Olfactory response of *Cryptolestes turcicus* (Grouvelle) to six varieties of grain

Hao, G.H.1, Zeng, L.1, Zeng, L.*#[2]
[1]Laboratory of Insect Ecology, College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510640, P. R. China
[2] Guangdong Institute for Cereal Science Research, Guangzhou 510050, P. R. China

*Corresponding author, Email: zengling33@163.com
#Presenting author, Email: zengling33@163.com

DOI: xx.xxxx/xxx.2014.xxx.xxx.xxx

Abstract

Cryptolestes turcicus (Grouvelle), a worldwide distribution insect pest of stored grain, is one of the major insect pests that cause serious damage to stored grains in China. Grains containing volatile compounds stimulate insect feeding, spawning, and other activities. To compare olfactory response of *C. turcicus* to different grains, we used a Y-shaped olfactometer and a four-arm olfactometer to confirm the chemotactic response and selection preference of adults among six major grain varieties, i.e. wheat, brown rice, soy, oat, corn and mung bean. The results show that adults had strong chemotactic responses to all varieties of grain and there was no significant differences between males and females. Oat was the most attractive grain for the adults, with the selection rate as high as 41.1%, in contrast to mung bean, which was only 11.1%.

Keywords : *Cryptolestes turcicus*, olfactory response, chemotactic response, selection preference

1. Introduction

The smaller rusty grain beetle, *Cryptolestes turcicu* (Grouvelle), is a member of Laemophloeidae, Coleoptera. It has a worldwide distribution, consuming flour, broken rice, bran, and cereal powder (Bai, 2007; Upadhyay et al., 2011). Over the past decade, widespread appearance of this pest has occurred in high temperature and high humidity grain storage areas in China, increasing its pest status from a secondary pest to a main pest of stored grain. *C. turcicu* can damage grain and is difficult to control with traditional chemicals. Thus, it represents a serious threat to grain storage security. Suitable alternatives and environment-friendly control methods are needed immediately.

Grains containing volatile compounds stimulate insect feeding, reproduction, and other activities. Willis and Roth (1950) reported that wheat flour volatiles were more attractive to adults of red flour beetle *Tribolium castaneum* (Herbst) and this work was the first to confirm that grain beetles can locate grain resources via olfactory response. Bekon (1988) studied chemotactic responses of red flour beetle to wheat, rice, maize, sorghum and other crops and found that wheat and sorghum had attraction to for red flour beetles. Pierce et al. (1990) documented that oat volatiles were important for host searching and selection of sawtoothed grain beetle *Oryzaephilus surinamensis* (Linne) and merchant grain beetle *Oryzaephilus mercator* (Fauvel), and that oats could be used as an alternative grain attractant. Phillips et al. (1993) and Trematerra et al. (2000) reported that the rice weevil *Sitophilus oryzae* (Linnaeus) and other pests responded intact and undamaged grains, whereas red flour beetle and other secondary pests responded to damaged cereals. These data suggest differences in pests' olfactory responses to different grain ingredients. Some studies suggest that butanol in cereals can attract insects such as red flour beetle, sawtoothed grain beetle, rust red grain beetle and foreign grain beetle *Ahasverus advena* (Waltl) to find and select hosts (De Cristofaro et al., 2000; Germinara et al., 2007). Wakefield et al. (2005) studied carob odor components with EAG techniques, gas chromatography and biometrics and they concluded that 2-nonyl ketone and methyl nonyl ketone components of carob odor lured foreign grain beetle. Collins et al. (2007) found that carob and peanut extracts could be used to attract grain weevil adults, and a mixture of volatile contents of cereals helped the grain weevil to locate hosts. Thus, insects can use volatiles from grain to locate hosts and this can be exploited for integrated pest management.

To find grain compounds that have attraction effects on smaller rusty grain beetle and explore pesticide measures, we studied olfactory response of this insect to six grain varieties and describe how these data can be used for pest management.

2. Materials and Methods

2.1. Insects

Smaller rusty grain beetle were provided by the Guangdong Institute for Cereal Science Research and were reared in artificial climate chamber at Laboratory of Insect Ecology, College of Natural Resources and Environment, South China Agricultural University. Insects were collected from grain depots in Guangdong, and then cultured for several generations at 30 ± 1℃ and 70 ± 5% RH, using medium consisting of whole wheat and oat flour and yeast extract (5:4:1). Experimental insects were 2–3 day-old adults.

2.2. Grains

Whole wheat, oat, brown rice, corn, soy and mung bean in flours were purchased from a local supermarket. All of grain flours were passed through an 80-mesh sieve and stored in 500 ml wide-mouth bottles, at 75 ± 5% RH for 7 days. Grain combinations used in chemotactic response test with a Y-shaped olfactometer were wheat – air (control), oat – air (control), brown rice – air (control), corn – air (control), soy – air (control) and mung bean – air (control). Grain combinations used in selection preference test with a four-arm olfactometer were created through random combination, as Table 1.

