The major achievements of grain storage in P. R. China

Wang J.-J.*#1, Deng, Y.-X.[1], Dou, W.[1], Yang, Z.-L.[2], Jiang, T.-K.[2]
[1] Chongqing Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection, Southwest University, Chongqing 400715, P. R. China
[2] Tongliang State Grain Reserve, Chongqing 402560, P. R. China

* Corresponding author
# Presenting author

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Abstract

During the past several decades, China has made great progress in grain storage research, which greatly improved and promoted grain storage techniques and facilities. Especially in the 1990s, modern grain storage depots were constructed, in which four new technologies of grain inspection automation, machinery aeration, grain cooling, and phosphine recirculating fumigation were popularized. The main technologies to phase out methyl bromide in national depots are under-film phosphine recirculation fumigation and mixing fumigation of phosphine and carbon dioxide. In grain storage scientific research, research programs from applied to molecular fields have been implemented in institutes and universities. The 4th national survey of stored grain insects showed 270 species including 44 natural enemies were recorded. Among these stored grain insect pests the psocid, *Liposcelis bostrychophila* is generally more prevalent in stored grain with high moisture content. Hence, bionomics, ecologies, control measures, and molecular biology have being conducted since 1990s, and many achievements have been obtained. Efficacy of new grain protectants such as diatomaceous earth and spinosad against the main stored grain insects were evaluated. Researches on effective concentration of phosphine, application technique, recirculation fumigation, combination phosphine with carbon dioxide, and phosphine resistance mechanism were executed. Other fumigants tested included ethyl formate, carbon disulfide, and sulfuryl fluoride against stored grain insects were evaluated, or reevaluated. The fumigation activities and mechanisms of ethyl formate to the main stored product insects were systemically studied and the results showed ethyl formate controlled Sitophilus oryzae, *Tribolium castaneum*, *Rhyzopertha dominica* and *Liposcelis bostrychophila* effectively in a very short time, and the fumigation efficacy at relatively low temperature was better than that at relatively higher temperature. With the rapid progress of technology and scientific research, the objectives of high quality, high nutrition, high benefit, low waste, low pollution, and low cost in grain storage will be achieved, even during prolonged storage.

Keywords: Review, Grain storage, Insect pest control, Fumigants, Psocids

1. A retrospect of grain storage in China

Chinese grain storage technology has a long history. Research showed that Chinese ancestors began to store grain and other seeds at least 7000 to 10000 years ago (Li and Jin, 1999; Zhu, 2008). Fence-style depots were used to aerate and store grain in southern China 7000 years ago and underground vaults were adopted to store grain in Central China 5000 years ago. Plant-source medicines were utilized to control stored grain insect pests about 2000 years ago. China was one of the oldest cradles of low temperature, controlled atmosphere and fumigation technology (Zhu, 2008). However, few fumigants for grain storage had been used in China before the People's Republic of China was founded in 1949. Most of them were still in the process of experimentation. Since the 1950s, fumigants such as chloropicrin, carbon, bisulfide, methyl bromide, aluminum phosphide, calcium phosphide, cyanhydric acid, dichloroethane and dichlorvos have been put into production and practice one after the other (Wang and Bian, 2004).

During the past 60 years, China has made great progress in grain storage research, which highly improved and promoted the grain storage technique and facilities. The Chinese government has been paying attention to the development of grain storage science and technology. In the middle of 1950s, institutions for grain science and research were set up. In the middle of the 1960s, the Chinese Research Institute of Grain Storage was established and the grain bureaus of various provinces, cities, and autonomous regions also began to set up grain research organizations. Since the 1970s, the research has been made more thorough, especially the 'modern grain storage technique', which was listed in the key science and technique programs for the 6th to 11th ‘five-year plans’, and gained great achievements (Li et al., 1999). In the 1990s, China constructed some modern grain storage depots, which featured large-scale grain bulks, high-level grain layers. In these depots, China has popularized four new technologies such as grain inspection automation, machinery aeration, grain cooling, and phosphine recirculation fumigation.

2. Four new technologies and ecological grain storage and environmentally friendly grain storage

Since 1998, with the loans from the World Bank and China’s national debt, some modern grain storage depots, which included large warehouse, squat silos and vertical silos, have been constructed. Each single depot capacity ranged from 5000 to 10,000 tonnes. These depots combined modern grain storage technology to popularize four new technologies which were grain inspection automation, machinery aeration, grain cooling, and phosphine recirculation fumigation. The objectives of these four new technologies were to adjust grain physical and chemical properties, to decrease grain temperature, to control mold and insect pest infestations. These newly-constructed depots were demanded to implement “Technical Regulations for Grain and Oil Seed Storage”, “Technical Orders for Grain Electronic Monitoring and Analysis System”, “Technical Orders for Grain Mechanical Areation”, and “Technical Orders for Grain Storage in Large Warehouses”. The four new technologies have made Chinese grain storage occupy a forefront place in the world (Zhu, 2008).

