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Frequency and Distribution of *rph2* Gene in *Tribolium castaneum* Collected from Grain Supply Chain of Coimbatore, Kangeyam and Theni

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A B S T R A C T

Resistance to insecticides is a worldwide issue for the major stored insect pests. Among several insecticides, phosphine (PH_3) gas is the most commonly used fumigant to protect the stored product from pests. Conventionally, a discriminating-dose bioassay is conducted to determine the phosphine resistance in stored insect pests. In the present study, the frequency of resistance is estimated in *Tribolium castaneum* (Herbst) populations collected from grain supply chain *viz.,* (Grain processing unit, bulk grain storage godowns, ration shop, wholesale shop, retail shop and household) from three locations of Tamil Nadu, India. Based on a cleaved amplified polymorphic sequence (CAPS) method, each individual insect is analysed using restriction enzyme to detect strong resistance allele. Among the populations collected from grain supply chain of Theni, Coimbatore and Kangeyam, the samples (*T. castaneum*) from bulk grain storage showed the highest frequency of phosphine resistant allele of *viz.,* 85.71, 78.33, 89.13%, respectively while as the lowest frequency of phosphine resistant allele was observed in household samples of Theni, Coimbatore and Kangeyam *viz.*,18.75, 34.48, 40.74%, respectively. Thus, strong phosphine resistance detected in beetle with the help of CAPS marker could be used for the improvement of management strategies for the given pest populations.

Introduction

India is the second largest producer of food grains and also a major exporter of cereals globally. Around 50 per cent of produce is stored by the farmers for their consumption and remaining surplus grains are procured by the government based stored grain agency like Food Corporation of India (FCI) and state warehouses. However, the wastage of food grains is witnessed due to lack of storage infrastructure and primitive grain handling mechanism. It is reported that post harvest grain losses in India is upto 12 to 16 million metric tons of food grains each year. The major loss is due to stored product insect pests which plays a key role in food grain deterioration and wastage is upto 5-10%.

Because of stored grain insect pest infestation the grains nutrients are degraded and makes it tasteless and valueless (Jood and Kapoor, 1996). Globally, several insect pests have been reported. Among them, *Tribolium castaneum* (Herbst) (Coleoptera; Tenebrionidae) is one of the most important secondary insect pests though it feeds on flour and other milled products (Good, 1936).

Phosphine (PH_3) gas fumigation is by far the most commonly used method for the management of insect pests in stored grains. This fumigant is efficient to use as it is toxic to all stored insects pests and their life stages (Chaudhry, 1997).

Ease of application, no residue, less cost and effectiveness are the advantages of phosphine (Nath *et al.,* 2011). Continuous application and exposure of phosphine has led to the development of strong resistance in stored insect pests which includes *Rhyzopertha dominica (F.), Tribolium castaneum (Herbst)* and *Cryptolestes ferrugineus* (Collins *et al.,* 2002; Benhalima *et al.,* 2004; Lorini *et al.,* 2007; Opit *et al.,* 2012; Nayak *et al.,* 2013). Resistance level has been categorized into two factors (*i.e.*) strong and weak resistance (Collins, 1998; White and Lambkin, 1990).

Phosphine resistance in *Tribolium castaneum* is identified and it is coded by two major autosomal genes *rph1* and *rph2* which combined together to produce strong resistance. *Rph1* is responsible for weak resistance, while *rph2* provides strong resistance (Schlipalius *et al.,* 2002; Jagadeesan *et al.,* 2012). Schlipalius *et al.,* (2012) reported that a mutation was found in Dihydrolipomide dehydrogenase (dld) gene at the *rph2* locus in the *R. dominica* and *T. castaneum* which lead to the development of resistance against phosphine. Variation in amino acid sequence in amplified 368bp fragment of *dld* gene and to confer resistance, a cleaved amplified polymorphic sequence (CAPS) analysis was performed using the *MboI* restriction enzyme which cleaves the *dld* gene into two fragments 296bp and 72bp in lengths (Schlipalius *et al.,* 2012; Kaur *et al.,* 2015). Schlipalius *et al.,* (2018) developed an assay for detecting strong phosphine resistance allele genes in *dld* gene of *R. dominica* using next-generation sequencing.