Table 1 Grain combinations used in olfactory response test.

Treatments	1	2	3	4
	Whole wheat	Oat	Whole wheat	Corn
Grain	Brown rice	Corn	Brown rice	Soybean
	Soybean	Mung bean	Oat	Mung bean
	Blank	Blank	Blank	Blank

2.3. Olfactory measuring device

A Y-shaped olfactometer was designed according to that described by Du et al. (1996). The device consisted of a filter bottle for activated carbon, a flavor source tube, a Y-shaped tube, flow meters, and a suction pump connected with silicone tubing. The four-arm olfactometer was similar to that described by Hou et al. (1996).

2.4. Testing methods

To measure smaller rusty grain beetle chemotactic response for to six varieties of grain and controls, using the Y-shaped shaped olfactometer, we placed 20g test grain in the flavor source tube (air control). During the test, the suction pump was set at 200ml/min and the bioassay time was 8:00-16:00 hours. Smaller rusty grain beetle adults were introduced into the main branch of the Y-shaped tube and observed for 5 min. If the beetle crawled more than the half of distance of the branch and did not return within 1 minute this was a “response”. If the beetle had not responded within 5 minutes, this was “no response”. After 5 tests, the direction of the Y-shaped tube was reversed and after 10 tests, the inner and outer walls of the Y-shaped tube were cleaned with ethanol and dried with a hair dryer to eliminate position error. Selection preference of smaller rusty grain beetle for six varieties of grain were measured by the four-arm olfactometer, adding 20g test grain to the flavor source tube (air control). During the test, the flow of each arm in the four-arm olfactometer was 200ml/min and the bioassay time was 8:00-16:00 hours. Adult insects were introduced into olfactometer test chamber after a 10s pump. Then, 50 adults in each group were tested for each smell source. Next, 10 min after testing, the selection preferences were documented. The chambers were cleaned and the olfactometer was rotated and reconnected to the flavor source bottle and control.

Individual response rates for male and female insects were documented. A selection rate was used to describe the strength of tropism to different grains. They were calculated by the formulae below. Individual response rates = Number of insects in the flavor source branch or blank branch /The total number of tested insects × 100%

Selection rate = (Number of insects in the grain chamber e –Number of insects in the blank chamber)/ The total number of tested insects × 100%

2.5. Data Analysis

Chi-square analysis and Duncan’s analysis was performed. SPSS 17.0 statistical software was used for new multiple range (Duncan’s) analysis.

3. Results and Discussion

3.1. Chemotactic response of smaller rusty grain beetle to six varieties of grain

Data from chemotactic response tests are depicted in Figures 1, and 2. Male insects responded to all grain types (Chi-square value for male insects for each grain exceeded $\chi 20.01 = 6.63$) and females were not significantly different with respect to preference. The results show that adults showed chemotactic response strongly to all of six varieties of grain and there was no significantly difference between the males and females.

