Tab. 3 *Tribolium castaneum adults* attracted by different food oils, pheromone or kairomone.

Source	Trapping in Treatment (%)*	Trapping in Control (%)
Olive oil	29edc**	15**
Rice bran oil	21ed	25
Coconut oil	52ab**	8**
Kairomone	44abc**	5**
Pheromone	53ab**	17**
Sunflower oil	36bdc	17
Mee oil	59a**	14**
Mustard oil	29ecd**	26**
Gingelly oil	22ed	21

* Percentage trapped followed by the same letter in a column are not significantly different at p=0.05 according to Tukey's test.

**denotes significant difference from the control.

The aggregation pheromone 4, 8 DMD released from *T. castaneum* adults is dispersed effectively up to 60 cm from the trap. Air flow increases the beetle orientation towards the source. Coconut oil and Mee oil equally attract adult beetles as the synthetic pheromone and kairomone.

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The responses of *Tribolium castaneum* to wheat germ oil and fungal produced volatiles

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Abstract

The red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) is a significant pest affecting a wide variety of different stored products around the Globe. Despite its economic impact, there is evidence that the lures currently used in traps to monitor for this species are largely ineffective. Based on the evolutionary history of *T. castaneum*, and the ecological niche it occupies, the volatiles of wheat germ oil and volatiles produced by grain-associated fungi have the potential to act as attractants for this species. We used electroantennography (EAG) to measure the electrophysiological response elicited by sixty-eight volatile compounds found in wheat germ oil and/or grain-associated fungi in two *T. castaneum* strains; an established lab population (CTC12 strain) and a recently caught wild population. Many volatile compounds from both sources elicited strong antennal depolarisations, and the responses of both strains were highly correlated. We then tested whether the compounds that triggered the strongest antennal depolarisations also elicited behavioural responses by using Y-tube olfactometer bioassays and identified several compounds attractive to both strains. The discovery of novel compounds that elicit strong EAG signals and behavioural responses could prove useful in the design of

improved lures for *T. castaneum* and other stored product pests. Our future research will identify how effective these attractive volatiles might be when used in combination, and when used under conditions that more closely replicate a stored product environment.

Key words: *Tribolium castaneum*, electroantennography, Y-tube olfactometer, fungal volatiles, wheat germ oil

Introduction

Tribolium castaneum and its sister species *T. confusum* are both economically significant pests of the stored product industry. They are particularly damaging owing to their global distribution and the wide variety of food products that they can infest, including nuts, milled grains and dried cereal products (Bell, 2014). Severe *Tribolium* infestations can also produce a "conditioning" effect in the medium they infest, which is characterised by depletion of the nutrient value of the medium, the accumulation of toxic benzoquinones secreted by the beetles and a build-up of debris such as larval casts and dead adults (Ghent, 1963). *Tribolium* infestations are a particular problem in stored product warehouses where damage by insects, mites and other microorganisms accounts for an estimated global annual post-harvest loss of 10-15% (Neethirajan *et al*., 2007). This problem is especially severe in developing countries, where post-harvest losses can be as high as 20% (Phillips and Throne, 2010).

One of the main ways that infestations of *Tribolium* species and other stored product pests can be detected and monitored is using lure baited traps. These lures typically contain insect pheromones in a slow-release formula and are commercially available for over 20 species of stored product insect, including *T. castaneum* and *T. confusum* (Phillips and Throne, 2010). Multispecies lures, containing the pheromones of different insects, are also available and are a common feature of integrated pest management strategies as they overcome the need to have multiple different lures for the different pest species encountered (Cox and Collins, 2002). Many of these lures combine insect pheromones with a food based kairomone such as wheat germ oil (Campbell, 2012), and this can make up 90% of the concentration by weight of these products. Wheat germ oil appears to be attractive to *T. castaneum* (Phillips *et al*., 1993), but the specific compounds in this mixture that elicit this attraction have not yet been identified. Despite the widespread use of these lures, there are reports from users in the stored product industry that they are not very effective (Semeao *et al*., 2011). This is supported by experimental data showing that the responses of *T. castaneum* to these pheromone baited traps is limited in ideal conditions and can be minimal in an environment with no air-flow (Campbell, 2012). In a simulated warehouse experiment, less than 2% of *T. confusum* released within a 60 cm distance from a pheromone trap were caught (Hawkin *et al*., 2011). As a result of this there has been a focused effort towards improving the efficacy of lures for *T. castaneum* and other stored product pests (Cox and Collins, 2002).