The distribution and frequency of resistance variants were estimated in thousands of individual insects in single run. In 1435 individuals collected from grain storage sites including farms and central storage across eastern Australia, resistance alleles were detected in 49% samples (38% farms, 60% of central storages). Two resistance variants (P49S and K142E) were widely found among other resistance alleles.

The present study investigated the frequency and distribution of phosphine resistance in *T. castaneum* collected from grain supply chain (Processing unit, bulk grain storage godowns, ration shop, wholesale shop, retail shop and households) of Coimbatore, Tiruppur (Kangeyam) and Theni districts. The *rph2* resistance allele frequency was measured by genotyping the insect population using cleaved amplified polymorphic sequence (CAPS) analysis. Based on this the resistance frequency was compared among three districts of Tamil Nadu.

Materials and Methods

Insect culture and survey

T. castaneum insect samples were collected from grain supply chain (Processing unit, bulk grain storage godowns, ration shop, wholesale shop, retail shop and households) of three different districts [Coimbatore, Tiruppur (Kangeyam) and Theni] of Tamil Nadu. These samples were cultured in whole wheat flour

and maintained at 30 °C and 55% relative humidity.

Bioassay for phenotype characterization and Statistical Analysis

T. castaneum adult insects were fumigated with two discriminating concentration of phosphine gas, low concentration (0.03 mg L^{-1}) (White and Lambkin, 1990) and high concentration (0.25mg L^{-1}) (Daglish and Collins, 1999).To detect weak and strong phosphine resistance the insects were exposed for about 20hrs. Fumigation was performed at a relative humidity of 30± 2°C and 60 ± 5 percent. Each test was replicated three times in which 50 insects/replication were maintained in Control and Test.

After exposure, insects were transferred on wheat flour to feed on and maintained in the culture room. Mortality was calculated after seven days from the end of the exposure time as reported by FAO. Statistical data analysis (RBD) was carried out using WASP 2.0 software for the bioassay replications (R1 to R4, respectively) and the overall SEd and CV values were also obtained (Table 1-3).

Genomic DNA extraction

Genomic DNA was extracted from *T. castaneum* samples using CTAB method (Doyle and Doyle 1987). The DNA extraction buffer contained 100mM Tris. HCl (pH 8), 10 mM EDTA, 1.4 M NaCl, 2% CTAB and 5% β-mercaptoethanol. Individual insect samples were homogenized with 200 µl of DNA extraction buffer and incubated at 65°C for 1 h. The tubes were removed from the water bath and allowed to cool at room temperature. Chloroform: isoamyl alcohol mixture (24:1, v/v) (0.8 volumes) was added and mixed by inversion for 10 min. to form an emulsion. It was centrifuged at 12,000 rpm for 10 min. and the clear aqueous phase was transferred to a new sterile tube. Ice-cold iso-propanol (0.7 volumes) was added and mixed gently by inversion and it was stored at -20^{o}C for overnight. It was then centrifuged at 12,000 rpm for 10 minutes to pellet the DNA and the supernatant was discarded. The DNA pellet was washed with 70% ethanol. After washing, DNA pellet was air dried and dissolved in 20-40 µl of TE buffer depending on size of the pellet and stored at -20°C until use. The isolated DNA was checked for its quality by separating in 0.8% agarose gel electrophoresis and quantified by spectrophotometer.