Ecological grain storage means fully using and controlling the ecological conditions which are favorable to stored grain quality, such as low temperature, low oxygen, therefore, ensuring safe grain storage. Environmentally friendly grain storage means that technologies leave no residues on stored grain. The two concepts are consistent with each other (Zhu, 2008). The theoretic principle for environmentally friendly grain storage is stored grain ecology, especially the stored grain ecosystem. Integrated pest management (IPM), which focuses on controlling ecological factors in a stored grain ecosystem, is the fundamental technology of environmentally friendly grain storage (Jiang, 2002). In China, the major technology for ecological environmentally friendly grain storage is low temperature storage combined with controlled atmosphere storage in the suitable climatic zones.

Low temperature grain storage means that average temperature of a grain bulk in the depot is below 15ºC and the highest temperature is not over 20°C in China, whereas quasi-low temperature storage means that the average temperature of grain bulk is below 20ºC with highest temperature not over 25ºC. The major methods to obtain low temperature and quasi-low temperature are natural low temperature, machinery aeration, and grain cooling. Controlled atmosphere technologies include natural low-oxygen, application with deoxidizers, double-low and three-low storage, controlled atmosphere with carbon dioxide and nitrogen enriching, bin sealing materials and technologies. China has made great progress in controlled atmosphere grain storage. In 2000, the Sichuan Mianyang CO2 controlled atmosphere grain depot was completed. Since then, some other CA grain depots have been constructed (Zhu, 2008).

3. Methyl bromide alternatives

Due to destruction of the ozone layer in the atmospheric stratosphere, and an overall negative effect on living conditions for all mankind on Earth, methyl bromide (MB) has been labeled an ozone deleting substance and is restricted or banned for application by the United Nations Environment Program. The Chinese Government attached great importance to methyl bromide substitute technology research. The Chinese government approved the Copenhagen Amendment of the Montreal Protocol. Since December 31, 2006, MB has not been allowed as a fumigant by any grain depots in the Chinese grain storage industries. Since January 2007, MB was completely prohibited in Chinese grain storage industries, in order to make proper contribution for carrying out the amendment of the Montreal Protocol completely in China. By the year 2015, MB should be phased-out completely except for use in quarantine fumigation. The substitute technology of the industry has been confirmed. The situation of methyl bromide application in grain industry has been grasped. For stored grain protection, tarped phosphine recirculation fumigation and phosphine and carbon dioxide mixing fumigation were confirmed as the main alternative technology to phase out methyl bromide (Liang, 2005; Li et al., 2007).

4. Stored-grain insect fauna and Chinese grain storage ecological zone

Four national surveys of stored-grain insects were conducted from 1955 to 2005. The 1st national survey started in 1955 and ended in 1958. One hundred and nine species of insect pests and 2 species of natural enemies were found. Between 1974 and 1975, the 2nd survey was implemented and 126 insect pest species and 6 natural enemy species were found. Between 1980 and 1982, the 3rd survey found 161 insect pest species and 10 natural enemies. In the 4th survey, 270 species of stored grain insects (including 226 species of insect pests and 44 species of natural enemies) were recorded (Yan et al., 2008). In the conventional two-low (low oxygen and low pesticide storage environments) national depots of Chongqing municipality and Sichuan Province, the investigations showed that the mite Tyrophagus putrescentiae (Schrank) and the psocid Liposcelis bostrychophila Badonnel were the two dominant species (Deng et al., 1995). By means of GJ89 type of probe trap, investigation and analysis of community composition and structure of stored grain insects in newly-constructed large warehouses, which were equipped four new grain storage technologies, were carried out in Beibei State Grain Reserve Depot, Chongqing. The results showed the main pests were *T. putrescentiae*, *L. bostrychophila*, *Cryptolestes ferrugineus* (Stephens), and *Sitophilus zeamais* Motschulsky in large warehouse and the dominant species was *L. bostrychophila*. The occurrence numbers of the four kinds of stored grain insect pests were the most at 0-50 cm grain depth, but a few were common at 100-150 cm grain depth. The four indexes of population richness, dominance, diversity and evenness were higher at 0-50 cm deep than the other depth. The occurrence of stored grain insects at horizontal levels had no obvious difference (Cui et al., 2006).

