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Behavioural responses of Callosobruchus maculatus to volatiles organic compounds found in the headspace of dried green pea seeds

Agnes Ndomo epse. Moualeu[1]*, Christian Ulrichs[2], Cornel Adler[3]

[1]University of Hannover, Germany
[2]Humboldt Universität Berlin, Germany
[3]Julius Kühn-Institut Berlin, Germany
*Presenting author: ndomonaf@yahoo.fr
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There is growing evidence that insects rely on chemical cues to locate food, hosts, predators, and potential mates. The pulse beetle Callosobruchus maculatus has been recognised for decades as the major post-harvest insect pest of legume seeds. In a previous study, we identified five volatile compounds in the headspace of dried green pea seeds as electroantennographically active in C. maculatus antennae: 1-pentanol, 1-octen-3-ol, (*E*)-2-octenal, nonanal and 3-carene. Volatile compounds are generally perceived by insects as blends, we hypothesized that *C. maculatus* might particularly show attraction to different mixtures of the aforementioned compounds. To test this we examined the behavioural response of *C. maculatus* towards volatile mixtures in a dual choice Y-tube olfactometer. The results showed that females were attracted to five mixtures while males were attracted only to two binary mixtures consisting exclusively of aldehydes. The other mixtures caused *C. maculatus* to move away. Further investigations with the attractive mixtures should be done in real storage conditions with the aim of developing a trap for the pulse beetle, *C. maculatus*.

Investigation on the Species and Distribution of Stored Grain Insects in Northwest China

Dandan Li*, Zhenya Mu, Daolin Guo, Xiaoping Yan, Qing Zhou

Chengdu Grain Storage Research Institute of SinoGrain, Building 32, No.239 Guangfu Road, Qingyang District, Chengdu P.R. China
*Corresponding author: dandanli@126.com
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Abstract

To understand the diversity of stored grain insects in northwest China, we have fulfilled insect collection in 56 grain storage enterprises, 60 grain, oil and feed processing plants and 65 farmers situated in 26 cities of five provinces (Shaanxi, Gansu, Qinghai, Ningxia and Xinjiang) in northwest China from 2016 to 2017. After systematical identification, totally 83 species of stored grain insects have been found in this investigation, belonging to five orders, namely Class Insecta Order Zygentoma, Order Coleoptera, Order Lepidoptera and Order Hymenoptera, as well as Class Arachnida Order Chelonethida, in which Order Coleoptera owns 74 species in 22 families, Order Lepidoptera owns six species in four families, Order Zygentoma and Order Hymenoptera own one species in one family respectively, and Class Arachnida Order Chelonethida has one species in one family. After the statistics of four insect investigations in northwest China during 1955-2017, this paper has analyzed the results of four insect investigations and the representative stored grain insects in northwest China.

Key Words: northwest China, stored grain insects, species, distribution, investigation

1. Introduction

Located in the hinterland of the Eurasian continent, the northwest region covers the first (high-cold and dry stored grain region), second (low temperature and dry stored grain region) and fourth

(medium-temperature and dry stored grain region) of China's seven stored grain ecological regions, and consists of Shaanxi, Gansu, Qinghai, Ningxia Hui Autonomous Region, and Xinjiang Uygur Autonomous Region. Wide area with scarce rainfall, the northwest region, as a traditional grain deficit area, has the most serious soil erosion, land drought and desertification problems in China. Main grains planted and stored in northwest region include wheat, corn, rice, highland barley, soybean, buckwheat, pea, naked oat, proso millet, flax and other small grains.