Amplification and Genotyping

A single nucleotide polymorphism (SNP) present in the gene sequences of the *dld* is identified using a cleaved amplified polymorphic sequence (CAPS) marker assay in the insects collected. In *T. castaneum,* the *dld* gene fragment is amplified using PCR in a reaction volume of 25µl containing 2.5µl of 10× PCR buffer, 1µl of 10µM forward (5′-GCCCTGACTGTCTTCCACCA-3′) and reverse (5′-AGCCTTGACAGCATTTTCCT-3′) primer, 0.5 µl (1.5 U) of Taq polymerase and 2 µl (50 ng) of template DNA. The PCR conditions consisted of 5 min at 95°C, followed by 35 cycles of 95 °C for 1 min, 55 °C for 30 s and 72 °C for 1 min and a final extension at 72 °C for 7 min. Amplified products were screened using agarose gel electrophoresis (1.5%). The amplified 368-bp product was digested with 1 U of *MboI* at 37 °C for 4 h in a reaction volume of 15 µl containing 5 µl of PCR product, 1 µl of 10 × buffer, 0.5 µl of *MboI* enzyme and 8.5 µl of nuclease-free water. The digested product was visualized using 3% agarose gel electrophoresis. Thus, presence of the resistant variant results in cleavage of the PCR product into 296 bp and 72 bp fragments.

Results and Discussion

Grains are stored and distributed to the market

through grain supply chain. These stored grains are affected by both biotic and abiotic factors. Comparatively, a biotic factor such as insects, mites, rodents, fungi etc., causes severe damage to the grains. Fumigation method is used for controlling these insect pests throughout the storage period. 80% of the insect pests are controlled by phosphine gas which is the only reliable fumigant (Chaudhry, 2000). Among these insect pests, resistance to phosphine is increasing day-by-day due to several factors like improper handling and frequent use of phosphine fumigant (Rafter *et al.,* 2017). Especially, high level of phosphine resistance is reported in *T. castaneum* (Rajendran, 1998). However, the comprehensive molecular study of development of resistance against pesticide imparts the data towards the development of resistance management strategy. It was reported that strong resistance to phosphine in stored grain insect pests was mainly regulated by two major genes (Ansell, 1992, Schlipalius *et al.,* 2002). Weak-R phenotype in stored insect pests is controlled by single major gene, *rph1*. In addition, the strong-R phenotype is developed by the combination of *rph1* and a second factor, *rph2* (Collins *et al.,* 2002; Schlipalius *et al.,* 2008). The present study was carried out to determine the frequency and distribution of phosphine resistance allele in key stored insect pests viz., *Tribolium castaneum* at molecular level using *rph2* gene, which was related to strong phosphine resistance in storage pests. From grain supply chain, only few reports are available on frequency and distribution of phosphine resistance allele at *rph2* in key stored insect pests.

In this study, total of 473 individuals of *T. castaneum* were examined for the frequency and distribution of phosphine resistance allele (RR) at *rph2* in grain supply chain of Coimbatore, Tiruppur (Kangayam) and Theni districts of Tamil Nadu, India. The phosphine resistance allele (RR) frequency varied from 85.71 to 18.75 per cent in Coimbatore, whereas it was 78.33 to 34.48 per cent in Kangayam and 89.13 to 24.07 per cent in Theni across grain supply chain.

Among the population collected from Coimbatore grain supply chain, the highest frequency of phosphine resistance allele were recorded in bulk grain storage (85.71% for FCI) and Ration shop (63.46%). The moderate frequency of phosphine resistance allele (48.33%) was observed in the wholesale shop. Retail shop and household samples recorded low frequency of phosphine resistance allele (37.50 and 18.75 %, respectively) and high frequency of susceptible allele (81.25 and 62.05%, respectively).

Individuals having atleast one “R” allele was calculated for all the individuals from grain supply chain. Samples collected from ration shop and bulk grain storage showed 96.15 and 89.28 % having one “R” allele. Wholesale shop and retail shop samples were recorded with 80 and 71.42 % having atleast one “R” allele. However, the lowest number of individuals having at least one “R” allele was observed in household samples (37.5 %) (Table 4).

