the experiment by counting the emergent adults and immediately removing them from the glass jar until no further insects emerged. Developmental time was calculated by averaging the time to adult stage across all offspring in each treatment. Fecundity was calculated as the number of offspring produced per female from the 3 day period of egg laying. Three replications of the experiment were undertaken separately at one month interval.

2.4. Insect response to phosphine

Phosphine gas (PH_3) was generated in a Valmas chamber from aluminium phosphide tablets (Fumitoxin®) submerged in 5% sulfuric acid (Valmas and Ebert, 2006). Purity of phosphine was determined by a Clarus® 580 gas chromatograph (Perkin Elmer, Waltham, MA) using a thermal conductivity detector with nitrogen as the standard (Winks and Waterford, 1986). Mortality response to phosphine of the tested strains was measured against a range of phosphine concentrations i.e. 0.002–0.03 mg L^{-1} for the S-strain, 0.01–0.15 mg L^{-1} for the WR-strain and 0.15–1.0 mg L^{-1} for the SR-strain. Fumigation was undertaken by placing three batches of 50 adult beetles (3 weeks old) in 30 mL ventilated plastic cups without food inside the gas tight desiccators and injecting phosphine through a rubber septum in the lid using a gas-tight syringe. Control (non-fumigated) and phosphine treatments were kept at 25 °C, 60% r.h for 48 h. After fumigation, insects were fed with the same type of grain on which they had been cultured and were left to recover at 25 °C, 55% r.h. Mortality was assessed after seven days to allow complete recovery. Phosphine fumigations were replicated three separate times.

2.5. Data analysis

2.5.1. Analysis of rate of development and fecundity

The analysis of variance of two factors (two-way ANOVA) was used to evaluate the significance of diets, strains and interaction between diet and strain on developmental time and fecundity. The analysis of variance (ANOVA) was performed using GenStat statistical package version 16 (GenStat, 2015). When statistical analysis revealed significant differences, comparisons of each pair were conducted by a post hoc multiple pair-wise comparison method using Fisher's Least Significant Difference (LSD) test. If the absolute value of the deviation between two means was $\geq$ LSD, then the means were significantly different at $\alpha = 0.05$.

2.5.2. Analysis of insect mortality in response to phosphine

Mortality data were adjusted using Abbott's formula to correct for control mortality (Abbott, 1925) prior to probit analysis. The analysis was performed using GenStat (2015). In this GenStat package, the fiducial limits for LC_{50} and $LC_{99.9}$ are estimated using Fieller's theorem, which has been shown to provide better accuracy