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How to Effectively Control Phosphine-resistance Development in Stored Grain Insects by Integrated Pest Management

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Abstract: The paper elucidated how to effectively control phosphine-resistance development in stored grain insects by integrated pest management. This is achieved by detecting phosphine-resistance in major stored grain insects in Fujian province, comparing with investigation results of phosphine-resistance in stored grain insects in the same ecological area in recent years, and taking into account insect pest control experience of grass-root grain storage units in vertical administration system of State Grain Reserves. This offered some valuable information for the development of integrated pest management in grass-root grain storage units.

Key words: stored grain insects, phosphine, resistance, integrated pest management

1 Introduction

The insect resistance to insecticide inevitably results from the continuous use of pesticides whose selective action might induce gene mutation, bringing about insect resistance and pesticides gradually become ineffective. As a major fumigant, PH_3 has been extensively used worldwide for over 60 years, and its excellent fumigation effect has been absolutely affirmed. However, many stored grain insects have produced serious resistance due to the objective factors, and its unscientific and irrational use during its long application period. According to the investigation of grain storage workers in China, the major stored grain insects, such as *Sitophilus zeamais* Motschulsky, *Rhyzopertha dominica* (Fabricius), *Tribolium castaneum* (Herbst) and *Cryptolestes ferrugineus* (Stephens), etc., have produced strong resistance to phosphine, furthermore, increasing with time, in southwest China, central China and south China where stored grain insects severely occur. In the 1980s, PH_3 resistance coefficient of an *R. dominica* strain in Guangdong province unexpectedly reached 1160 times. The enhanced PH_3 resistance led to incomplete fumigation effect, and even no effect. About 50% stored grain requires two PH_3 fumigations, which is very common in high temperature and humidity areas in China. A global investigation from 1972 to 1973 showed that about 10% tested insects collected from 40% countries produced PH_3 resistance, which has been found in Australia, Bangladesh, U. S. A., India, east Asia, Africa and America. Therefore, the increasing insect resistance has become a global issue, augmenting the control cost and threatening the continuous PH_3 use.

In order to understand the major grain insects phosphine resistance in Fujian, supervise and offer valuable information for the future stored grain insects control, the stored grain insects resistance degree to phosphine in Fujian province was detected.

2 Materials and Methods

2.1 Insects and Their Incidental Grain

Test insects were collected from deputy depots and depots in Fujian branch company, directly under China Grain Reserves Corporation (in the remainder of this paper referred to as Fujian branch company). Several stored grain insects, which are difficult to control in common management, were selected based on practical experience of grass – root grain storage units. Three species: *Cryptolestes ferrugineus* (Stephens) *Sitophilus zeamais* Motschulsky, and *Rhyzopertha dominica* (Fabricius) were selected, including 6 samples (Table 1). The grain collected with the insects has been stored for 2 – 4 years in Fujian. The grains were produced in both local field and other provinces of China and from foreign field. The tested insect have propagated for several generations, and basically adapted to the stored grain environment in Fujian province.

1. *Quanzhou Depot State Grain Reserves*, *Quanzhou* 362113)
2. *Fujian Branch of China Grain Reserves Corporation*, *Fuzhou* 350001)
3. *Putian Depot State Grain Reserves*, *Putian* 351158)
4. *Fuzhou Depot State Grain Reserves*, *Fuzhou* 350101)

Table 1. The basic information of the tested insects

No.	Insect	Collecting place	Incidental grain kind	Grain producing time	Grain producing place
1	*C. ferrugineus*	Xiamen grain purchasing and storage company	Wheat	2004	Henan
2	*C. ferrugineus*	Pucheng county grain trade company	late indica rice	2006	Fujian
3	*R. dominica*	Quanzhou Depot Directly Under Central Grain Reserves	late indica rice	2004	Anhui
4	*R. dominica*	Pucheng county grain trade company	late indica rice	2004	Fujian
5	*S. zeamais*	Putian Depot Directly Under Central Grain Reserves	Wheat	2004	Import
6	*S. zeamais*	Putian Depot Directly Under Central Grain Reserves	Corn	2005	Liaoning

2.2 Measurement of the Resistance to Phosphine

The standard FAO recommended method[7] was used to measure resistance to phosphine. Phosphine was prepared from reaction of zinc phosphide and 10% H_2SO_4 solution, and the insects were exposed for 20 h and 72 h.

