Donahaye, E.J., Navarro, S. and Leesch J.G. [Eds.] (2001) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, Fresno, CA. 29 Oct. - 3 Nov. 2000, Executive Printing Services, Clovis, CA, U.S.A pp. 609-616

RELATIONSHIP BETWEEN PHOSPHINE RESISTANCE AND NARCOTIC KNOCKDOWN IN *TRIBOLIUM CASTANEUM* (HERBST), *SITOPHILUS ORYZAE* (L.) AND *S. ZEAMAIS* (MOTSCH.)

YANG CAO* AND DIANXUAN WANG

Zhengzhou Grain College, Zhengzhou, Henan, 450052, P. R. China
*[*e-mail: caoyangw@public2.zz.ha.cn]*

ABSTRACT

This paper examines the relationship between LC_{50} of adult insects exposed to phosphine (PH_3) according to the FAO method and time to 50% knockdown (KT_{50}) at 2 mg/L. The aim of this study was to provide a basis for developing a rapid resistance test for practical situations. This study differs from other published studies by investigating this relationship across strains within three different species: *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.) and *S. zeamais* (Motsch.) There was a strong positive linear relationship between log LC_{50} and KT_{50} : log LC_{50} = 1.5245 log KT_{50} - 3.869 (r^2 = 0.9646). Although adults are easier to kill than eggs or pupae, resistant strains can be characterised by the response of their adults. This means that the resistance level of a field strain can be estimated from its response in the knockdown test. On the basis of these results, managers in depots could determine the minimum concentration and exposure time required for effective fumigation, or to choose alternative control methods if PH_3 fumigation is unlikely to control these insects. Therefore, this rapid knockdown test has potential as a tool in the management of PH_3 resistant grain insects.

INTRODUCTION

Phosphine (PH_3) is one of the best fumigants at present and is used widely for controlling insects during storage of agricultural products. For example, it is reported that about 90% of grains are fumigated with PH_3 in China, (Wang and Cao, 1999) and the figure is about 80% in Australia. Phosphine can penetrate deeply, spread quickly, is very effective, convenient, inexpensive, and has low residue characteristics. In short, PH_3 plays a very important role in stored-grain pest management.

Phosphine has been used for over fifty years, and because of prolonged usage and continuous selective pressure, major stored-grain insects have developed considerable resistance to PH_3 in many countries (Champ and Dyte 1976; Attia and Greening, 1981; Mills 1983 and 1986). In China, major stored-grain insects such as *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), *Cryptolestes* spp., and *Tribolium*

castaneum (Herbst) have all developed relatively substantial PH_3resistance (Liang, 1994; Liang 1998). Using the FAO Method (Anon. 1975), their highest resistance factors were determined as 606, 1160, 60 and 180-fold respectively. Fumigation operators and depot managers often complain that PH_3 cannot kill insects completely. The consequences are bad or even futile and over 50% of stored grain needs to be fumigated at least twice a year. Similarly, there is serious resistance to PH_3 in Australia, the USA, India, Pakistan, East Asia, Africa and Latin America (Rajendran 1994; Taylor, 1989; Zettler 1990). The resistance developed to PH_3 actually threatens its continued use. For this reason, the undertaking of PH_3 anti-resistance management should become an important part of the integrated stored grain pest management system, now and in the future.

The key to an effective strategy is the detection of resistant insects so that an appropriate dosage schedule can be applied to control them. The FAO (Anon, 1975) recommended method can be used to detect and measure resistance, and to derive the resistance factor relative to susceptible insects. Since 1975, this method has played a central role in PH_3 resistance laboratory research, but it is unable to meet the requirement of commercial depot management due to its relatively complicated operation and the prolonged delay (at least 15 days) until results are obtained. Therefore there is an urgent need for a convenient, practical and rapid method for the detection of resistance to PH_3 for the on-site depot level so as to enable grain storage operators to carry out anti-resistance management of infestations.