Since the founding of the people's Republic of China, there have been seven national insect investigations related to the grain system, in which Gansu, Ningxia, Xinjiang and other northwest provinces were not included in the first and second investigations (Qizong Chen, 1994; Xiaoping Yan, et al., 2008). Besides the national insect investigations, some provinces have carried out their own investigation independently. The coleoptera pest investigation in Shaanxi commercial warehouses carried out by He Jinyan, et al. in 1983-1984 discovered 30 species of insects in eight families, Coleoptera Order (Jinyan He, et al. , 1993), Gao Duping reported the main stored grain pests in Pingliang, Gansu, composed of 19 species in 10 families, three orders (Jinyan He, et al. , 1993), the stored grain pest investigation in Ningxia Hui Autonomous Region carried out by Zhu Desheng, et al. in 1983-1984 found 47 species of pests in 19 families, two orders (Desheng Zhu,1987); the stored grain pest investigation in Changji Hui Autonomous Prefecture, Xinjiang carried out by Li Mingshan, et al. in 1981 found 39 species of pests in 22 families, seven orders (Mingshan Li, et al.,1994); and the stored grain insect investigation in Tibet Autonomous Region carried out by Chen Qizong, et al. in 1987 found 73 species of stored product insects (Qizong Chen,1990) Over the past 20 years, the farming mode and storage environment in northwest region have changed greatly. Therefore, regular investigation and research on pest species, location, distribution area and object should be carried out for effective stored grain pest control. Considering 2015 Grain Public Welfare Industry Research Project and *Notice of State Administration of Grain on the Seventh National Stored Grain Insect and Mite Investigation* [GLBC (2016) No.95], under the great support of grain administrations and local grain bureaus of Shaanxi, Gansu, Qinghai, Ningxia and Xinjiang as well as relevant enterprises, an investigation on the species and distribution of stored grain insects has been carried out in grain storage enterprises, grain, oil and feed processing plants, farmers and other relevant places within three stored grain ecological regions, namely high-cold and dry stored grain region (the first region), low-temperature and dry stored grain region (the second region), and medium-temperature and dry stored grain region (the fourth region), for a better understanding of the stored grain insect diversity in the northwest region and pest control.

2. 2. Investigation Method and Scope

2.1 Sampling Site

Within the scope of five northwest provinces (Shaanxi, Gansu, Qinghai, Ningxia and Xinjiang), besides some representative cities selected in the light of each stored grain ecological region, some relevant enterprises in the east, west, south, north and middle of each province were also selected as sampling sites. Totally, 56 grain storage enterprises, 68 grain, oil and feed processing plants, and 65 farmers from 26 cities in the first, second and fourth stored grain ecological regions, participated in this investigation. (See Table 1)

Table 1 Number of Cities, Relevant Enterprises and Farmers in Five Provinces.

Stored Grain Ecological Region	Province	Number			
		City	Grain Storage Ent.	Processing Plant	Farmer
First: High-cold and Dry	Qinghai	4	7	7	4
Second: Low-temp. and Dry	Xinjiang	6	10	14	1
	Gansu (partial)	4	14	6	19
	Ningxia	3	3	8	1
Fourth: Med.-temp. and Dry	Gansu (partial)	3	12	5	15
	Shaanxi	6	10	20	25

2.2 Sampling Method

Field sampling and screening were adopted by this investigation, and the durations from July to September, both 2016 and 2017, were selected as its sampling time. Collected samples were preliminarily classified and processed on site with original information registration, such as host of pest, sampling time, sampling site, etc. After then, a series of follow-up processes were carried out at the laboratory, including further separation, processing, preliminary species naming, classification and preservation, as well as specimen preparation.

2.3 Insect Identification Method

In terms of traditional morphological characteristics of insects, the species of each sample was identified, named and then reviewed by an expert team composed of researcher Zhang Shengfang from China Academy of Inspection and Quarantine, Professor Bai Xuguang and Professor Zhou Yuxiang from Henan University of Technology, in case of any preliminary naming error.

3. 3. Investigation on Insect Species and Distribution

3.1 Catalogue of Stored Grain Insects of Five Provinces in Northwest China

Through this investigation on stored grain insects in the northwest region, 83 species of stored grain insects were identified, respectively belonging to 29 families in five orders, two classes (Class Insecta: 74 species in 22 families of Order Coleoptera, six species in four families of Order Lepidoptera, and one species in one family of Order Zygentoma and Order Hymenoptera respectively; and Class Arachnida: one species in one family of Order Chelonethida). There were 16 species of undetermined species, in which seven were natural enemies of stored grain pests.

3.2 Insect Distribution Difference in Different Grain Storage Environments

Main pests found in this insect investigation in northwest China totaled eight species, including *Sitophilus zeamais* (Motschulsky), *Sitophilus oryzae* (Linnaeus), *Rhyzopertha dominica* (Fabricius), *Tenebroides mauritanicus* (Linnaeus), *Bruchus rufimanus* (Boheman), *Araecerus fasciculatus* (Degeer), *Sitotroga cerealella* (Olivier) and *Plodia interpunctella* (Hübner). It is reported that *Sitophilus zeamais* (Motschulsky), *Sitophilus oryzae* (Linnaeus) and *Rhyzopertha dominica* (Fabricius) are the main wheat, corn and rice pests in many temperate and tropical countries [8, 9], while *Rhyzopertha dominica* (Fabricius) is more common in warm and dry wheat producing areas of China, Australia, India and Pakistan[10].