The method to measure knockdown resistance[4]: 10 one-week adults were put into a fumigation flask with a known volume. Phosphine was injected into the flask corresponding to dosage of 2.0 mg/L, then the response of tested insects was observed. The adult was confirmed as "knocked down" by phosphine when it exhibited convulsion symptoms. The adult knockdown time was recorded. Each strain was tested 3 times.

The results of exposing for 20h and 72h were analysed by Probit analysis, and the results were analysed by DPS software.

3 Results

3.1 Measurement of the Phosphine Resistance

The results of the phosphine resistance measurement are provided in Table 2, which showed that the phosphine resistance of *C. ferrugineus* in Fujian province significantly increased as compared with historical resistance data in the same ecological area. In particular, the tested insects collected from Xiamen grain purchasing and storage company reached 449 times, which increased 2.8 times compared to 160 times of *C. ferrugineus* in Jianyang, Fujian province detected by Yan Xiao-ping in 2004[3]. The results accorded with the great difficulty of *C. ferrugineus* control in grass – root grain storage units at the present time. The phosphine resistance level of *R. dominica* and *S. zeamais* was slight low, exhibiting susceptible or moderate, which basically equaled with the historical record

Table 2. The results of the phosphine resistance of stored grain insects in Fujian branch company

No.	Insect	Regressione quation	LD 50.00	Range value	Resistance coefficient	Grain producing place
1	*C. ferrugineus*	Y = 2.04X + 3.58	4.94	4.16 – 6.12	449.14	Henan
2	*C. ferrugineus*	Y = 2.31X + 3.64	3.86	2.90 – 17.48	350.51	Fujian
3	*R. dominica*	Y = 0.70 X + 5.95	0.043	0.0007 – 0.12	5.39	Anhui
4	*R. dominica*	Y = 0.52X + 5.30	0.26	0.016 – 0.50	32.64	Fujian
5	*S. zeamais*	Y = 0.65X + 6.55	0.004	0.000 – 0.014	0.6	Import
6	*S. zeamais*	Y = 0.95X + 5.84	0.12983	0.00004 – 0.39	18.55	Liaoning

The phosphine resistance of the tested insect species were different. *C. ferrugineus* was the most resistant among stored grain insects in Fujian, and its resistance coefficient reached 449 times, the phosphine resistance of *R. dominica* and *S. zeamais* was comparatively low. As far as the origin of the gain was concerned, the phosphine resistance level of the stored grain insects in other provincial and imported grain was lower than that from local grain. For the incidental grain kind, the phosphine resistance level of the stored grain insects in paddy rice was stronger than that in corn, wheat and other grains. For the management condition, the phosphine resistance level of the stored grain insects in depots directly under Central Grain Reserves was lower than that in local deputy depots.

3.2 The Reasons about Origin of the Phosphine Resistance

3.2.1 Geographical environment factor Since the climatic condition of high temperature and humidity offered favorable environment for occurrence and reproduction of the stored grain insects, in addition to long-term pesticide selection, the phosphine resistance level of a portion of insects in Fujian was higher that in other provinces. Therefore, the phosphine resistance level of the stored grain insects in grain from other provinces was lower than that in local grain. Furthermore, because the *R. dominica*, *S. zeamais* and other insect species diffused into Fujian in recent years, their adaptability to climatic environment were slight weak, and their resistance level was instead lower than that of the same ecological area, and easy to control.

3.2.2 Storage equipment factor. The storage equipment of local deputy storage corporations was very old, and its gas-tightness condition was poor. The lack of gas-tight structures resulted in a great deal of fumigant leaking out before evenly distributing deep within the grain mass after fumigation application, unevenly distributing among grain mass, or not keeping effective fumigant gas concentration for enough time, and so on, then incomplete fumigation effect against insects among every portion of the grain mass.

Sealing of the doors and windows of the warehouse before the fumigation did not satisfy the requirements for an effective fumigation.