Winks (1985) and other researchers found that under high PH_3 concentrations, stored-grain insects could be knocked down in a short time (termed narcosis by some experts). Price (1985) reported that PH_3 resistant *R. dominica* could actively exclude PH_3. Reichmuth (1991) developed a rapid method to detect PH_3 resistance based on the knockdown time. Waterford *et al.,* (1994) and Bell *et al.,* (1994) also researched the relationship between the PH_3 killing time and knockdown time respectively. They both thought that there was a relationship between the killing time or knock-down time of different resistant strains, and their resistance levels. Moreover this relationship might be used for measuring PH_3 resistance. Mills and Athie (1998) studied the knockdown times of *S. oryzae* and *Oryzaephilus surinamensis* (L.) exposed to non-narcotic concentrations of PH_3 in the range 0.393 - 0.466 g m^{-3}. Cao *et al.,* (1998) investigated a rapid resistance test method for *T. castaneum*. This paper further researches the relationship between the knockdown time and resistance and also attempts to find a correlation that would apply to more than one species. We hope this method can provide a scientific basis for a rapid test method of PH_3 resistance that can be used in storage depots.

MATERIAL AND METHODS

Insect strains and culturing methods

The following species and strains were used in these experiments:

T. castaneum: HZZTc from Zhengzhou Grain College, Henan Province, China; QTC300 from the Queensland Department of Primary Industries, Australia; JSTc

from Siping, Jilin Province, China; AYTc from Anyang, Henan Province, China; ZXTc from Zhengzhou, Henan Province, China;

S. oryzae: SMGSo from Chengdu Grain Storage Research Institute, Sichuan Province, China; QSO335 from the Queensland Department of Primary Industries, Australia; GDSo from Guangzhou, Guangdong Province; SQSo from Shangqiu, Henan Province, China.

S. zeamais: ZZSz from Zhengzhou Grain College, Henan Province, China.

T. castaneum was cultured on a mixture containing 10% yeast and 90% whole wheat flour. *S. oryzae* and *S. zeamais* were reared on sterilized whole wheat (14±2% m.c.). All species were cultured at 25±2ºC and 70±5% r.h.

Generating phosphine and determining concentration

Zinc phosphide, instead of aluminum phosphide, was reacted with dilute sulfuric acid to generate a stock of PH_3 according to the recommended FAO bioassay method for PH_3 (Anon., 1975). The concentration of PH_3 was determined by colorimetry according to the Chinese standard GB5009 36-89.

LC_{50} test

The resistance status of each strain was established by using the recommended FAO bioassay method for PH_3 resistance (Anon., 1975). There were five concentrations in each bioassay, and the bioassay was repeated two to three times for each strain. Bioassays were performed in 6 L glass desiccators using 100 adult insects. Phosphine was injected through a septum using a gas tight syringe. After a 20 h exposure the adults were held for two weeks before the number of dead adults was recorded. The LC_{50} was obtained using probit analysis (Finney 1971).

Knockdown test

Ten adults were put into a 140 mL glass bottle sealed with a gas-tight rubber stopper. A gas-tight syringe was used to transfer PH_3 gas into the bottle and a concentration of 2□mg/L was obtained. Then the response of the adults was closely observed continuously. When the adults were not able to climb or walk properly, they were recorded as knocked down. The knockdown time (KT) of each adult was recorded until all of adults were knocked down. An average of KT_{50} from three replicated tests was obtained for each strain.

Data analysis

The results from the FAO method were processed by probit analysis (Finney, 1971). The knockdown test results and the correlation between LC_{50} and KT_{50} were analyzed by regression analysis method using Microsoft Excel 97 software.

RESULTS

FAO test

Data on the response of test strains in the FAO test are given in Table 1. Of the five *T. castaneum* strains JSTc and HZZTc were both judged to be susceptible strains according to the FAO recommended LC_{50} range for susceptible strains. HZZTc was used as susceptible reference strain for calculating resistance factors (Rf). AYTc and ZXTc were very resistant with Rf's of 217.1 and 245.1 respectively. QTC300 had a low level of resistance with a Rf of 13.1.

Of the four *S. oryzae* strains tested, SMGSo was judged to be susceptible according to the FAO recommended LC_{50} range for susceptible strains. GDSo was a high level resistant strain with a Rf of 549. QSO335 and SQSo were low level resistant strains with Rf's of 3.6 and 3.2 respectively.

The single strain of *S. zeamais* was found to be susceptible to PH_3 according to the FAO recommended LC_{50} range for susceptible strains.