One major barrier in improving the ability of these lures to attract *T. castaneum* and other stored product insects is the lack of knowledge about the specific odours that attract these insects to stored product environments. This is particularly true for *T. castaneum* where little is known about its attraction to specific food related volatiles (Campbell, 2012). As the common name of *T. castaneum,* the red flour beetle, implies, flour and other milled grains are a major food source for this species. However, experimental data show that the odours of flour are only marginally attractive to this species (Campbell, 2013). *Tribolium castaneum* appears to be more attracted to cereal grains that exhibit signs of damage from decay or pest infestation (Trematerra *et al*., 2000). They are also more attracted to wheat kernels with other insects present, and to those that have been damaged by other pest species (Trematerra *et al*., 2000). *Tribolium castaneum* also exhibits attraction to fungal odours, specifically the volatiles of fungi associated with cotton seed lint (Ahmad *et al*., 2012). Indeed, they were shown to be more attracted to these odours than to the odours of conventional food sources such as wheat (Ahmad *et al.,* 2012). These preferences could be explained by the fact that *T. castaneum* is a 'secondary pest' which primarily feeds on grains that are rotten or have been damaged by the infestation of other insects, or mechanically processed by humans, i.e. milled

(Trematerra and Sciarretta, 2004). The presence of wheat germ and fungal volatiles would therefore be an indicator that the grains are in a suitable condition for *T. castaneum* to feed on.

Despite the knowledge that *Tribolium* species are generally attracted to the odours of both wheat germ oil and fungi the specific volatiles underpinning this attraction have not been clearly identified. As many current lures contain wheat germ oil as a food based attractant there is the potential to improve the efficiency of these traps by only including the specific volatiles in wheat germ oil that attract *T. castaneum*. The incorporation of attractive fungal volatile compounds also has the potential to improve the attractiveness of lures. To identify specific volatiles of wheat germ oil and grain-associated fungi that are attractive to *T. castaneum* we have used a combination of electroantennography (EAG) and behavioural bioassays. We have used EAG as an efficient method of identifying which compounds can be detected by the antennae of *T. castaneum* and have determined whether *T. castaneum* are attracted to the volatiles that they can detect using Y-tube bioassays. A variety of different compounds have already been tested for *T. castaneum* using EAG (Balakrishnan *et al.*, 2017; Verheggen *et al.*, 2007), but this is the first screen focusing specifically on the volatiles found in wheat germ oil and produced by grain associated fungal species.

Methods

Tribolium husbandry

Two *T. castaneum* strains were used in the experiments; the CTC12 strain and a wild captured Zimbabwean population. The CTC12 strain originates from an organophosphate resistant strain from Australia (Champ and Campbell, 1970) that has since been cultured in the laboratory. This strain was used to represent an established laboratory population. The wild captured population were cultured from a population found inside a shipment of infested grain from Zimbabwe in 2017. Cultures for both strains were maintained at 30°C in containers of 200 g of whole grain flour with the addition of 10 g yeast powder (as an additional protein source) and 1 g of the antimicrobial agent Fumagillin (to inhibit fungal growth in the cultures). All beetles used in the experiments were aged between 4 and 8 weeks post-emergence.

Volatile compounds

Sixty-eight volatile organic compounds that are either present in wheat germ oil or produced by grain-associated fungi were used (Table 1). The 34 wheat germ oil volatiles used in these experiments had been previously identified through headspace-solid phase microextraction of a sample of wheat germ oil (Niu *et al.*, 2013). Fungal compounds (28) were identified from a review listing the volatiles produced by common fungi grown on cereal and grain substrates (Magan and Evans, 2000). Six compounds were identified as being found in both wheat germ oil and produced by grain-associated fungi. Synthetic DMD (4,8-Dimethyldecanal), the *Tribolium* spp. aggregation pheromone, was used as a positive control as it is known to be behaviourally attractive and elicit strong antennal depolarizations in *T. castaneum* (Levinson and Mori, 1983). All odorants were diluted to working concentration using hexane, an established solvent for use in insect olfactory behavioural experiments that has previously been shown to not elicit significant EAG depolarisations or behavioural attraction in *T. castaneum* (Verheggen *et al.,* 2007). All compounds were obtained from commercial suppliers.