Similarly, Kangayam population collected from grain supply chain were analysed for frequency of phosphine resistance allele. Among them the highest frequency of phosphine resistance allele were recorded in bulk grain storage (78.33 % for TNCSC) (Plate 1&2), processing unit and ration shop (62.96 and 60 %) respectively.

The moderate frequency of phosphine resistance allele (55.76 and 53.70 %, respectively) was observed in wholesale shop and retail shop. The samples collected from household were recorded with low frequency of phosphine resistance allele (34.48%) and

high frequency of susceptible allele (65.51%). Bulk grain storage and wholesale shop were recorded 96.66 and 92.30 % of individuals having at least one "R" allele.

Table.1 Bioassay and statistical data analysis of *T. castaneum* populations collected from Coimbatore region

S.No.	Location	Percent Resistance (Mean ± SE)	
		Low conc. (0.03mg/L)	High conc. (0.25mg/L)
1	Storage Godown (FCI)	100.00±00 (89.09)[a]	81.00±2.94 (64.50)[a]
2	Ration Shop	84.88±2.94 (68.02)[b]	54.65±5.88 (47.67)[b]
3	Whole Sale shop	79.78±2.94 (63.52)[c]	40.45±3.85 (39.20)[c]
4	Retail Shop	71.59±3.85 (58.46)[d]	29.55±2.94 (33.23)[d]
5	House Hold	68.97±3.85 (56.65)[d]	26.44±4.84 (31.15)[e]
	SEd	0.27	0.15
	CV %	1.76	1.56

[a, b, c, d] **Figures in the parentheses are arcsine transformed values.**

Table.2 Bioassay and statistical data analysis of *T. castaneum* populations collected from Kangeyam region

Sr.No	Location	Percent Resistance (Mean ± SE)	
		Low conc. (0.03mg/L)	High conc. (0.25mg/L)
1	Storage Godown (TNCSC)	86.52±2.94 (68.47)[a]	67.42±3.85 (55.58)[a]
2	Rice Mill	85.39±2.22 (67.55)[a]	51.69±4.84 (46.19)[b]
3	Ration Shop	82.02±2.94 (64.94)[b]	47.19±3.85 (43.38)[c]
4	Whole Sale shop	75.28±2.94 (60.19)[c]	42.70±2.22 (41.03)[d]
5	Retail Shop	67.42±3.85 (55.20)[d]	33.71±3.85 (35.74)[e]
6	House Hold	59.55±2.94 (50.50)[e]	17.98±2.94 (25.44)[f]
	Sed	0.24	0.14
	CV%	1.91	1.61

[a, b, c, d] **Figures in the parentheses are arcsine transformed values**

Table.3 Bioassay and statistical data analysis of *T. castaneum* populations collected from Theni region

S.No	Location	Percent Resistance (Mean ± SE)	
		Low conc. (0.03mg/L)	High conc. (0.25mg/L)
1	Storage Godown (CWC)	98.89±1.11 (84.25)[a]	85.56±2.94 (67.69)[a]
2	Rice Mill	87.36±2.22 (69.21)[b]	68.97±3.85 (56.55)[b]
3	Ration Shop	80.90±3.85 (64.49)[c]	59.55±4.01 (50.86)[c]
4	Whole Sale shop	78.89±2.94 (63.02)[c]	57.78±5.88 (49.47)[c]
5	Retail Shop	78.16±2.94 (62.39)[c]	51.72±5.09 (46.55)[d]
6	House Hold	68.97±3.85 (56.51)[d]	45.98±2.94 (43.02)[e]
	Sed	0.37	0.20
	CV%	2.74	1.90

[a, b, c, d] **Figures in the parentheses are arcsine transformed values.**

Table.4 Frequency of phosphine resistance gene (*rph2*) in *T. castaneum* populations collected from grain supply chain of Coimbatore region