TABLE 1
Values of LC_{50}, KT_{50}, and resistance factors of strains of *Tribolium castaneum* and *Sitophilus oryzae* and *S. zeamais* to phosphine

Species	Strain	FAO test		Knockdown test	
		LC_{50} (95% FL) (mg/L)	Resistance factor	KT_{50} min	Resistance factor
T. castaneum	HZZTc	0.0051 (0.0042-0.0064)	-	7.8	-
	JSTc	0.0073 (0.0065-0.0081)	1.4	15	1.9
	QTC300	0.067 (0.062-0.072)	13.1	70	9.0
	AYTc	1.1 (0.98-1.2)	215.7	378	48.5
	ZXTc	1.3 (1.2-1.3)	254.9	469	60.1
S. oryzae	SMGSo	0.0115 (0.010-0.0125)	1	27	-
	SOSo	0.037 (0.035-0.039)	3.2	45	1.7
	QSO335	0.041 (0.037-0.046)	3.6	57	2.1
	GDSo	6.314 (5.39-7.89)	549.0	642	23.8
S. zeamais	ZZSz	0.00462 (0.00298-0.00590)		7	

Knockdown test

Data on the response of test strains in the knockdown test are given in Table 1. Of the five *T. castaneum* strains JSTc and HZZTc were susceptible strains and their adults were knocked down easily (KT_{50} = 7.8 and 15 min respectively). HZZTc was

used as the reference strain. The KT_{50} and Rf of ZXTc were 469 min and 59.1-fold respectively. The KT_{50} of this strain was the longest one among the five strains tested. The KT_{50} and Rf of AYTc were 378 min and 48.5-fold this being the second longest KT_{50}. ZXTc and AYTC appeared to have a relatively high tolerance to knockdown of 2mg/L PH_3. The KT_{50} and Rf of DPITc were 70 min and 9-fold representing a mid-way position among the five strains and a low level of resistance. From this it can be seen that the order of resistance of the five strains to PH_3 according to the KT_{50} criterion, was consistent with the results by the FAO test method.

The susceptible strain of *S. oryzae* (SMGSo) had a KT_{50} of 27 min. Of the three resistant strains SOSo had the shortest KT_{50} of 45 min and a Rf of 1.7-fold, and GDSo had the longest KT_{50}.

The single strain of *S. zeamais* was susceptible and it had a KT_{50} of 7 min.

Relationship between LC_{50} and KT_{50} in ten strains

For these 10 strains belonging to 3 species the results of the FAO test and the knockdown test were strongly correlated (Fig 1.). The regression equation was:

$$\log (LC_{50}) = 1.5245 \log (KT_{50}) - 3.869 \ (R^2 = 0.9646)$$

Therefore, it is deemed feasible to estimate the resistance level of test insects in practical situations by using the faster knockdown test, and then use the KT_{50} in the regression equation to establish the LC_{50}.

DISCUSSION

The insect knockdown phenomenon

Winks (1985) found that stored grain insects were subject to knock-down in a short time under high PH_3 concentrations. They called this narcosis because although insects were knocked down, if they were removed from the PH_3 most of them would recover and survive. In contrast, at lower concentrations, the insects took longer to be knocked down and most did not recover after removal from the PH_3. Based on this phenomenon, experts thought that when the insects were exposed to relatively high PH_3 concentrations, they would respond by entering narcosis and this would result in a decrease in inhalation of PH_3, thus reducing the effect of the fumigation. For this reason, experts suggested that high PH_3 concentrations and short exposure times should be avoided while fumigating.

Other reports also showed that under the same PH_3 concentration, the knockdown times of different resistant insect strains were different. Price (1984) proved that resistant *R. dominica* adults could actively exclude PH_3, and also found a correlation between PH_3 resistance or knockdown time and the active exclusion. So it was inferred that the stronger the resistance to PH_3, the stronger was the ability of the insects to actively exclude the PH_3, and therefore the longer the time to knockdown. Reichmuth (1991), Waterford *et al.,* (1994), Bell *et al.,* (1994) and Cao *et al.,* (1998)

have further confirmed this correlation. Waterford *et al.,* (1994) showed a positive linear relationship for several *T. castaneum* strains between the log-transformed time to kill all the insects at 0.1 mg/L PH_3 and the log KT_{100} at 2 mg/L PH_3.