There exist obvious differences in species numbers collected in different storage environments. Grain, oil and feed processing plants usually have suitable temperature and humidity and difficulty in complete cleaning [11], especially the small flour and rice mills in rural area without any pest control measures, where more (72 in total) species of stored grain insects were found. As for grain storage enterprises, 50 species of stored grain insects were found, belonging to 24 families, four orders, in which 25 species were found in Sinograin depots, and 43 species were found in local grain depots. Relying on less stored grain types, standard management, regular fumigation and better storage conditions, the species number of pests founded in grain depots is less than that of processing plants. In recent years, few farmers store grains by themselves, and their grain storage environment is improved after the wide application of small steel barns, hence the occurrence of stored grain pests is reduced. As a result, only 26 species of stored grain insects were found in farmers' barns in northwest China. (See Table 2).

Table 2 Information of Stored Grain Insect Distribution in Different Environments.

Classification	Sinograin Depot	Local Grain Depot	Grain, Oil and Feed Processing Plant	Farmer
Order	3	4	5	4
Family	15	23	26	17

Species	25	43	72	26

3.3 Insect Distribution in Stored Grain Ecological Regions

The investigation on stored grain insects in northwest China involves three stored grain ecological regions, the first, second and fourth ones, covering five provinces. The species number of stored grain insects in each ecological area remains in a range between 46 and 60 (Tab. 3). Wang Dianxuan, et al. [12] discovered 16, 59, 34, 23 and 59 species of stored grain pests respectively in the flour mills of the third, fourth, fifth, sixth and seventh stored grain ecological regions. According to the overall data, the species numbers of stored grain pests in low-temperature and high-humidity stored grain region (the third region), medium-temperature and high-humidity stored grain region (the fifth region) and medium-temperature and low-humidity stored grain region (the sixth region) is less, which may be related to the limited numbers of sampling site, sampling point and sampling time.

Table 3 Species Numbers of Stored Grain Insects Collected in Different Stored Grain Ecological Regions (SGER).

Classification	High-cold and Dry Stored Grain Region (First)	Low-temp. and Dry Stored Grain Region (Second)	Medium-temp. and Dry Stored Grain Region (Fourth)
Order	4	5	4
Family	21	23	23
Species	53	60	46

4. Discussion

4.1 Analysis on Investigations on Stored Grain Insects in Northwest China

Totally, seven national investigations on the species and distribution of stored grain insects and stored product insects have been carried out within Chinese grain system, and because of partial overlapping, investigations in 1955, 1957 and 1956-1958 were recognized by this paper as an investigation, in which date related to investigation on stored grain insects in northwest China were sorted out in this paper. According to statistics, 199 species of stored grain insects were found in total, belonging to 40 families, eight orders, Class Insecta and Class Arachnida. Among then, Class Insecta: Order Coleoptera owns 174 species in 26 families, Order Lepidoptera owns 15 species in six families, Order Blattidae owns three species in two families, Order Hymenoptera owns two species in two families, Order Hemiptera, Thysanura, Diptera own one species in one family respectively; and Class Arachnida Order Chelonethida has two species in one family, comprehensively summarizing the stored grain insects in northwest China.

Zhao Yangchang, et al. carried out comprehensive investigation on pests in stored grain, oilseeds, livestock products, aquatic products, medicinal materials, archives, timber and other stored products in northwest China with an emphasis on stored grain pests in 1955-1960, discovering 113 species of store product insects in 33 families, six orders [13]. According to the data of the fourth national stored grain pest investigation organized by the Ministry of Commerce in 1974-1975 [14], the insect species distribution investigation group found 94 species of stored grain insects in 29 families, four orders in northwest China. The sixth national stored grain insect investigation organized by the State Grain Administration in 2004-2005 found 133 species of stored grain insects in 40 families, eight orders in the northwest region. Following the task stipulated in the 2015 Grain Public Welfare Industry Research Project – "Stored Grain Insect and Mite Region System Investigation and Pest Monitoring and Forecasting Technology Research" and the arrangement of the State Grain Administration's "Seventh National Stored Grain and Mite Investigation", a project team carried out investigation on stored grain insects in the northwest region from 2015 to 2017, indentifying and recording 83 species of stored grain insects in 29 families, five orders. According to previous investigation results, the species number of stored grain insects remained stable basically. However, due to the limitation of time, site and scope of these investigations, the species of stored

grain insects may not be comprehensive.

