



## ATTRACTIVENESS OF INFESTED AND UNINFESTED WHOLE WHEAT GRAIN AND COARSE WHEAT MEAL ODORS TO COLEOPTERAN STORAGE INSECT PESTS

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**ABSTRACT:** Using a two-way olfactometer, the effects of the whole wheat grains and coarse wheat meal odors on the primary *Sitophilus granarius* (L.), *S. oryzae* (L.) and *S. zeamais* (Motch.) and secondary *Tribolium castaneum* (Herbst) and *T. confusum* (Du Val) stored-product pests behavior were examined. Whole wheat grains and coarse wheat meal were used in uninfested and infested form. Whole wheat grains were infested with *S. oryzae* adults and coarse wheat meal with *T. castaneum* adults. The odors of infested wheat grains depending on the insect species were 1.9 to 3 times more attractive than the uninfested wheat grains odors, except for the *T. castaneum* for whom the odors of infested and uninfested grains were equally attractive ( $p = 0.641$ ). The greatest difference in the attractiveness of the infested and uninfested wheat grains odors was found for *T. confusum* (3 times longer in the arm with the odors of infested grain), and the smallest for *S. granarius* (1.9 times). We found that all primary and secondary stored-product pests, depending on the species, were 1.4 - 2.4 times more attracted to the odors of infested compared to the odors of uninfested wheat meal. The greatest difference in the attraction of infested and uninfested coarse wheat meal odors was recorded for the *T. confusum*, whose adults were 2.4 times longer in the arm with the odors of infested coarse wheat meal than in the arm with odors of uninfested wheat meal, while the smallest difference (1.4 times) was recorded for *S. oryzae*. The results of this study give a significant contribution to a better understanding of the storage insects behavior, which could be used in the design of new methods of monitoring of their presence in stored products.

**Key words:** *Tribolium sp.*, *Sitophilus sp.*, olfactometer, pest behaviour, food odours

## INTRODUCTION

Stored-product insect pests inflict heavy damage on stored plant products intended for human and domestic animal consumption. It is estimated that the losses resulting from their activity at the annual level range from 9% in developed to > 20% in developing countries (Phillips and Thorne, 2010). However, over 90% of all losses are due to the activity and feeding of primary pests (Rees, 2004). The storage insects are divided into two groups ac-

ording to the diet: primary that attack and damage the whole grain and secondary that attack and damage already damaged grain and various grain products. Primary pests are usually developing inside the grain, whether the female lay the egg into the grain or on its surface, the first instar larvae enter the grains, feed and continue their development inside the grains, while the secondary feeders are developing outside the grain, feeding on the damaged

grains, or grain products (Rees, 2004; Mahroof and Hagstrum, 2012; Mason and McDonough, 2012).

In addition to damaging whole grains and creating favorable conditions for infestation, primary pests also affect the behavior of secondary pests (Trematerra et al., 2015; Stevenson et al., 2017). In this context, in some previous studies it has been documented that odors of grain damaged by primary pests *Sitophilus oryzae* (L.), *Sitophilus zeamais* (Motch.) and *Rhyzopertha dominica* (F.), were significantly more attractive to secondary pests *Oryzaephilus surinamensis* (F.), *Tribolium castaneum* (Herbst) and *Tribolium confusum* Jackuelin du Val than the odors of mechanically damaged grains (Trematerra et al., 2000, 2015). However, the odors of uninfested stored products (whole or mechanically damaged grain) express different attractive potential for storage insects and significantly affect their behavior (Phillips et al., 1993; Trematerra et al., 1999). For example, the results obtained by Phillips et al. (1993) indicate that *S. Oryzae* and *T. castaneum* can quite differently respond to the same grain-related odors. On the other hand, Barrer (1983) found that whole grains of wheat added to the traps, beside *Rhyzopertha dominica*, attracted both *Cryptolestes ferrugineus* (Stephens) and *T. castaneum*.