Table 1. The environmental volatiles used in our experiments and whether they were identified as being found in wheat germ oil, produced by grain-associated fungi or as both.

Compound	Source	Compound	Source	Compound	Source
5-methyl-3-heptanone	Wheat germ oil	3-octanone	Fungal	3-methyl-1-butanol	Both
trans-2-heptenal	Wheat germ oil	butyl acetate	Fungal	hexanal	Both
ethyl hexanoate	Wheat germ oil	benzaldehyde	Fungal	1-octen-3-ol	Both
Limonene	Wheat germ oil	3-methylanisol	Fungal	1-hexanol	Both
trans-trans-2,4,-heptandinal	Wheat germ oil	2-methylacetophone	Fungal	nonanal	Both

2-heptanone	Wheat germ oil	1-pentanol	Fungal	ethanol	Both
trans-2-pentenal	Wheat germ oil	trans-2-hexen-1-al	Fungal		
iovaleraldehyde	Wheat germ oil	2-methyl-2-butanol	Fungal		
Octanal	Wheat germ oil	damascenone	Fungal		
amyl acetate	Wheat germ oil	3-octanol	Fungal		
trans-2-octene	Wheat germ oil	dimethyl benzene	Fungal		
1-penten-3-one	Wheat germ oil	styrene	Fungal		
ethyl benzene	Wheat germ oil	2-butanol	Fungal		
trans-2-pentanal	Wheat germ oil	naphthalene	Fungal		
1-octene	Wheat germ oil	1-butanol	Fungal		
trans-cinnamaldehyde	Wheat germ oil	2-methyl-1-propanol	Fungal		
ethyl octanoate	Wheat germ oil	2,2,4-trimethylhexane	Fungal		
Pentane	Wheat germ oil	2-nonanone	Fungal		
2-pentylfuran	Wheat germ oil	acetone	Fungal		
trans-2-octenal	Wheat germ oil	butyl acetate	Fungal		
Undecane	Wheat germ oil	2-methylfuran	Fungal		
1-heptene	Wheat germ oil	octyl acetate	Fungal		
Nonane	Wheat germ oil	2-pentanone	Fungal		
trans-5-decene	Wheat germ oil	1-phenylethanol	Fungal		
Toluene	Wheat germ oil				
trans-3-octene	Wheat germ oil				
2-methyl-2-butene	Wheat germ oil				
p-anisaldehyde	Wheat germ oil				
trans,trans-2,4-decadienal	Wheat germ oil				
trans-2-decenal	Wheat germ oil				
4-allylanisol	Wheat germ oil				
octanoic acid	Wheat germ oil				
Tridecane	Wheat germ oil				
Hexane	Wheat germ oil				

Electroantennography

The electroantennography protocol was adapted from the Syntech Electroantennography manual (Syntech, 2004). Only female beetles were used, as in preliminary experiments (not reported) we found there were no significant differences between the responses of male and female beetles. The same finding has recently been reported by Balakrishnan *et al.* (2017). For each strain, a live female beetle was carefully positioned on a glass slide with adhesive tape to restrict movement and allow EAG recordings to be taken (N=8). A thin strip of double-sided adhesive tape was placed under the head of the beetle. This was sufficient to prevent movement of the antenna with the addition a small drop of cyanoacrylate glue to stick down the head of the beetle. Care was taken to not get any glue on the antenna of the beetles. Small holes were pierced into the tip of the antenna and through the eye of the beetle with an electrolytically sharpened tungsten wire to allow glass capillary electrodes filled with Ringer solution, in contact with silver wire, to be inserted. Filtered air continuously flowed over the restrained beetle and the test odorants were delivered by an air-puff from a Syntech stimulus controller. When triggered the stimulus controller delivered a one second puff of air to the end of a Pasteur pipette pointed at the head of the restrained beetle. Strips of Whatman filter paper with 5 µl of 20% vol/vol dilution in hexane of each volatile compound were inserted into this pipette to present the beetles with the different volatiles used in the experiments. Every 10 volatiles the responses of the beetles were tested against DMD (positive control) and hexane (negative control) and the responses of the preceding 10 volatiles were normalised against the DMD response. The EAG potential was recorded on a computer using a signal amplifier, IDAC convertor and EAG 2000 software.