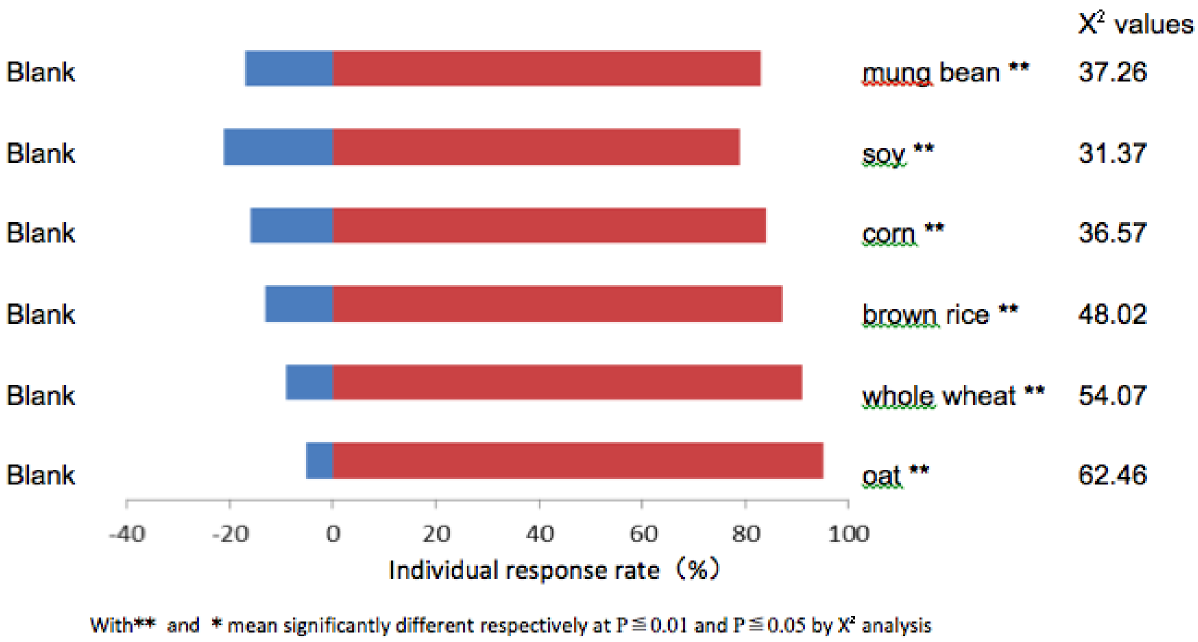

Figure 2 Chemotactic response of male adults to six varieties of grain.

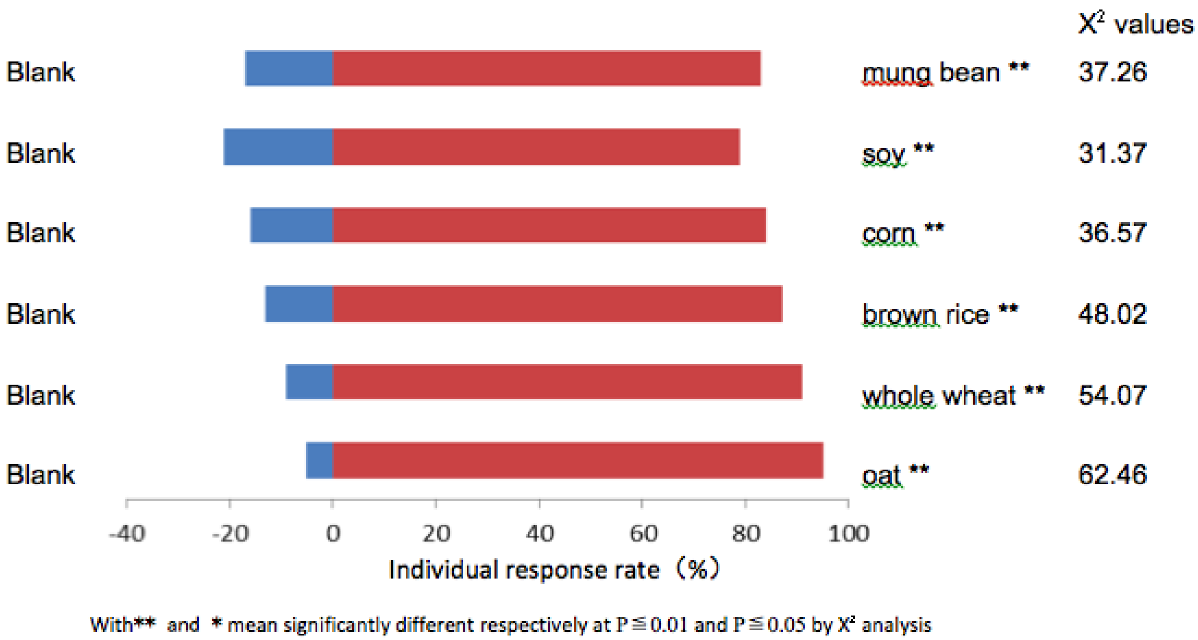

Figure 3 Chemotactic response of female adults to six varieties of grain.

3.2. Selection preference of smaller rusty grain beetle to six varieties of grain

We measured smaller rusty grain beetle selection preference to six varieties of grain and found that they preferred these grains to controls (Table 2). In the first group, the number of insects selecting wheat and brown rice were 9.67 and 8.67s, respectively and these data were not different. Insects selecting soy was 6.33les. In the second group, insects preferred oat (12.33 beetles), significantly more than the corn (6.67 beetles) and mung bean (3.33 beetles). In the third group, oat flour was most preferred (11.33 beetles) followed by wheat (8.67 head), and brown rice (7.67 beetles). In the fourth group, corn was preferred (10.67 beetles) over soy (9.00 beetles) and mung bean (7.00 beetles). Thus, insects preferred oat (Selection rate 37.78–41.11). Mung bean was the least preferred (Selection rate 11.11–23.23).

The results show that oat was the most attractive grain for the adults, with the selection rate as high as 41.1%. In contrast to mung bean flour which was only 11.1%.

Table 2 Selection preference of smaller rusty grain beetle to six varieties of grain.