Monitoring is a vital component of integrated pest management programs. Early stored-product pests detection is essential for the rational use of insecticides, safe storage of goods and protection of their quality. Sampling from commodity and traps are the most commonly used methods for monitoring insects in storage (Hagstrum et al., 1998). The advantage of the trap is that they allow early detection of initial infestation, and so far, aggregation and sexual pheromones are used as attractants in the traps (Hussain et al., 1994; Phillips, 1997; Hagstrum et al., 1998). In order to improve the attractive potential of the traps, laboratory, semi-field and field experiments showed that odor attractants from whole kernels, mechanically damaged kernels and insect damaged kernels or their combinations can improve potential of the traps and instead of capturing

just one species or only one sex of the species, these components can be attractive for several species of storage insects (Phillips et al., 1993; Olsson, 2001; Wakefield et al., 2005; Stevenson et al., 2017). Athanassiou et al. (2016) found that in some cases, prior capture insects were more determinative than the presence of pheromone for trap performance. However, Athanassiou et al. (2017) found that relative cogeneric species, being present either dead or alive, may moderate trap performance. To our knowledge, the data available on the co-occurrence of relative species (species of the same genus) on behavioral interaction as well as between primary and secondary pests in the same commodity are few.

In this context, the intention of this study was to examine and compare the impact of whole wheat grain previously infested with *S. oryzae* and coarse wheat meal previously infested with *T. castaneum* on the primary (*S. zeamais*, *S. oryzae* and *Sitophilus granarius* (L.)) and secondary (*T. castaneum* and *T. confusum*) pest behaviour.

## MATERIALS AND METHODS

### Test insect

In the tests we used laboratory populations of *S. granarius*, *S. oryzae*, *S. zeamais*, *T. castaneum* and *T. confusum* reared by the procedures described by Harein and Soderstrom (1966), and Bry and Davis (1985). Weevils, were reared in 2.5 L glass jars containing whole-grain soft wheat with moisture content below 12%, while *S. zeamais* was reared on whole-grain maize with moisture content below 13%. *Tribolium* species were reared in 2.5 L glass jars on white wheat flour with 5% yeast. Air temperature in the insectary was 25±1 °C, and relative humidity 60±5%. Unsexed 2-4 week old adults of all tested species were used and starved 24 hours before the experiment.

### Substrates used in the experiment

In the experiment we used whole wheat grain NS 40S variety and coarse wheat meal produced by milling whole wheat grains of the same variety. Uninfested substrates were prepared following the

methods described by Đukić et al. (2016). Infested substrate were prepared following the modified methods described by Trematerra et al. (2015), by exposing whole wheat grain and coarse wheat meal to adults of *S. oryzae* and *T. castaneum* (5 adults per 1g of the substrate), respectively. After 15 days insects were removed and substrates were kept in the laboratory on the room temperature for 24h before used in the experiment.

### Olfactometer bioassay

Modified four-arms olfactometer (Ninkovic et al., 2013) was used to test the effects of the uninfested and infested substrates odors on the insects' behavior. For both arms, plastic container with mesh lid was connected with tubes in order to allow undisturbed crossing of the air over the substrates. In the tubes, there was a filter (cotton wool and activated carbon) to purify the air, and thus prevent suction of the flour particles into the olfactometer. Air circulation was provided by a vacuum pump. The air crossed over the substrate and, together with its odors, entered the arms, and went to a central zone which was connected to the vacuum pump by a tube.

Experiments were performed in a darkened room with light just above the olfactometer to exclude the influence of all the other stimuli that could affect the behavior of the insects. One insect was placed in the central zone of the olfactometer and left for three minutes to adapt to the new environment. After adjusting, the insect position was recorded, for next 10 minutes, whether and how long it remained in one or the other arm. After each insect,

the olfactometer was cleaned with 96% alcohol. Each experiment was done in 20 repetitions (one insect one repetition). After every repetition, in order to increase the reliability of the results, the containers with the substrates were rotated to the opposite side. We compared the *S. granarius*, *S. oryzae*, *S. zeamais*, *T. castaneum* and *T. confusum* preferences for odors from:

1. Uninfested versus whole wheat grains infested with *S. oryzae*
2. Uninfested versus coarse wheat meal infested with *T. castaneum*

### Data analysis

Data of the length of the insect stay in one or the other olfactometer arm were statistically analyzed using the Wilcoxon pair test (StatSoft, 2011) setting a significance level of  $P \leq 0.05$ .

