Session 1：Emerging Global Issues in Stored Product Protection

An emerging international picture of phosphine resistance: opportunities for global cooperation

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Abstract

Phosphine resistance in stored grain pests has been increasing in severity internationally for several decades and is a threat to the continued use of phosphine fumigation as an effective control method. Important insights into the development of resistance that will help to maintain the viability of phosphine as a fumigant in the future have come from molecular genetics. The recent discovery of genes responsible for resistance has enabled relatively rapid, high-throughput monitoring and detection of resistance alleles in several major pest species. Use of these markers is revealing how resistance arises and is selected within populations, information essential for effective, sustainable resistance management. Knowledge of the genes has also provided us the keys to understanding the mechanisms of phosphine toxicity and resistance. These insights are being used to discover side-effects of resistance that result in physiological weaknesses (i.e. 'fitness costs'). The few molecular surveys that have been conducted to date have shown us that while the genes that give rise to resistance are highly conserved, i.e. always the same genes, the pattern of resistance allele selection, distribution and frequency are quite different between regions. This situation could be due to differing environmental conditions, storage and transport systems and pest management practices. The differences between patterns of resistance gene allele distribution and frequency will likely reveal factors important in the process of selection for resistance. The common genetic basis of phosphine resistance will facilitate the gathering and exchange of resistance data between regions and storage systems, providing new opportunities for international cooperation and comparison of data.

Keywords: phosphine, resistance, insect pest, stored grain

1. Introduction

Phosphine has been used as s grain fumigant since the 1930s. Detection of resistance led to a global survey sponsored by the FAO (Champ and Dyte, 1977) that detected at least weak resistance in many countries. Strong resistance was first reported in a *Rhyzopertha dominica* strain from Bangladesh in 1991 (Chaudhry and Price, 1991). Strong resistance has since been found in the major pests on every continent that grows and stores grain (Benhalima et al., 2004; Chaudhry, 1997; Collins et al., 1993; Lorini et al., 2007; Pimentel et al., 2009; Song et al., 2011; Zettler and Keever, 1994). It has become apparent that strong resistance to phosphine is increasing in frequency and possibly in the level of resistances found. To gain an

understanding of what phosphine resistance means, how it occurs and how it is selected, research into the molecular genetic basis of resistance has been undertaken.

2. What is phosphine resistance?

Phosphine resistance is operationally defined as survival of insects at a dose that would kill all individuals of a susceptible strain of the species. A dose that discriminates resistance is ususally set at the $LD_{99.9}$ of a reference susceptible strain that has never been selected with phosphine. This differs between species, but is ususally in the range of 0.03-0.07 mg/L for a 20 hour exposure period (Champ and Dyte, 1977). In Australia, it became apparent that field-collected strains of insects could survive doses much higher than the standard discriminating dose. These strains were labelled 'strong resistance' and were characterized by survival of doses that would kill 99.9% of typical weakly resistant strains. It was also noted that 20 hour exposures were not long enough to give accurate resistance factors, and so it was recommended that 48 hour exposures be used when testing for strong resistance (Collins et al., 2002).

3. Genetics of phosphine resistance

To better understand the basis for the 'weak' and 'strong' resistance traits, genetic analyses of resistance was undertaken. Through genetic crosses and phenotype analysis, Collins et al. (2002) determined that 'weak' resistance in *Rhyzopertha dominica* was mostly due to a single gene, whereas strong resistance was due to two or more genes. This was also determined to be the case in *Tribolium castaneum* (Bengston et al., 1999; Jagadeesan et al., 2012; Jagadeesan et al., 2013).

Linkage analysis using genetic markers in *R. dominica* confirmed that resistance was due to two major loci *rph1* and *rph2* (i.e. *r*esistance to *ph*osphine *1* and *2*) that acted in synergy to produce strong resistance (Schlipalius et al., 2008; Schlipalius et al., 2002). This was also found to be the case for *T. castaneum* (Jagadeesan et al., 2013). Comparative and combinatorial analysis of resistance in multiple independent strains of *R. dominica* revealed that the same two genes contributed to strong resistance in every instance and that crossing the strains did not result in a super strong resistance trait (Mau et al., 2012a; Mau et al., 2012b).