Y-tube bioassay

The Y-tube olfactometer apparatus consisted of a 20 cm long, 6 cm in diameter, glass cylinder that branches in the middle to form a two-armed (Y-shaped) glass tube. The Y-tube was connected by PTFE tubing to a vacuum pump, which drew an air-flow through each of the two Y-arms at a rate of 0.2 L/min. Each arm was in turn connected by PTFE tubing to three sealed vials, the first containing Whatman paper disks to which 5 µl of a 200 ng/µl dilution of the test volatile was added, the second

containing activated charcoal and the third containing water. The Y-tube olfactometer had a sealable hole on the main stem that allowed for insects to be inserted while the vacuum pump was running. A single beetle was inserted into the Y-tube through this hole for each trial and its movements were observed for five minutes. Once a beetle had walked 2 cm down one of the two branches of the Y-tube it was recorded as having chosen that arm of the olfactometer. If no choice was made within five minutes the beetle was deemed to be non-responsive and was discarded. The odorants connected to each arm of the olfactometer were switched over every 10 trials to prevent the direction of the arms from biasing the choices of the beetles. Only females were used as other researchers have suggested that aggregation pheromone could be produced by males within the olfactometer, which could influence the behaviour of beetles used in subsequent trails (Ahmad et al. 2012). All trials were conducted in a 20°C controlled temperature room.

Statistical analysis

Differences between the EAG responses of the two strains to the different volatiles tested were analysed with a two-way mixed ANOVA. Where significant differences were found they were followed up with pairwise paired t-tests (for within-strain differences) or unpaired t-tests (for between-strain differences). To correct for the error associated with number of statistical tests the significances were adjusted using a false discovery rate method. All statistics were performed using IBM SPSS Statistics 24.

Results

Electroantennography

The average antennal depolarisations elicited by the volatiles presented to female *T. castaneum* after being normalised against the response to the DMD positive control are shown in Figure 1. It was noted that the absolute depolarisations of the CTC12 strain were much larger than that of the wild strain for all volatiles tested with the compounds on average eliciting depolarizations of twice the response from the CTC12 strain as from the wild strain. However, after normalisation of the data, the overall trend in the responses of both strains to the different volatiles was remarkably similar. A two-way mixed ANOVA revealed a highly significant effect of the volatiles ($F_{1,66}$= 10.59, p< 0.001), and a significant effect of the interaction between strain and volatile ($F_{1,66}$= 1.362, p= 0.032). This indicates that the size of the antennal response changed depending on the volatile tested and that there were some differences between responses of the two strains to the same volatile. However, no significant effect of strain alone was found ($F_{1,66}$= 1.384, p= 0.259Post hoc paired-t tests with a false discovery rate applied were performed to identify volatiles that were significantly different from the hexane control. The relationship between the responses of the two strains is also shown by a bivariate plot of the normalised responses of the two strains to the different volatile compounds (Fig. 2), which revealed a strong correlation between the responses of both strains to the different volatiles ($r = .809$, $n = 68$, $p < .001$).