## RESULTS

### Behavioral responses of primary and secondary storage pests to odors of uninfested and whole wheat grains infested with *S. oryzae*

All tested insect species were statistically significantly more attracted to the odors of wheat grain infested with *S. oryzae* than the odors of uninfested grain, except for *T. castaneum*, which was equally attracted to odors of grain in both conditions ( $p = 0.641$ ). Infested grain was equally more attractive for species *S. oryzae*, *S. zeamais* and *T. confusum* ( $p = 0.0001$ ) and slightly weaker for *S. granarius* ( $p = 0.0004$ ) compared to uninfested (Table 1).

**Table 1.**

Mean number of seconds  $\pm$  SE which insects spent in the arm with odors of infested and arm with uninfested whole wheat grains

Insect species	Whole wheat grains		p*
	Uninfested	Infested	
<i>Sitophilus granarius</i>	124.50 $\pm$ 12.78	234.75 $\pm$ 19.21	0.0004
<i>Sitophilus oryzae</i>	109.50 $\pm$ 14.67	312.00 $\pm$ 15.72	0.0001
<i>Sitophilus zeamais</i>	104.00 $\pm$ 9.55	265.75 $\pm$ 17.94	0.0001
<i>Tribolium castaneum</i>	173.25 $\pm$ 16.54	212.25 $\pm$ 24.08	0.6407
<i>Tribolium confusum</i>	101.25 $\pm$ 11.43	305.25 $\pm$ 29.83	0.0001

\* Wilcoxon paired test, statistically significant differences if  $p \leq 0.05$

**Table 2.**

Mean number of seconds  $\pm$  SE which insects spent in the arm with odors of infested and arm with uninfested coarse wheat meal

Insect species	Coarse wheat meal		p*
	Uninfested	Infested	
<i>Sitophilus granarius</i>	143.50 $\pm$ 14.20	297.50 $\pm$ 24.30	0.0007
<i>Sitophilus oryzae</i>	176.00 $\pm$ 10.89	249.75 $\pm$ 16.36	0.0269
<i>Sitophilus zeamais</i>	145.25 $\pm$ 11.01	249.25 $\pm$ 21.23	0.0034
<i>Tribolium castaneum</i>	125.50 $\pm$ 15.33	289.75 $\pm$ 22.76	0.0005
<i>Tribolium confusum</i>	86.50 $\pm$ 10.51	207.25 $\pm$ 22.54	0.0007

\* Wilcoxon paired test, statistically significant differences if  $p \leq 0.05$

### Behavioral responses of primary and secondary storage pests to odors of uninfested and coarse wheat meal infested with *T. castaneum*

All tested species were statistically significantly more attracted to the odors of coarse wheat meal infested with *T. castaneum* compared to the odors of uninfested coarse wheat meal. The most significant difference in the attractiveness of infested and uninfested coarse wheat meal was recorded for *T. castaneum* ( $p = 0.0005$ ), followed by *T. confusum* and *S. granarius* ( $p = 0.0007$ ). The least significant difference was recorded for *S. oryzae* ( $p = 0.0269$ ) (Table 2).

## DISCUSSION

Depending on the insect species, the odors of the whole wheat grains infested with *S. oryzae*, attracted primary pests from 1.9 to 2.9 times more than the uninfested wheat grain odors. This is in line with the studies carried out by Trematerra et al. (2007, 2013), which have found that *S. zeamais* was 1.6 times more attracted to the odors of wheat grains infested with the same species than to the fresh uninfested wheat grain odors. *Tribolium confusum* was attracted 3 times more to the odors of the whole wheat grains infested with *S. oryzae* than to the uninfested wheat grains odors. Trematerra et al., (2000) came to very similar results, and found that the odors of whole wheat grains infested with *S. oryzae* was 3.2 times more attractive than the intact whole grains odors for the same species. However, it was surprising that in this experiment, *T. castaneum*, as a species of the same genus, did not show the preference

for the wheat grains infested with primary pests over the uninfested wheat. This is opposite to the results of a study conducted by Trematerra et al., (2000, 2013) which have found that *T. castaneum* was statistically significantly more attracted to the odors of wheat grains infested with *S. oryzae* and *S. zeamais* than to the uninfested grains odors. However, recent studies have even determined the repellent effect of grains infested with *S. granarius* for *T. castaneum* (Stevenson et al., 2017). They explain this fact by the possibility that *T. castaneum* adults are seeking early infestations rather than potentially overcrowded late-stage infestations.