3.2.3 Human factor During the past, insect pest control practices, the human factors, such as insect pest control conception and incorrect fumigation manipulation, etc., occasionally resulted in the fumigation failure, accidentally enhancing the phosphine resistance.

(1) Unreasonable fumigationSome grass-root grain storage units immediately implemented fumigation once insects appeared, even if grain temperature was less than 15℃. Under these conditions, the insects reproduce very slowly, fumigant could not adequately diffuse among the grain mass, and the fumigation effect is very poor at too low temperature.

(2) Unreasonably reduce dosage Some people think that low phosphine concentration is in favor of enhancing fumigation effect, meanwhile, keeping low phosphine concentration in grain mass for a long period could kill the existing insects and prevent insects occurring. However, they ignored that only appropriate high phosphine concentration could kill some resistant insects. Because of low dosage and poor gas-tightness, it is difficult to get the effective lethal concentration. Even if the effective lethal concentration is obtained, the CT value is too low, resulting in not killing the insects, instead inducing insect resistance.

(3) Unreasonably increase dosage Some people mistakenly thought that application dosage was too low when they could not obtain the expected fumigation effect using preventative fumigation method and dosage at some places. In order to kill the insects, they unreasonably increased dosage next time, resulting in hidden insects receiving over-high phosphine dosage or concentration. The insect could revive after implementing fumigation for a period of time, and not be 100% killed.

(4) Fumigation seal time was not enough In early phosphine application instruction, some made suggestions to seal for 3 to 5 days, later, seal for 7 to 10 days after fumigant application. The seal time in these suggestions is far from enough at present concept. Many factors, such as grain mass temperature, application dosage, insect pesticide tolerance, etc., can impact phosphine fumigation seal time required. Consequently, the fumigation seal time cannot regulate in one criterion.

(5) Lack of concentration detecting measures. A lot of phosphine fumigation were implemented according to fumigant dosage. The grain quantity, dosage and gross application amount were taken into account during the fumigation. Owing to lack of instruments to measure gas concentration, a series of issues, such as if the effective fumigation concentration quickly reached in the warehouse or grain mass after application, if there were existing fumigation dead zone or local low concentration, and if the effective fumigation concentration could be kept for enough time, etc., might inevitably bring unreasonable fumigation.

(6) It is very common that many depots directly implemented fumigation without investigating stored grain insects stage and resistance before fumigation. This only killed adults and

susceptible strains, then the other stage insects and resistant individuals survived, and the more resistant insects soon again occurred after fumigation.

(7) The single use of aluminium phosphide as a fumigant for a long time has induced insect resistance. In addition, most depots did not consider the effect of micro-airflow in grain mass and applied the fumigant at same place. This also is one reason responsible for insect resistance.

After the establishment of vertical administration system of State Grain Reserves in 2000, the insect control concept has changed. The human factors noted above have basically disappeared during the course of insect control in depots directly under Central Grain Reserves now. This is one reason for the phosphine resistance level of the stored grain insects in depots directly under Central Grain Reserves lower that in local deputy depots.

4 Discussion

4.1 Coincident Degree Between Resistanceand Practical Management Difficulty

C. ferrugineus was the most resistant in view of the results, and the phosphine resistance of *R. dominica* and *S. zeamais* was not severe. The phosphine resistance of *R. dominica* and *S. zeamais* from other depots was comparatively weak except Shaowu depot with resistance coefficient 32.64 times of *R. dominica*. There is a huge difference from other provincial detecting results, but it is consistent with daily storage management practice in Fujian.

In the early of 1980s, *R. dominica* was seldom found in Fujian. At that time Fujian Province was self-sufficient for grain. After then, with the adjustment of agriculture plant structure, more and more grain was transferred into Fujian, along with *R. dominica*. Although the warehouse conditions were very poor, mortality of *R. dominica* was very high after fumigation, the common application dosage basically killed all of the insects. However, since the late 1980s, many depots have reported that *C. ferrugineus* could survive the fumigation. Because of poor warehouse condition and technique at that time, the issues could be resolved only by increasing the dosage, but it was not completely resolved. Because repeat fumigation screened *C. ferrugineus* resistance, the resistant strain continuously reproduced, and the resistance increased stage by stage.