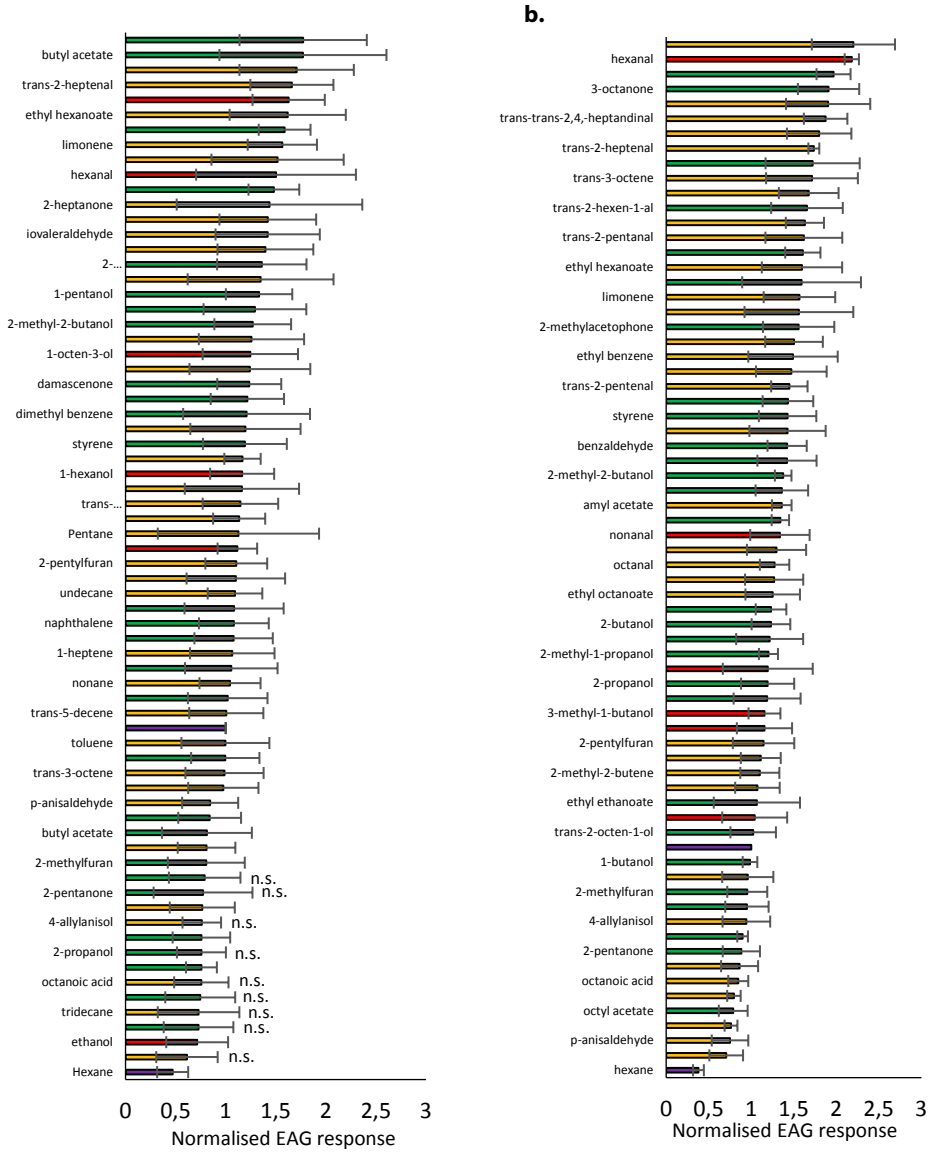

Figure 1 Average EAG responses of 8 CTC-12 strain (a) and 8 wild captured *Tribolium castaneum* (b), normalised against a DMD control, to 68 volatile organic compounds found in wheat germ oil or produced by grain associated fungi, plus the *Tribolium* aggregation pheromone, DMD as a positive control. Columns, arranged by descending EAG response, represent the average depolarization across eight individuals, and the error bars represent the standard deviations of the mean. Yellow bars represent volatile compounds found in wheat germ oil, green bars represent compounds identified as being products of grain associated fungi, red bars represent compounds we identified as being produced by both sources, and purple bars represent the two control compounds, DMD (positive control) and hexane (negative control). Compounds that did not elicit a significantly different EAG response compared to the hexane control are indicated with "n.s"; no compounds were found to be significantly different in the wild population beetles.

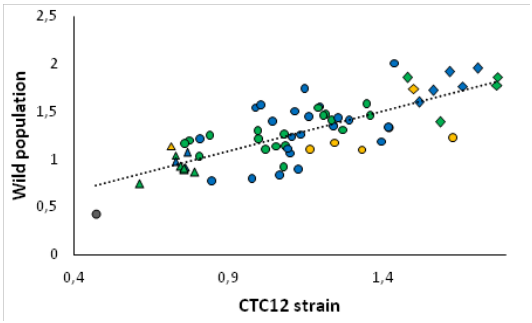

Figure 2 A bivariate plot showing the correlation between females of the CTC12 strain and females of the wild population strain in their average (n=8) normalised EAG responses to 68 different volatile organic compounds found in wheat germ oil or produced by grain associated fungi. Blue points represent volatile compounds found in wheat germ oil, green points represent compounds identified as being produced by grain associated fungi, yellow points represent compounds we identified as being produced by both sources, the grey point represents the control compound hexane. Pearson product-moment correlation indicated a strong positive correlation between the responses of the two strains to the different volatiles ($r = .809$, $n = 67$, $p < .001$). The ten compounds that elicited the largest average responses across both strains are indicated with diamond shaded points, and the ten compounds that ellicited the smallest average EAG responses are indicated with triangle shaped points. The responses to these indicated volatiles was also tested behaviourally using a y-tube olfactometer.