S. No	Location	Total number of samples	(RR)	(RS)	(SS)	'R' Alleles (%)	'S' Alleles (%)	Percentage of individuals having at least one " R" allele
1.	Bulk grain storage FCI	28	23	2	3	85.71	14.28	89.28
2.	Public Distribution Shop (Ration shop)	26	8	17	1	63.46	36.53	96.15
3.	Wholesale shop	30	5	19	6	48.33	51.66	80.00
4.	Retail shop	28	1	19	8	37.50	62.5	71.42
5.	House hold	24	0	9	15	18.75	81.25	37.50

***RR – Resistance; RS – Heterozygote; SS – Susceptible ; FCI – Food Corporation of India.**

Table.5 Frequency of phosphine resistance gene (*rph2*) in *T. castaneum* populations collected from grain supply chain of Kangeyam

S. No	Location	Total number of samples	(RR)	(RS)	(SS)	'R' Alleles (%)	'S' Alleles (%)	Percentage of individuals having at least one " R" allele
1.	Bulk grain storage TNCSC	30	18	11	1	78.33	21.66	96.66
2.	Grain processing unit (Rice mill)	27	10	14	7	62.96	51.85	88.88
3.	Public Distribution Shop (Ration shop)	30	12	12	6	60.00	40.00	80.00
4.	Wholesale shop	26	6	17	2	55.76	44.23	92.30
5.	Retail shop	27	5	19	2	53.70	42.59	88.88
6.	House hold	29	1	18	10	34.48	65.51	65.51

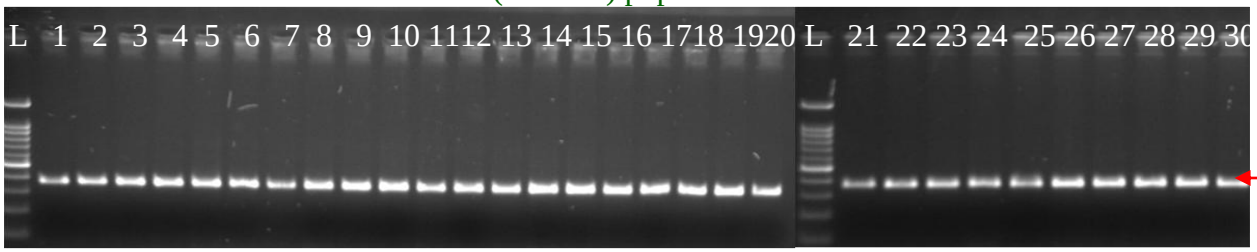
***RR – Resistance; RS – Heterozygote; SS – Susceptible; TNCSC - Tamil Nadu Civil Supply Chain**

Table.6 Frequency of phosphine resistance gene (*rph2*) in *T. castaneum* populations collected from grain supply chain of Theni region

S. No	Location	Total number of samples	(RR)	(RS)	(SS)	'R' Alleles (%)	'S' Alleles (%)	Percentage of individuals having at least one " R" allele
1.	Bulk grain storage CWC	23	20	1	2	89.13	10.86	91.30
2.	Grain processing unit (Rice mill)	30	16	13	1	75.00	25.00	96.66
3.	Public Distribution Shop (Ration shop)	29	15	13	1	74.13	25.86	96.55
4.	Wholesale shop	30	15	14	1	73.34	26.66	96.67
5.	Retail shop	29	1	13	15	25.86	74.13	48.28
6.	House hold	27	2	9	16	24.07	75.92	40.74