During the national survey of stored grain insects, bionomics and control for the main stored-grain insect pests including *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), *Tribolium confusum* Jaquelin du Val, *Sitotroga cerealella* (Olivier) were researched. Southwest University (the former Southwest Agricultural University) in Chongqing also did a lot of surveys on stored grain mites. Especially for stored food mites, systematic investigations were conducted and 79 species of food mites, including 18 new species and 21 species of new Chinese records, were found. The research on ecology and control measures for T. putrescentiae showed essential oils combined with controlled atmosphere could obtain satisfactory control (Li et al., 2009). Chinese scientists also summarized a stored grain ecosystem theory to guide grain storage and depot construction. It included: reasonable partition of Chinese grain storage ecological zones; reasonable depot type selection and design in different zones, grain depot equipment in different depot types and zones, rational configuration of machinery and special safety-ensuring grain storage equipment for different depot types and zones; evaluation indices and systems on safe grain storage technology (Zhu, 2008; Li et al., 2009).

5. Psocids

In the last two decades, a series of papers about psocids have been published about their bionomics (Dong et al., 2007; Jiang et al., 2008; Wang et al., 2009), ecology (Wang et al., 2004), control measures (Wang et al., 2001), and molecular biology (Wang et al., 2006; Dong et al., 2007; Jiang et al., 2008; Tang et al., 2009) by Key Laboratory of Entomology and Pest Control of Chongqing Municipality (Southwest University, China). The main differences between *L. bostrychophila* and *Liposcelis entomophila* (Enderlein) about biochemical and toxicological characteristics of detoxification, protection, target enzymes and energy sources were clarified (Wang et al., 2004; Cheng et al., 2004; 2007). The activity of carboxylesterase (CarE) extracted from *L. entomophila* was significantly higher than that from *L. bostrychophila*, while for acid phosphatase (ACP), alkaline phosphatase (ALP) and glutathione S-transferases (GSTs) (Cheng et al., 2007), *L. bostrychophila* had more activities. To inhibition by dichlorvos, acetylcholinesterase (AChE) and CarE played the most important roles, followed by GSTs or superoxide dismutase (SOD) (Cheng et al., 2007). Besides, on the basis of GSTs (Dou et al., 2006, 2009) and AChE (Xiao et al., unpublished) purification of psocids (*L. bostrychophila*, *L. entomophila*, *Liposcelis paeta* Pearman) through the affinity chromatography method, the biochemical and toxicological characterizations of purified enzymes from different resistant strains and field populations were systematically analyzed. Compared to the susceptible strain, the specific activities of GSTs in resistant strains were significantly higher. The comparison analysis of in vitro inhibition of insecticides revealed that compared to the counterparts in resistant strains GSTs from susceptible strain were most sensitive and sensitivity difference to inhibitors existed for GSTs in resistant strains.

At molecular levels, gene cloning of detoxification and target enzymes (such as P450, AChE, CarE, etc.), further mRNA expression level and heterogeneous expression of functional genes were carried out, mainly from *L. bostrychophila*, *L. entomophila*, *Liposcelis decolor* (Pearman), and *L. paeta* (Jiang et al., 2008; Tang et al., 2009). To date, two full length cDNAs encoding AChE were all cloned from *L. bostrychophila*, *L. entomophila*, *L. decolor*, respectively, by the methods of RT-PCR and RACE. The mRNA expression levels of two ace genes from *L. bostrychophila* in different strains, development stages, and insecticide treatments were studied using Real Time PCR. The results showed that the expression levels of two ace genes in resistant strains were significantly higher than those of a susceptible strain. After treated by dichlorvos or phosphine, the expression levels of two ace genes were all significantly increased over the control. The highest expression level of two ace genes was detected at the second stadium and the lowest was at the first stadium and adult stage. The Real Time PCR determination of ace genes from other psocids has also been conducted. Furthermore, two full length cDNA encoding CarE were cloned from L. bostrychophila by the methods of RT-PCR and RACE, named Lb est1 (GenBank Accession No.: EU854151) and Lb est2 (GenBank Accession No.: EU854152). The followed Real Time PCR analysis showed that the expression level of Lb est2 in dichlorvos and phosphine strains were 1.91 and 1.42 fold- higher than that of susceptible strain, respectively. For P450, a number of genes from CYP4 and CYP6 families were cloned and their mRNA expression levels were studied by real time PCR among different developmental stages (strains or treatments by insecticides). Meanwhile, some housekeeping genes were also cloned gradually from different psocids species. These results not only fastened the acquaintance of psocid molecular toxicology and enriched the content of evolution and genetic variation, but also contributed to development of a molecular diagnostic technique for psocid resistance in the field and paved the way for designing new insecticides and developing new strategies for pest management.

6. Grain protectants

Grain protectants are applied either as a liquid or a dust to specific areas or stored grain to suppress insect populations for a period of time ranging from months to a year or more. Grain protectants have been thoroughly researched since 1950s. During 1970s and 1980s, some protectants including malathion, fenitrothoin, pirimiphos-methyl, deltamethrin and mixture of organophosphorous with deltamethrin were studied as grain protectants. In China, grain protectants which are being used at present include: malathion, fenitrothion, deltamethrin, piperonyl butoxide, mixture of malathion and deltamethrin, mixture of fenitrothion and deltamethrin, and pirimiphos-methyl.