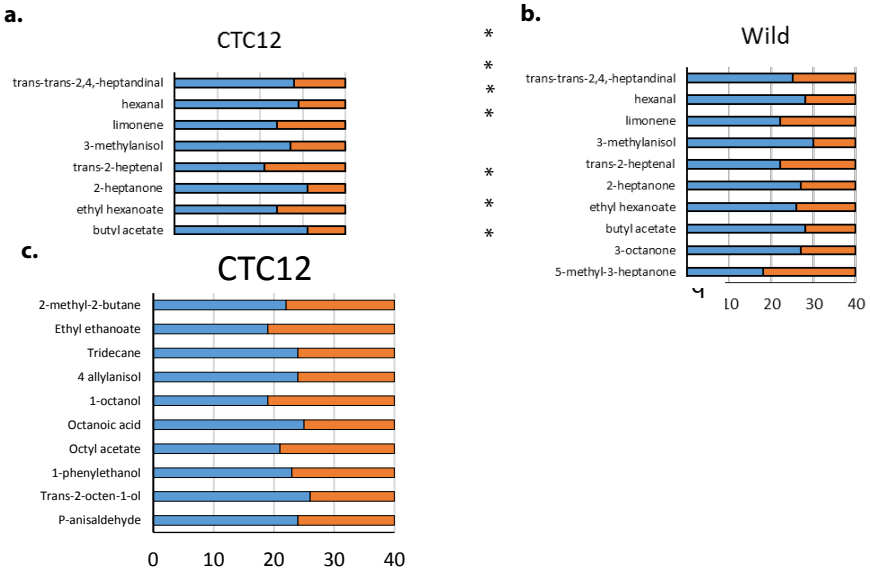

Figure 3 The attraction of female CTC-12 (a.) and wild population (b.) *T. castaneum* beetles to the ten volatile compounds that elicited the largest average EAG responses across both strains (see Fig. 2). The blue bars indicate the number of beetles (out of 40 individuals tested) that chose the Y-tube arm containing the test volatile, the orange bars indicate the number that chose the arm containing the hexane control solvent. Asterisks indicate a statistically significant bias for one arm of the Y-tube over the other (chi-square of goodness-of-fit <.05)

Y-tube olfactometer

Figure 3 shows the behavioural responses of female CTC12 (a) and wild (b) strain beetles to the ten compounds that elicited the highest EAG responses across both strains (indicated with diamond shaped points in Figure 2) and the responses of CTC12 strain females to the 10 compounds that elicited the smallest average EAG response across both strains (c) (indicated with diamond shaped points in Figure 2). The results show that, of the ten most attractive compounds, seven of the compounds elicited a statistically significant attractive response in the CTC12 strain (chi-square of goodness-of-fit $p < .05$), whereas five were shown to be significantly attractive to the wild population with all the compounds that were behaviourally attractive to the CTC12 strain also attractive to the wild population. None of the 10 compounds that elicited the smallest average EAG responses were found to be significantly attractive to the CTC12 strain.