All tested primary and secondary pests were statistically significantly more attracted to the odors of coarse wheat meal infested with *T. castaneum* than to the uninfested coarse wheat meal odors. Although there is very little available data about behavior of secondary pests influenced by odors of food that is infested with the same group of pests, a high attraction of infested coarse wheat meal that attracted *T. castaneum* and *T. confusum* 2.3 and 2.4 times more, respectively than uninfested coarse wheat meal, was expected. Previous studies have found that secondary pests are more attracted by the odors of damaged, elderly and decaying grain and grain products than the odors of the fresh grains (Phillips, 1993; Cox and Collins, 2002; Athanassiou et al., 2017). By examining the intraspecific behavior of *T. castaneum*, Stevenson et al., (2017) have also found the attraction of the flavor infested with adults of this species for its larvae and adults. On the other hand, surprisingly high attraction of odors of coarse wheat meal infested with

secondary pest for primary pests (1.4 to 2.1 times more attractive than uninfested), was unexpected. In contrast to our research Phillips et al. (1993) found that primary pests were more attracted to the fresh intact grain odors. However, recent studies conducted by Trematerra et al. (2015) have found that the primary pest (*S. zeamais*) was 1.5 and 1.8 times more attracted to the odors of the wheat grains infested with adults or larvae of *T. castaneum* than to the uninfested grains odors, which leads to the conclusion that the first colonizer can play the role of the primary colonizer.

## CONCLUSION

The results of these studies showed the high attractiveness of infested substrates for primary and secondary pests and provide insight into the still insufficiently known stored-product insect pests behavior and their intraspecific and inter-specific interaction. The high attractiveness of the infested substrates can be used in the future to improve traps for monitoring the occurrence and number of these pests, but that would require further more detailed research.

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## АТРАКТИВНОСТ МИРИСА ИНФЕСТИРАНЕ И НЕИНФЕСТИРАНЕ ПШЕНИЦЕ У ЗРНУ И ПРЕКРУПЕ ЗА СКЛАДИШНЕ ТВРДОКРИЛЦЕ

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**Сажетак:** Помоћу двокраког олфактометра испитиван је утицај мириса пшенице у зрну и пшеничне прекрупe на понашање примарних штеточина *Sitophilus granarius* (L.), *Sitophilus oryzae* (L.) и *Sitophilus zeamais* (Motch) и секундарних штеточина *Tribolium castaneum* (Herbst) и *Tribolium confusum* (Du Val). Пшеница у зрну и пшенична прекрупа коришћени су у неинфестираном и инфестираном облику, с тим да су зрна пшенице била инфестирана адултима *S.oryzae* а прекрупа адултима *T. castaneum*. Мирис инфестиране пшенице у зрну је био у зависности од инсекатске врсте од 1,9 до 3,0 пута атрактивнији од мириса неинфестиране пшенице, осим за адулте *T. castaneum* за које је био подједнако привлачан ( $p=0,641$ ). Највећа разлика у атрактивности мириса инфестиране и неинфестиране пшенице је забележена код *T. confusum* (3 пута више времена у краку са мирисом инфестираног зрна), а најмања код *S. granarius* (1,9 пута). Све примарне и секундарне штеточине су у зависности од врсте биле од 1,4 – 2,4 пута више привучене мирисом инфестиране, него мирисом неинфестиране пшеничне прекрупe. Највећа разлика у атрактивности мириса инфестиране и неинфестиране прекрупe, утврђена је код *T. confusum*, чији су адулти провели 2,4 пута више времена у краку са мирисом инфестиране прекрупe него у краку са мирисом неинфестиране, а најмања разлика (1,4 пута) забележена је код *S.oryzae*. Резултати ових истраживања дају значајан допринос бољем познавању понашања складишних инсеката, што би могло да се употреби у осмишљавању новог начина праћења њиховог присуства у ускладиштеним производима.

**Кључне речи:** *Tribolium sp.*, *Sitophilus sp.*, олфактометар, понашање штеточина, мириси хране

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