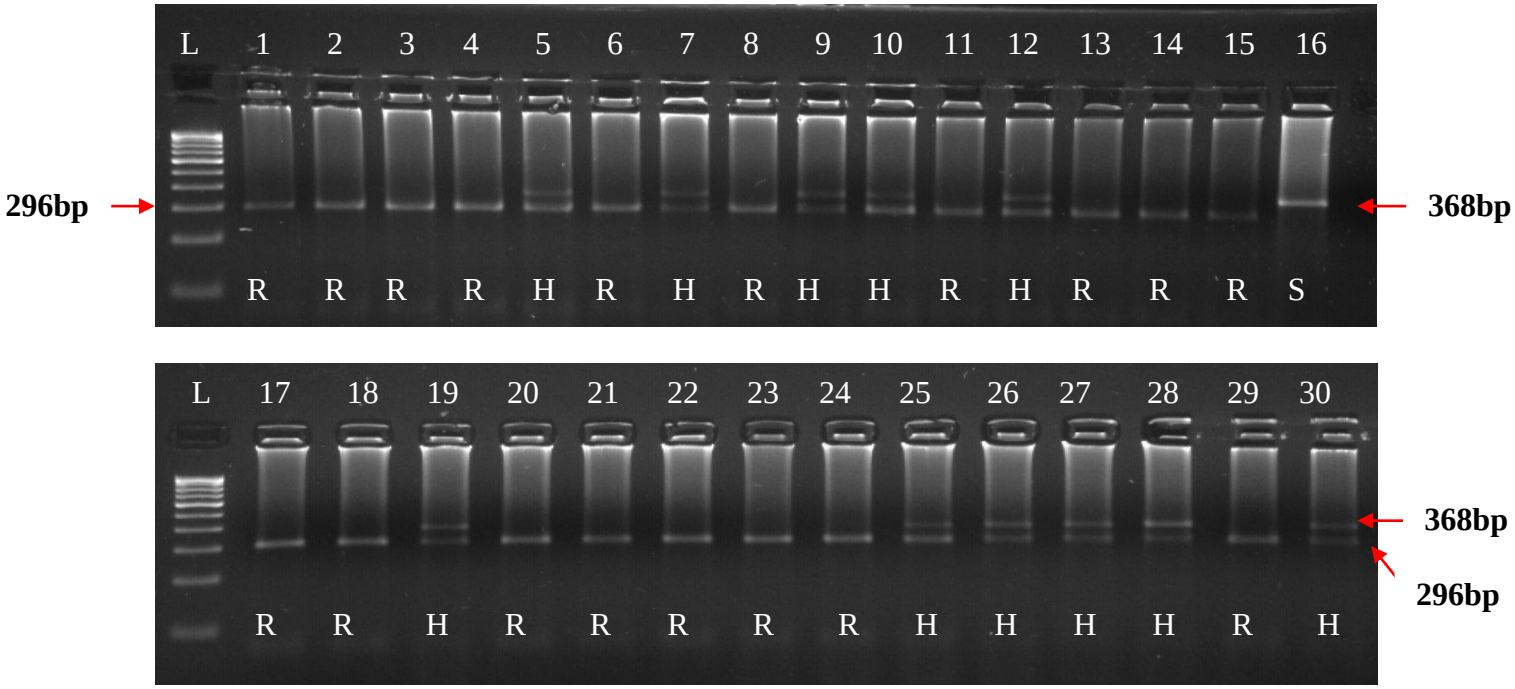
***RR – Resistance; RS – Heterozygote; SS – Susceptible ; TNCSC - Tamil Nadu Civil Supply Chain**

Plate.1 Amplification of rph2 gene in individual samples of *Tribolium castaneum* Kangeyam (TNCSC) population



Lane L:100 bp ladder
Lane 1 to 30: Individual *T. castaneum* samples

Plate.2 PCR - RFLP analyses of individual sample of *Tribolium castaneum* Kangeyam (TNCSC) population



Lane L:100bp ladder ; R- Resistance ; H- Heterozygote ; S- Susceptible

Around 88.88% of individuals having at least one "R" allele were observed in the samples collected from grain processing unit and retail shop and samples collected from ration shop were observed with 80% individuals having one "R" allele were 80%. The lowest number of individuals having at least one "R" allele was observed in household samples (65.51 %) (Table 5).