The major stored grain insect resistance in Fujian is basically equal with national average level in China. But it also appeared some local characteristics, for instance, *R. dominica* showed low resistance, or even no resistance in Fujian, however prominent resistance in other provinces, particularly, Guangdong, Anhui, etc., locating southern high temperature and humidity areas. The phenomenon is very hard to understand. In our opinion, most paddy rice in Fujian was bought from Anhui and Jiangxi, and after the *R. dominica* came into Fujian along with the grain, most stored grain temperature due to low and quasi-low temperature grain storage implemented by Fujian branch company, Central Grain Reserves, was too low to be suitable to *R. dominica* development.

4.2 About Fumigation Management of Stored Grain Insects

Insecticide resistance results from continuous selection with insecticides. Insecticide kinds used in stored grain insects control are very limited, and fumigant kinds are even rarer. Therefore, insect resistance is unavoidable. However, as long as deeply understanding about insect resistance, taking scientific and rational control measures, the development of insect resistance can be delayed. The following major measures are summarized based on recent several years practical experience.

4.2.1 Execute integrated control Carefully execute the control principle "prevent first, integrated control", seriously improve storage condition, enhance the quality of stored grain, reinforce daily management, and prevent insect infestation. Timely, harmonious using various methods, to the best avoiding apply insecticides or not using insecticide, is the precondition of prevent insect resistance development.

Firstly, strictly check on the grain quality before storage, intentionally reduce the kinds and quantity of stored grain insects during the grain entering warehouses, prevent the outer insect entering warehouses, concentrate several extensively distributed important insects into some portion avoiding to diffuse into other warehouses with no or a few insects. Meanwhile, various manual or mechanical equipments can be used to clean out grain impurity and outer insects.

Secondly, change the insect reproduction condition. Try to deteriorate the living environment condition of stored grain insects, so as to prevent and restrain stored grain insects happening. During the grain entering warehouses, try to decrease grain impurity and moisture, some depots with good storage equipments can implement low and quasi-low temperature grain storage.

Cleanliness and sanitation control. Thoroughly and frequently maintain cleanliness and sanitation inside and outside the warehouses, carefully disinfect empty warehouses before putting the grain into the warehouses, and then apply defending line avoiding insects diffusing.

Strengthen behavioural control. Behavioural control can be taken when the insect density is lower than common insect grain standard. For example, moths can be controlled by sealing the grain surface with plastic film or other materials from late winter to early spring. All kinds of insects can be trapped and killed based on their up-climbing, chemical tropism, photokinesis, etc., generally, including probe trap and kill, lamplight trap and kill, noxious bait trap and kill, etc., subsequently, decreasing insect density and insecticide application times.

4.2.2 Enhance control quality Improving insecticide application environment, selecting rational insecticide application method, dosage and formulation, trying one fumigation to completely kill all kinds of insects, development stages, leaving no living insects, are the foundation of preventing insect resistance. The action characteristics of phosphine should be fully understood before fumigation, taking scientific, rational and effective application techniques, avoiding non-effective fumigation due to unreasonably increasing dosage, executing fumigation under poor sealing condition, and over-high concentration at the beginning of the fumigation and too low concentration towards the end of the fumigation. For fumigant selection strategy, phosphine should be protected as a priority and used, not being substituted by other fumigants. For phosphine application, we should develop scientific application techniques; implement fumigation according to actual phosphine concentration and fumigation effect, not simply based on dosage and experience.

Keeping rational phosphine concentration is extremely important. The effective phosphine concentration differs depending on warehouse, insect species, insect stage, resistance degree in practical fumigation. Generally speaking, in order to get ideal fumigation effect, phosphine concentration must be carefully detected, and ensure keeping effective phosphine concentration in each portion of the grain mass, which is very important for avoiding fumigation failure and inducing insect resistance. In other words, phosphine fumigation should be implemented based on concentration and fumigation effect. The reference parameters, 100 – 350mL/m^3 fumigation concentration and sealing over 14 – 28 days, were recommended in LS/T1201 – 2002 *Fumigation Regulation of Phosphine Recirculation* issued in 2002 according to different insects condition and grain temperature, etc.. In recent years, application practices of grass-root grain storage units, the reference parameters can basically successfully guide practical fumigation practices. But for some especially high resistant or endurance insects, such as *C. ferrugineus*, the practical fumigation concentration should be higher, and the sealing time should extend for 30 – 45 days.