4. Identification of resistance genes

Schlipalius et al (Schlipalius et al., 2012) identified that resistance due to the *rph2* gene was conferred by specific amino acid variants in critical regions of the enzyme dihydrolipoamide dehydrogenase (DLD). DLD contributes to multiple enzyme complexes of energy metabolism (pyruvate dehydrogenase (PDH), α-ketogluarate dehydrogenase (KGDH), branched chain amino acid dehydrogenase (BCKDH), and glycine cleavage system (GCS)) mostly within the mitochondria. This discovery has formed the basis of molecular markers that can be used to unambiguously identify phosphine resistant insects (Kaur et al., 2013).

The characterization of DLD from multiple strains of *R. dominica* collected from various geographic locations across Australia showed that there are at least five different variants at the *rph2* locus that confer resistance, all of which occur at or near the active site of the protein.

5. Monitoring and surveys

Since identification of the *rph2* gene, several regional surveys have been conducted using molecular markers designed to identify specific variants.

In one case, Kaur et al. (Kaur et al., 2013) conducted a survey of a single *rph2* resistance variant in *R. dominica* collected from farm storages in south-east Queensland. One advantage of this survey over monitoring by bioassay is that changes in resistance allele frequency could be monitored over time. That is, insects were analysed that had been collected from the same locations in 2006 and then in 2011. There was an increase in the frequency of the resistance variant on farms that had not used phosphine during that period, providing evidence for beetle movement between farms and storages. This survey demonstrates one aspect of the power of using DNA to test for resistance. The insects do not have to be alive to be tested for the resistance variant, and DNA can be retained for years and retested. The ability to collect many samples over a long period of time without having to culture them or immediately test them provides flexibility in how a resistance survey can be conducted.

A different resistance variant (P49S), originally found in *R. dominica* in Australia (Schlipalius et al., 2012) has been found to be very abundant in India and Turkey in both *R. dominica* (P49S) and *T. castaneum* (P45S). Though not as extensively studied in other countries, it is also present in these species in the USA, Turkey, Vietnam, and Brazil. This makes it the most common allele detected globally. The P45/49S variant appears to have arisen independently in each region and is not found in any other of the hundreds of Eukaryotic organisms in which the *rph2* gene has been sequenced. This means that it was specifically selected as a phosphine survival mechanism. We know that it could only have been at a very low frequency in insect populations prior to phosphine selection as strong resistance only developed many years after the resistance variant of *rph1* had become widespread, despite extremely strong selective pressure due to the sole reliance on phosphine fumigation for the control of insect pests. The lack of this variant among eukaryotes except in insects that had been under many years of extreme selective pressure suggests that there may be a hidden metabolic weakness or susceptibility associated with the resistance variant that is selected against in the absence of phosphine.

One such associated susceptibility identified is arsenic: strongly phosphine resistant strains of insects are more sensitive to arsine gas than phosphine susceptible strains (Chaudhry and Price, 1991; Schlipalius et al., 2012). This sensitivity may result from oxidized arsenic derivatives covalently bonding to the lipoic acid substrate of DLD. This specific biochemical susceptibility may also be affected by several alternative compounds that would be suitable for commercial use. This is only one example of the kinds of information that come from identifying the resistance genes and mode of action of phosphine that would also be very useful for resistance management strategies. Other metabolic weaknesses remain to be identified.

The overall picture emerging is one of increasing *frequency* of strong resistance, but not one of increasing *strength* of the resistances. So far, no major third gene (‘*rph3*’) for resistance has been detected (Mau et al., 2012a), especially one that confers a synergistic jump in resistance similar to that seen for *rph1* and *rph2*. This suggests that the strength of the resistances may have a limit and protocols developed to manage strongly resistant strains have international relevance. This also means that resistance management strategies that take into account both *rph1* and *rph2* are less likely to fail due to additional genetic factors.

6. Opportunities for international cooperation

Molecular markers for phosphine resistance now have application in industrial monitoring, ecological research (testing trap catches) and evaluating resistance management practices as well as informing management decisions. We have found that the same genes are responsible for strong phosphine resistance globally, so this enables us to conduct surveys and experiments that are internationally comparable without the requirement to develop local bioassays or maintain live cultures of insects. Information on the frequency of resistance, the actual alleles present and their geographical distribution can be delivered in a relatively short amount of time, with a lower overall cost. It is anticipated that the value that this information provides for pest mangement plans and strategic decision making will extend the use of phosphine as a routine treatment.