Also, the frequency of phosphine resistance allele were evaluated in Theni grain supply chain in which the bulk grain storage showed the highest frequency of phosphine resistance allele (*i.e*) 89.13 per cent. Grain processing unit and ration shop and wholesale shop were recorded with moderate frequency of phosphine resistance allele of (75, 74.13, 73.34%, respectively). Relatively, the lowest frequency of phosphine resistance allele was recorded in retail shop and household samples (25.86 and 24.07 %, respectively) and the high level of frequency susceptible allele (74.13 and 75.92 %, respectively). Bulk grain storage, grain processing unit, ration shop and wholesale shop were observed with (91.30, 96.66, 96.55, 96.67%, respectively) of individuals having at least one "R" allele. The lowest number of individuals having at least one "R" allele were recorded in retail shop and household samples (48.28 and 40.74%, respectively) (Table 6).

The present results indicated the presence of specific P45S allele of *rph2* which is responsible for the strong resistance and also it was previously reported in other regions of the world (Schlipalius *et al.*, 2012; Kaur *et al.*, 2015; Chen *et al.*, 2015; Kocak *et al.*, 2015) the bulk grain storage population of *T. castaneum* of these three locations showed high level of frequency. Though the bulk grain storage indicated high frequency of P45S resistance allele it was suggested that this allele may be the most widespread allele worldwide, although it is likely to occur independently in all the multiple regions. This also suggests that the P45S allele confer the

strongest phosphine resistance phenotype, and confers the least fitness cost. This would allow survival under selection pressures and maintenance in insect populations that breed outside of storages and are not exposed to phosphine. The pleiotropic enhancement of fitness has been observed in resistant *T. castaneum* individuals are extremely tolerant to starvation. In future, resistance markers combined with population genetic studies to assess patterns of gene flow, rates and patterns of selection for resistance will be able to be estimated, allowing us to enhance phosphine resistance management strategies.

References

Ansell, M. R. (1992). The mode of inheritance of resistance to phosphine in two species of stored product beetles, (PhD) Thesis. University of Reading, UK.

Benhalima, H., Chaudhry, M., Mills, K., and Price, N. (2004). Phosphine resistance in stored product insects collected from various grain storage facilities in Morocco. *Journal of Stored Products Research, 40*(3), 241-249.

Chaudhry, M (1997). A Review of the Mechanisms Involved in the Action of Phosphine as an Insecticide and Phosphine Resistance in Stored-Product Insects. *Pest Management Science,* 49(3), 213-228.

Chaudhry, M. (2000). Phosphine resistance. *Pesticide Outlook, 11*(3), 88-91.

Chen, Z., Schlipalius, D., Opit, G., Subramanyam, B., and Phillips, T. W. (2015). Diagnostic molecular markers for phosphine resistance in US populations of *Tribolium castaneum* and *Rhyzopertha dominica*. *PloS one,* 10(3), e0121343.

Collins, P. J., Daglish, G. J., Bengston, M., Lambkin, T. M., and Pavic, H. (2002). Genetics of resistance to phosphine in *Rhyzopertha dominica* (Coleoptera: Bostrichidae). *Journal of economic entomology, 95*(4), 862-869.

Collins, P.J. 1998. Resistance to grain protectants and fumigants in insect pests of stored products in Australia. In: Banks, H.J., E.J. Wright and K.A. Damcevski (eds.,). Stored grain in Australia. Proceddings of the Australian Post-harvest Technical Conference. CSIRO: Canberra. Australia, pp. 55 – 57.

Daglish, G.J. and P.J. Collins. 1999. Improving the relevance of assays for phosphine resistance. In: Proceedings of 7th International Working Conference on Stored- Product Protection, Beijing, China. Sichuan Publishing House of Science and Technology, Chengdu, China. 1998. pp. 584-593.