Keeping phosphine concentration effective and even distribution is closely relating to application techniques, methods, instruments. The application techniques have been helpfully explored in China, and the more practical techniques include slow-releasing application, phosphine generator application outside warehouse, cylinder fumigant containing $PH_3 : CO_2$ mixture application, and so on. Recirculation technique can effectively accelerate phosphine even and quick distribution in huge and deep grain mass.

4.2.3 Shift or change of insecticides According to current insect resistance development condition, selecting different kinds insecticides, and designedly periodically shifting insecticides is very important to control resistant insects, especially selecting new insecticides and intentionally shifting insecticides to avoid cross-resistance happening. It was reported that the adults of phosphine resistant *R. dominica* and *Sitophilus oryzae* strains did not produce cross-resistance to malathion, deltamethrin, etc.. thus, grain protectants can be used to control phosphine resistant insects. In addition, synergist also can be used to enhance toxicity. The common synergist is PBO (piperonyl butoxide). Pyrethroid mixing with synergist can increase control effect。

Furthermore, mixing different insecticides can enhance controlling effect. Different mechanism insecticides being used together can not only successfully overcome insect resistance, but also enhance controlling effect, reduce dosage and control cost. Zhengzhou Grain College validated that CO_2 could obviously enhance PH_3 toxicity to *Tyrophagus putrescentiae* by mixing different proportion PH_3 and CO_2 to control *T. putrescentiae*.

5 Prospect

Chemical control is inevitable to generate resistance, as well as grain and environment contamination. With the increasing concern about green food, non-chemical control are more and more highlighted. Therefore, most experts and enterprises are positively researching non – chemical control, such as controlled temperature storage, controlled atmosphere storage, biological control, etc. , in China, and this is a necessary development orientation for the future. The researchers have got some achievement, for example, China Grain Reserves Corporation implemented controlled atmosphere storage experiments in tens of depots in 2007. Ideal fumigation effect was realized by injecting CO_2 or nitrogen, integrating sealing and low oxygen storage, storage time was prolonged, and stored grain quality was maintained. Moreover, a portion of depots of China Grain Reserves system plan to use cold source from ambient liquefied natural gas storage stations to realize quasi – low temperature grain storage, so as to greatly reduce insect control cost. Most experts and scholars are researching plant materials and natural enemies to control insects in China. For example, garlic volatiles possesses strong inhibition or contact action against *R. dominica*, *Tribolium castaneum*, cereal psocid. Aloe extract had strong inhibition against *R. dominica* population, etc.. It can be reliably predicted that these non – chemical control methods must be popularized in practical production, bring about a revolution of insect control methods, arouse far – reaching effect on insect control.

References

[1] Wang Dianxuan, Yuan Kai, Wu Zeng-qiang, et al.. Comparison to relative phosphine resistance of *Cryptolestes ferrugineus* and other species of insect pests in stored product. Journal of zhengzhou institute of technology, 2004, 25(1): 4 – 8

[2] Cao yang. Survey of the resistance of stored-grain pests to phosphine in china. Journal of henan university of technology (natural science edition), 2006, 27(1): 1 – 6

[3] Yan Xiao-ping, Li Wanwu, Liu Zuowei, et al.. Investigation of phosphine-resistance in major stored-grain pests. Grain Storage, 2004, 32(4): 17 – 19

[4] Cao yang, Liu Mei, Zhong Yanchang. Measurement of the resistance to phosphine in eleven strains of five species of stored grain insects. Grain Storage, 2003, 32(2): 9 – 11

[5] Ma Ming-jun. Reasons and countermeasures of the resistance to phosphine of stored grain insects. Grain Knowledge, 2001. 15(5): 30 – 33

[6] Wang Dian-xuan, Bian Ke. Development trend of fumigants used in stored grain. Grain Storage, 2004, 32(5): 3 – 7

[7] Anonymous. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative method for adults of some major pest species of stored cereal, with methyl bromide and phosphine. FAO Method №16. FAO Plant Protection Bulletin. 1975, 23: 12 – 26