Spinosad is a commercial biological insecticide produced by fermentation culture of actinomycete Saccharopolyspora spinosa. To evaluate the effect of spinosad in controlling stored grain pests, the touch effects of 2.5% spinosad were tested in the laboratory on three main stored grain insects *L. entomophila*, *Oryzaephilus surinamensis* (L.), and *S. oryzae*. Results indicated that spinosad is effective in pest control for the three insects, more effective for *L. entomophila* than the other two insects (Cao et al., 2007).

7. Fumigants

Fumigants are toxic gases which penetrate into stored grain. Maintaining an adequate concentration of a fumigant for enough time kills most insect pests. The most popular fumigant in China is phosphine (PH3). A lot of research has been done since the 1960s. It included: PH3 efficiency, factors affecting PH3 efficacy (pest species, pest developmental stages, temperatures, grain absorbability to PH3, warehouse sealing condition, and airflow in grain bulks), gas diffusion, PH3 toxicity to human being, mixed fumigation with CO2, operational methods of different PH3 fumigation techniques (PH3 + CA fumigation, airflow fumigation, intermittent fumigation and slow releasing fumigation), dispensing apparatus, and combustion condition and prevention. Other fumigants involved were ethyl formate, carbon disulfide, carbonyl oxysulfide, ethylene oxide, and sulfuryl fluoride against stored grain insects and they were tested, evaluated, or reevaluated.

Since the 1970s, sulfuryl fluoride has been widely researched by Chinese scholars (Xu et al., 2006) and was registered for fumigating such materials as wood, official files, books, embankments and buildings, and was already taken into application in more ranges of buildings and quarantine ministries. Due to the notable pharmacodynamic action and excellent efficacy against stored products insect pests such as *Trogoderma granarium* Everts, *S. oryzae*, *S. zeamais*, *Tribolium castaneum* (Herbst), *Sitophilus granarius* (L.), *Callosobruchus chinensis* (L.), and *Lasioderma serricorne* (F.), temporary registration certification has been approved to take into practice in grain (Li et al., 2008). Ethyl formate (EtF) is a promising and environmental friendly fumigant, which was registered as a dry fruit fumigant in 2002 in Australia. In China, the fumigation activities and mechanisms of ethyl formate to main stored product insects were systemically studied and the results showed that ethyl formate controlled *S. oryzae*, *T. castaneum*, *R. dominica*, *L. bostrychophila* and *L. entomophila* effectively in a very short time period, and the fumigation efficacy at relatively lower temperature was better than that at relatively higher temperature (Tang et al., 2006 a b; Li et al., 2006; Deng et al., 2008).

8. Plant materials and essential oils

The bioactivities of plant materials and essential oils as fumigants, repellents, antifeedants, and insect growth inhibitory agents were researched because of pesticide residue and insect pest resistance to traditional pesticides. Fumigant toxicities of nine essential oils including *Citrus tangerina*, *C. limon*, *C. hongheensis*, *Litsea cubeba*, *Mentha spicata*, *Pinus tabulaeformis*, *Cinnamoum camphora*, *Melaleuca alternifolia* and *Eucalyptus globules* on adults of maize weevil, *S. zeamais* were investigated and the results proved that fumigation efficacies of the essential oils of *C. camphora*, *M. alternifolia*, *Citrus limon*, *M. spicata*, and *P. tabulaeformis* were better than those of the other four essential oils. Among the tested essential oils, *C. camphora* oil gave the best fumigation toxicity (Deng et al., 2004). The fumigant toxicities of the nine plant essential oils against adults of R. dominica showed that four plant essential oils of *E. globules*, *C. limonum*, *M. alternifolia* and *C. tangerina* resulted in higher adult mortality of *R. dominica* and *E. globules* oil obtained the best efficacy (Zhang et al., 2004). The population inhibition, repellency activity and contact toxicity of nine plant essential oils showed that *C. camphora* and *E. globules* were most effective (Zhang et al., 2004). The fumigation activity, contact toxicity, repellence activity, attractant activity and population inhibition effect of several prickly ash extracts against the adults of *S. zeamais*, *T. castaneum* and other stored-product pests were also systematically studied (Nie et al., 2006; 2007).

Although great progress has been achieved in China's grain storage, some new problems including farmer storage loss reduction, high moisture grain in Northern China, environmentally friendly storage and transportation need to be addressed. In the future, China should strengthen its scientific research and upgrade its grain storage technology. We believe the objectives of high quality, high nutrition, high benefit, low waste, low pollution, and low cost in grain storage will be achieved with the rapid progress of technology and scientific research in China.

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