References

Bengston, M., Collins, P.J., Daglish, G.J., Hallman, V.L., Kopittke, R., Pavic, H., 1999. Inheritance of phosphine resistance in *Tribolium castaneum* (Coleoptera : Tenebrionidae). Journal of Economic Entomology 92, 17-19.

Benhalima, H., Chaudhry, M.Q., Mills, K.A., Price, N.R., 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. Journal of Stored Products Research 40, 241-249.

Champ, B.R., Dyte, C.E., 1977. FAO Global Survey of Pesticide Susceptibility of Stored Grain Pests. FAO Plant Protection Bulletin 25, 49-67.

Chaudhry, M.Q., 1997. A review of the mechanisms involved in the action of phosphine as an insecticide and phosphine resistance in stored-product insects. Pesticide Science 49, 213-228.

Chaudhry, M.Q., Price, N.R., 1991. Comparative Toxicity of Arsine and Stibine to Susceptible and Phosphine-Resistant Strains of *Rhyzopertha dominica* (F) (Coleoptera, Bostrychidae). Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology 99, 41-45.

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 Economic Entomology 95, 862-869.

Collins, P.J., Lambkin, T.M., Bridgeman, B.W., Pulvirenti, C., 1993. Resistance to Grain-Protectant Insecticides in Coleopterous Pests of Stored Cereals in Queensland, Australia. Journal of Economic Entomology 86, 239-245.

Jagadeesan, R., Collins, P.J., Daglish, G.J., Ebert, P.R., Schlipalius, D.I., 2012. Phosphine Resistance in the Rust Red Flour Beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae): Inheritance, Gene Interactions and Fitness Costs. Plos One 7.

Jagadeesan, R., Fotheringham, A., Ebert, P.R., Schlipalius, D.I., 2013. Rapid genome wide mapping of phosphine resistance loci by a simple regional averaging analysis in the red flour beetle, *Tribolium castaneum*. Bmc Genomics 14.

Kaur, R., Daniels, E.V., Nayak, M.K., Ebert, P.R., Schlipalius, D.I., 2013. Determining changes in the distribution and abundance of a *Rhyzopertha dominica* phosphine resistance allele in farm grain storages using a DNA marker. Pest Manag Sci 69, 685-688.

Lorini, I., Collins, P.J., Daglish, G.J., Nayak, M.K., Pavic, H., 2007. Detection and characterisation of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera : Bostrychidae). Pest Management Science 63, 358-364.

Mau, Y.S., Collins, P.J., Daglish, G.J., Nayak, M.K., Ebert, P.R., 2012a. The rph2 Gene Is Responsible for High Level Resistance to Phosphine in Independent Field Strains of *Rhyzopertha dominica*. Plos One 7.

Mau, Y.S., Collins, P.J., Daglish, G.J., Nayak, M.K., Pavic, H., Ebert, P.R., 2012b. The rph1 Gene Is a Common Contributor to the Evolution of Phosphine Resistance in Independent Field Isolates of *Rhyzopertha dominica*. Plos One 7.

Pimentel, M.A.G., Faroni, L.R.D., Guedes, R.N.C., Sousa, A.H., Totola, M.R., 2009. Phosphine resistance in Brazilian populations of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Journal of Stored Products Research 45, 71-74.

Schlipalius, D.I., Chen, W., Collins, P., Nguyen, T., Reilly, P., Ebert, P., 2008. Gene interactions constrain the course of evolution of phosphine resistance in the lesser grain borer, *Rhyzopertha dominica*. Heredity 100, 506-516.

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.

Schlipalius, D.I., Valmas, N., Tuck, A.G., Jagadeesan, R., Ma, L., Kaur, R., Goldinger, A., Anderson, C., Kuang, J.J., Zuryn, S., Mau, Y.S., Cheng, Q., Collins, P.J., Nayak, M.K., Schirra, H.J., Hilliard, M.A., Ebert, P.R., 2012. A Core Metabolic Enzyme Mediates Resistance to Phosphine Gas. Science 338, 807-810.

Song, X.H., Wang, P.P., Zhang, H.Y., 2011. Phosphine resistance in *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) from different geographical populations in China. African Journal of Biotechnology 10, 16367-16373.

Zettler, J.L., Keever, D.W., 1994. Phosphine Resistance in Cigarette Beetle (Coleoptera, Anobiidae) Associated with Tobacco Storage in the Southeastern United-States. Journal of Economic Entomology 87, 546-550.