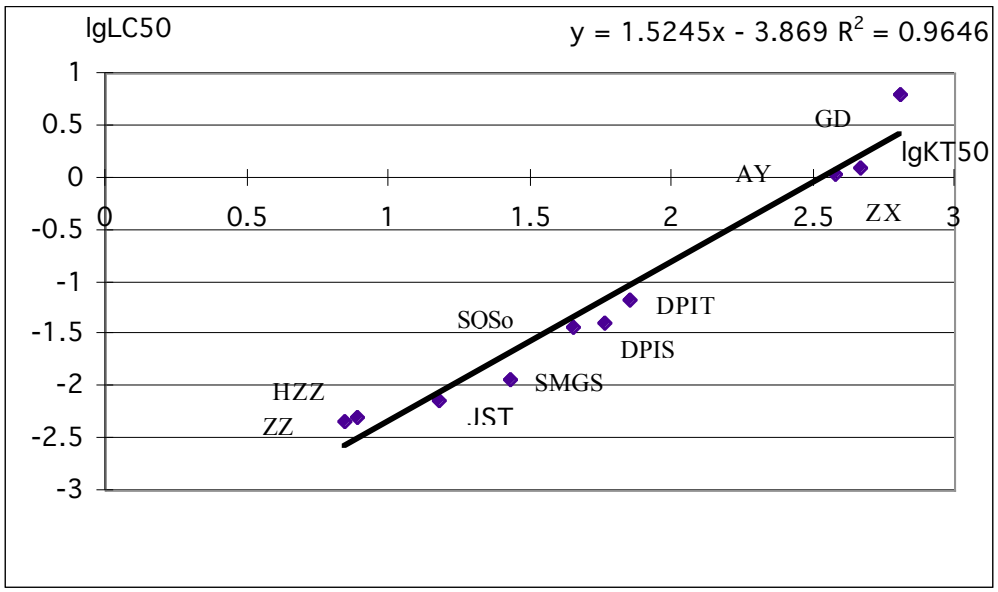

Fig. 1. The correlation between log (LC_{50}) and log (KT_{50}) of strains of *Sitophilus oryzae*, *Sitophilus zeamais* and *Tribolium castaneum*.

This paper has revealed a single positive linear relationship between the LC_{50} and KT_{50} for three different species (*T. castaneum*, *S. oryzae* and *S. zeamais*), the equation of which has been provided above.

By measuring the KT_{50}, we can estimate the corresponding $LC_{50,}$ and then estimate the degree of resistance of the test strain. The minimum concentration and exposure time required for effective PH_3 fumigation can then be determined for insects with this type of resistance, or alternative control decisions can be made.

The rapid method to determining phosphine resistance

Reichmuth (1991) introduced a rapid method to determine PH_3 resistance. If adults were still crawling normally after 30 min exposure to 1mg/L PH_3, then they were classified as a PH_3 resistant strain, but if they all were knocked down within 30 min they were classified as susceptible. However, this method can only be used to detect resistance but not to measure the strength of resistance.

This paper added the results of four *S. oryzae* strains and one *S. zeamais* strain to previously published results of *T. castaneum* (Cao *et al.,* 1998) to obtain a single equation relating KT_{50} to LC_{50}.

By measuring the LC_{50} and KT_{50}, of the test insects, the rapid method of using knockdown time to determine resistance was reconfirmed. According to the LC_{50} FAO recommended discriminatory concentration method of subjecting the test strain to 0.03 mg/L (is this R.dominica or other species as well???) for an exposure of 20 h, and mortality count at 14 d after fumigation, if more than 50% of the insects were still alive, it was regarded as a resistant strain (Anon. 1975). From our findings, if the LC_{50} = 0.03 mg/L is inserted into the regression equation, then KT_{50} = 35 min. In order to estimate the resistance, based on KT_{50} = 35 min we recommend the following conclusions to be drawn:

- If KT_{50} >30 min the test insect strain is resistant;
- If KT_{50} <30 min, the test insect strain is susceptible.

ACKNOWLEDGEMENTS

The State Administration of Grain Reserve, Ministry of Internal Trade, Beijing, China and the Australian Centre for International Agricultural Research (ACIAR) financially supported the work. Their support is gratefully acknowledged.

We also acknowledge the help of Dr. Greg Daglish and Dr. Pat Collins, Farming Systems Institute, DPIQ. Indooroopilly Research Centre, Qld. Australia who provided some valuable suggestions and made many modifications to this paper.

REFERENCES

Anon. (1975) Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative methods for adults of some major beetle pests of stored cereals with methyl bromide and phosphine. FAO method No 16. *FAO Plant Protection Bulletin*, **23**, 12 –25.