Treatments	Grain	Number of insects	Selection rate （%）
1	Wheat	9.67±1.53a	32.22±5.09
	Brown rice	8.67±1.16ab	28.89±3.85
	Soy	6.33±1.53b	21.11±5.09
	Blank	3.00±1.00c	0
2	Oat	12.33±2.08a	41.11±6.94
	Corn	6.67±1.15b	22.22±3.85
	Mung bean	3.33±0.58bc	11.11±1.93
	Blank	4.33±0.58c	0
3	Wheat flour	8.67±1.53b	28.89±5.01
	Brown rice	7.67±1.53b	25.56±5.0
	Oat	11.33±1.53a	37.78±5.01
	Blank	1.67±1.53c	0
4	Corn	10.67±1.54a	35.56±3.85
	Soy	9.00±1.00ab	30.00±3.33
	Mung bean	7.00±1.00bc	23.23±3.33
	Blank	1.67±1.53c	0

*Means±SE are given. Within the same treatment, means followed by same letters are not significantly different by the one-way ANOVA /Duncan test at $\alpha = 5\%$.

4. Conclusions

Smaller rusty grain beetle adults showed chemotactic response strongly to all of six varieties of grain. Oat was the most attractive grain for the adults. Using Oat, we can create pest traps that are safe and environmentally friendly for controlling smaller rusty grain beetle. These data will enable us to create more suitable alternatives and environment-friendly control methods for integrated pest management.

Acknowledgements

This work is supported by the Special fund for Grain Scientific Research in the Public Interest from the State Administration of Grains, China, Project No. 201313002-04.

References

Bai, X.G., 2007. Stored product insect pests and their control, Beijing Science Press, China, pp. 370-371.

Bekon, K.A., Fleurat-Lessard, F., 1988. Segregation Olfactive et Alimentaire Realisee par les Adultes de *Tribolium castaneum* (Herbst) (Col.:Tenebrionidae) entre les Grains de Six Especes Cerealieres. Acta OEcologica OEcol. Applic. 9 no2, 153-171.

Collins, L.E., Bryning, G.P., Wakefield, M.E., Chambers, J., Cox, P.D., 2007. Progress towards a multi-species lure: Identification of components of food volatiles as attractants for three storage beetles. Journal of Stored Products Research 43, 53-63.

De Cristofaro, A., Rorundo, G., Germinara, G.S., 2000. Electrophysiological and olfactory responses of *Ephestia kuehniella* Zeller adults to cereals' semiochemicals. IOBC-WPRS Bull 23, 189-194.

Du, Y.J., Poppy, G.M., Powell, W., 1996. Relative importance of semiochemicals from first and second trophic levels in host foraging behavior of *Aphidius ervi*. Journal of Chemical Ecology 22, 1591-1605.

Germinara, G.S., Rotundo, G., De Cristofaro, A., 2007. Repellence and fumigant toxicity of propionic acid against adults of *Sitophilus granarius* (L.) and *S. oryzae* (L.) Journal of Stored Products Research 43, 229-233.

Hou, Z.Y., Yan, F.S., Chen, X., 1996. Olfactory responses of *Lysiphlebia japonica* to volatile chemicals and fresh leaves of the host plants of cotton aphids in olfactometer Journal. Entomologia Sinica 3, 49-57.

Phillips, T.W., Jiang, X.L., Burkholder, W.E., Phillips, J.K., Tran, H.Q., 1993. Behavioral responses to food volatiles by two species of stored-product coleoptera, *Sitophilus oryzae* (curculionidae) and *Tribolium castaneum* (tenebrionidae). Journal of Chemical Ecology 19, 723-734.

Pierce, A.M., Pierce, H.D., Jr., Oehlschlager, A.C., Borden, J.H., 1990. Attraction of *Oryzaephilus surinamensis* (L.) and *Oryzaephilus mercator* (Fauvel) (Coleoptera: Cucujidae) to some common volatiles of food. Journal of Chemical Ecology 16, 465-475.

Trematerra, P., Sciaretta, A., Tamasi, E., 2000. Behavioural responses of *Oryzaephilus surinamensis*, *Tribolium castaneum* and *Tribolium confusum* to naturally and artificially damaged durum wheat kernels Journal of Entomology Experiment et Applicata 94, 195-200.

Upadhyay, R.K., Ahmad, S., 2011. Management strategies for control of stored grain insect pests in farmer stores and public ware houses. World Journal of Agricultural Sciences, 7, 527-549.

Wakefield, M.E., Bryning, G.P., Collins, L.E., Chambers, J., 2005. Identification of attractive components of caroh volatiles for the foreign grain beetle *Ahasverus advena* (waltl). Journal of stored product research 41, 239-253.

Willis, E.R., Roth, L.M., 1950. The attraction of *Tribolium castaneum* to flour. Journal of Economic Entomolo