Doyle, J. J and Doyle, J.L (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull Bot Soc Am, 19*, 11-15.

Good, N.E. 1936. The flour beetles of the genus Tribolium. USDA Technical Bulletin, 5: 27-28.

Jagadeesan, R., Collins, P. J., Daglish, G. J., Ebert, P. R., and 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*(2), e31582.

Jood, S., Kapoor, A., and Singh, R. (1996). Chemical composition of cereal grains as affected by storage and insect infestation. *Tropical agriculture*, 73(1996), 161-164.

Kaur, R., Subbarayalu, M., Jagadeesan, R., Daglish, G., Nayak, M., Naik, H. R., Ramasamy, S., Subramanian, C., Ebert, P. R., and Schlipalius, D. (2015). Phosphine resistance in India is characterised by a dihydrolipoamide dehydrogenase variant that is otherwise unobserved in eukaryotes. *Heredity,* 115(3), 188.

Koçak, E., Schlipalius, D., Kaur, R., Tuck, A., Ebert, P., Collins, P., and Yılmaz, A. (2015). Determining phosphine resistance in rust red flour beetle,

Tribolium castaneum (Herbst.) (Coleoptera: Tenebrionidae) populations from Turkey. *Turkish Journal of Entomology*, *39*(2).

Lorini, I., Collins, P. J., Daglish, G. J., Nayak, M. K., and Pavic, H. (2007). Detection and characterisation of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.)(Coleoptera: Bostrychidae). *Pest management science*, 63(4), 358-364.

Nath, N. S., Bhattacharya, I., Tuck, A. G., Schlipalius, D. I., and Ebert, P. R. (2011). Mechanisms of phosphine toxicity. *Journal of toxicology*, 1-9.

Nayak MK, Holloway JC, Emery RN, Pavic H, Bartlet J, Collins PJ (2013). Strong resistance to phosphine in the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae): its characterisation, a rapid assay for diagnosis and its distribution in Australia. Pest Manage Sci 69: 48–53.

Opit, G., Phillips, T. W., Aikins, M. J., and Hasan, M. (2012). Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from stored wheat in Oklahoma. *Journal of Economic Entomology*, 105(4), 1107-1114.

Rafter, M. A., McCulloch, G. A., Daglish, G. J., and Walter, G. H. (2017). Progression of phosphine resistance in susceptible *Tribolium castaneum* (Herbst) populations under different immigration regimes and selection pressures. *Evolutionary applications*.

Rajendran, S. (1998). *Phosphine resistance in stored grain insect pests in India*. Paper presented at the Proceedings of the 7th International Working Conference on Stored-Product Protection.

Schlipalius, D. I., Cheng, Q., Reilly, P. E., Collins, P. J., and 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(2), 773-782.

Schlipalius, D. I., Tuck, A.G., Pavic, H., Daglish,G.J., Nayak,M.K., Ebert, P. R., (2018). A high-throughput system used to determine frequency and distribution of phosphine resistance across large geographical regions. *Pest Management Science*, 75: 1091–1098.

Schlipalius, D. I., Valmas, N., Tuck, A. G., Jagadeesan, R., Ma, L., Kaur, R., Goldinger, A., Anderson, C., Kuang, J., Zuryn, S., and Zuryn, S. (2012). A core metabolic enzyme mediates resistance to phosphine gas. *Science*, 338(6108), 807-810.

Schlipalius, D., Chen, W., Collins, P., Nguyen, T., Reilly, P., and Ebert, P. (2008). Gene interactions constrain the course of evolution of phosphine resistance in the lesser grain borer, *Rhyzopertha dominica*. *Heredity*, 100(5), 506.

White, G.G. and T.A. Lambkin. 1990. Baseline response to phosphine and resistance status of stored grain pests in Queensland, Australia. Journal of Stored Product Research, 83(3): 1738 – 1744.

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