Attia, F.I. and Greening, H.G. (1981) Survey of resistance to phosphine in Coleopterous pests of grains and stored products in New South Wales. *General and Applied Entomology*, **4**, 98-101.

Bell, C.H., Savvidou, N., Mills, K.A., Bradberry, S. and Barlow, M.L. (1994) A same-day test for detecting resistance to phosphine. In: *Proc. 6th Int. Working Conf. on Stored-Product Protection* (Edited by Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R.) Canberra, Australia, 17-23 April 1994, CAB International, Wallingford, Oxon, UK, **1,** 41-44.

Cao Y., Zhang, J. and Bengston, M. (1998) Studies on a quick method to measure resistance of four strains of *Tribolium castaneum* (Herbst) to phosphine. In: *Proc. 7th Int. Working Conf. on Stored-product Protection,* (Edited by: Zuxun, J., Quan, L., Yongsheng, L., Xianchang, T. and Lianghua, G.), Beijing. Sichuan Publishing House of Science & Technology, Chengdu, China, **1,** 603-606.

Champ, B.R. and Dyte, C.E. (1976) Report of the FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant Prod. Prot. Ser., No. 5.

Finney, D.J. (1971) Probit analysis, 3rd edition. Cambridge University Press, London.

Liang, Q. (994) Meeting with the challenge of the insect pest resistance to phosphine. *Grain Storage*, **1**, 1-8 (in Chinese).

Liang, Q. (1988) The survey on the stored grain insects resistance to phosphine in Guangdong province and the management strategy. Proc. 3rd Academy of China Grain and Oils. 220-225 (in Chinese).

Mills, K.A. (1983) Resistance to the fumigant hydrogen phosphide in some stored-product species associated with repeated inadequate treatments. *Mitteilungen der Deutschen Gesellschaft für Allgemeine Angewandte Entomologie,* **4,** 98-101.

Mills, K.A. (1986) Phosphine dosages for the control of resistance strains of insects. GASGA Seminar on fumigation technology in developing countries, TDRI, Slough, 119-131.

Mills, K.A. and Athie, I. (1998) The development of a same-day test for the detection of resistance to phosphine in *Sitophilus oryzae* (L.) and *Oryzaephilus surinamensis* (L.) and findings on the genetics of the resistance related to a strategy to prevent its increase. In: *Proc. 7th Int. Working Conf. on Stored-product Protection,* (Edited by: Zuxun, J., Quan, L., Yongsheng, L., Xianchang, T. and Lianghua, G.), Beijing. Sichuan Publishing House of Science & Technology, Chengdu, China, **1,** 594-602.

Price, N.R. (1984) Active exclusion of phosphine as a mechanism of resistance in *Rhyzopertha dominica. J. stored Prod. Res.,* **20,** 163-168

Rajendran, S. (1994) Responses of phosphine-resistant strains of two stored-product insect pests to changing concentration of phosphine. *Pesticide Science,* **40,** 183-186.

Reichmuth, C. (1991) A quick test to determine phosphine resistance in stored products research. *GASGA Newsletter,* **15**, 14-15.

Taylor, R.W.D. (1989) Phosphine – a major grain fumigant at risk. *International Pest Control,* **1**, 10-14.

Wang, D. and Cao, Y. (1999) *Technology of phosphine fumigation*. Publishing Company of Chengdu University of Technology, p 78, (in Chinese).

Waterford, C.J. and Winks, R.G. (1994) Correlation between phosphine resistance and narcotic response in *Tribolium castaneum* (Herbst). In: *Proc. 6th Int. Working Conf. on Stored-Product Protection,* (Edited by Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R.) Canberra, Australia, 17-23 April 1994, CAB International, Wallingford, Oxon, UK, **1,** 221-225.

Winks, R.G. (1985) The toxicity of phosphine to adults of *Tribolium castaneum* (Herbst): Phosphine induced narcosis. *J. stored Prod. Res.*, **21**, 25-29.

Zettler, J.L. (1990) Phosphine resistance in stored product insect in the United States. In: *Proc. 5th Int. Working Conf. on Stored-product Protection,* (Edited by Fleurat-Lessard, F. and Ducom, P.), Bordeaux, France, 1990, Imprimerie Médocaine, Blanquefort Cedex, France, 1075-1081.