4.2 Representative Store Grain Insects in Northwest China

Among these four investigations in 1955-1960, 1974-1975, 2004-2005, and 2015-2017, 29 species of stored grain insects occurred in the northwest region in every investigation, basically covering the main stored grain pest species in China, namely *Cryptolestes ferrugineus* (Stephens), *Cryptolestes turcicus* (Grouvelle), *Tribolium castaneum* (Herbst), *Tenebroides mauritanicus* (Linnaeus), *Oryzaephilus surinamensis* (Linnaeus), *Ahasverus advena* (Waltl), *Stegobium paniceum* (Linnaeus), *Sitophilus granarius* (Linnaeus), *Bruchus pisorum* (Linnaeus), *Rhyzopertha dominica* (Fabricius), *Lyctus sinensis* (Lesne), *Ptinus japonicus* (Reitter), *Trogoderma variabile* (Ballion), *Cryptophilus integer* (Heer), *Carcinops pumilio* (Erichson), *Palorus ratzeburgi* (Wissmann), *Alphitophagus bifasciatus* (Say), *Alphitobius laevigatus* (Fabricius), *Tenebrio obscurus* (Fabricius), *Typhaea stercorea* (Linnaeus), *Migneauxia orientalis* (Reitter), *Thes bergrothi* (Reitter), *Holoparamecus ellipticus* (Wollaston), *Sitotroga cerealella* (Olivier), *Pyralis farinalis* (Linnaeus), *Ephestia cautella* (Walker), *Plodia interpunctella* (Hübner), *Tinea tugurialis* (Meyrick), and *Ctenolepisma villosa* Fabricius. Due to lack of data, poor test conditions, and other factors, difficulty in differentiating *Sitophilus zeamais* (Motschulsky) from *Sitophilus oryzae* (Linnaeus) may lead to an error in the 1955-1960 investigation. After combination of external genitalia anatomy and morphology was introduced into identification in 1975, differentiation between *Sitophilus zeamais* (Motschulsky) and *Sitophilus oryzae* (Linnaeus) and other allied species was finally achieved [1]. Therefore, all of the following three investigations in 1974-1975, 2004-2005 and 2015-2017 found *Sitophilus oryzae* (Linnaeus) in the northwest region, while *Sitophilus oryzae* (Linnaeus) found in the 1955-1960 investigation may be mistakenly identified as *Sitophilus zeamais* (Motschulsky).

The representative stored grain insects in the northwest region (incl. the first, second and fourth stored grain ecological regions) listed in the *Technical Specification for Grain and Oil Storage* (GB/T 29890-2013) total 13 species, namely *Sitophilus zeamais* (Motschulsky), *Sitotroga cerealella* (Olivier), *Plodia interpunctella* (Hübner), *Oryzaephilus surinamensis* (Linnaeus), *Tenebroides mauritanicus* (Linnaeus), *Tribolium castaneum* (Herbst), *Tribolium madens* (Charpentier), *Attagenus augustatus gobicola* (Frivaldszky), *Trogoderma variabile* (Ballion), *Niptus hololeucus* (Faldermann), *Gibbium psylloides* (Czenpinski), *Ptinus japonicus* (Reitter), and *Sitophilus granarius* (Linnaeus) (Xinjiang) [15]. In this investigation, *Sitophilus zeamais* (Motschulsky), *Sitotroga cerealella* (Olivier), *Plodia interpunctella* (Hübner), *Oryzaephilus surinamensis* (Linnaeus), *Tenebroides mauritanicus* (Linnaeus), *Tribolium castaneum* (Herbst), *Attagenus augustatus gobicola* (Frivaldszky), *Trogoderma variabile* (Ballion), *Niptus hololeucus* (Faldermann) and *Sitophilus granarius* (Linnaeus) were found, but *Tribolium madens* (Charpentier) *Gibbium psylloides* (Czenpinski), and *Ptinus japonicus* (Reitter) were not found. According to the newest *Stored Product Beetle*, *Tribolium madens* (Charpentier) *Gibbium psylloides* (Czenpinski), and *Ptinus japonicus* (Reitter) never occurs in China [16]. Therefore, the previous records may be naming errors. *Gibbium aequinoctiale* (Boieldieu) has been mistakenly recognized as the allied species of *Ptinus japonicus* (Reitter) in some domestic references, which coincides with the fact of discovery of *Gibbium aequinoctiale* (Boieldieu) rather than *Ptinus japonicus* (Reitter) in this investigation.

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