Discussion

The results of our EAG and Y-tube olfactometer experiments give new insights into the physiological and behavioural responses of *T. castaneum* to common environmental volatile compounds that could have relevance to its future pest management. The results of the EAG experiments reveal that many of the compounds tested elicited large EAG responses relative to the DMD positive control, with around two-thirds of them eliciting larger responses than to DMD when used at the same concentration. This is encouraging given that insect aggregation pheromones (i.e. DMD) form the basis of many current lures. Strong antennal and behavioural responses were observed following exposure to a subset of volatiles found in wheat germ oil. This is perhaps unsurprising, given what was already known from the literature, and the current composition of insect lures. Interestingly, we also observed strong antennal and behavioural responses to volatiles from grain-associated fungi. *Tribolium castaneum* is known to have been associated with humans for at least 4,500 years, having been found sealed within Pharaonic urns in Egypt (Dawson, 1977). However, prior to the existence of anthropogenic food stores, *Tribolium* species must have fed on a different source of food. As many species in the same *Tenebrionidae* family as *T. castaneum* primarily feed on rotting tree bark, and other decaying plant matter, it is possible that this was the original food source of this species, before it switched to feeding on anthropogenic stored products. It has also been theorised that *T. castaneum* may have first adapted to feed on rotting grains stored in the burrows of rodents, and other sources of rotten grains, before switching to feed on mechanically processed grains stored by humans (Dawson, 1977). Therefore, *T. castaneum* may have co-opted an ancient ancestral attraction to fungal volatiles, derived from rotting plant matter, to find human stored grain products. Our findings lend some tentative support to this idea.

Although *T. castaneum* was attracted to volatiles from both wheat germ oil and grain associated fungi, there was no clear pattern between the responses to these two sources, with both groups of volatiles containing within them individual compounds that elicited very strong antennal responses, as well as volatiles that did not elicit strong responses. There was also no clear relationship between the type of compound and the size of the antennal depolarizations recorded. Some alcohols, ketones and compounds with methyl groups were found to elicit EAG depolarisations, while other compounds of the same chemical group did not. This agrees with a previous large-scale EAG screen which found no clear pattern between the size of depolarisations and the chemical class of the compounds tested in *T. castaneum* (Balakrishnan *et al*., 2017). This suggests that this species is responding to very specific compounds associated with stored products, rather than to a broad range of chemically related compounds.

Before normalisation of the EAG responses, a striking difference was observed between the two *Tribolium* populations tested, with the depolarisations of the wild caught population typically being half the amplitude of the laboratory strain. However, after normalising the responses against a DMD positive control to correct for variation in antennal resistance over the course of taking recordings, the responses of both strains were found to be highly correlated. This demonstrates that the overall trend across the volatiles was the same in both strains, and could indicate that the composition of

odorant receptors is similar between both strains. However, to ensure we could record strong EAG responses, the concentration at which we tested these volatiles were much higher than would be encountered typically in a stored product environment, so it is possible that the responses to the two strains could still differ when they encounter these volatiles at more natural concentrations. It should also be noted that the results of the EAG on their own do not reveal how attractive these compounds are, only the degree to which they are detected at the insects' antenna. The amount of antennal depolarisation and the attractiveness of compounds are often not strongly correlated, and insects can have different responses when encountering blends of volatiles at different ratios (Bruce *et al.,* 2005). A previous study that examined behavioural differences between freshly caught and established laboratory populations of *T. castaneum* found little difference in their responses to traps baited with food and pheromone lures (Campbell, 2012). These results could suggest that fungal and wheat germ oil volatiles might elicit similar responses in *Tribolium* populations from diverse ecological backgrounds, which is important if these volatiles are to be used in a general-purpose lure and suggest that the results of previous behavioural studies conducted in lab strains should be applicable to wild populations, and vice versa. However behavioural experiments testing the responses to these compounds under more natural conditions would be needed to confirm this idea.

Owing to the very small response elicited to the hexane control volatile, all the volatiles tested in the wild population, and almost all the volatiles tested in the CTC12 strain, were found to elicit significantly different electrophysiological responses compared to this control. This is similar to the results of previous EAG experiments in *T. castaneum*, which found almost all of the compounds tested gave "a measurable EAG response" (Balakrishnan *et al.*, 2017). However, it was not clear from the study by Balakrishnan *et al*. (2017) what level of antennal depolarisation would predict a significant behavioural response from the beetles. When the ten volatiles that elicited the highest average EAG responses in the current study were tested for behavioural responses in the CTC12 strain, seven of them were found to be significantly attractive. In contrast, when the ten volatiles that elicited the smallest average EAG responses were tested, none of them were found to be significantly attractive. This would suggest that a certain threshold depolarisation must be reached before compounds become behaviourally attractive or that these compounds elicit a response that the Y-tube olfactometer does not measure, e.g. they are repellent or arrest the beetles by stimulation oviposition. However the results also show that even compounds that elicit relatively large EAG responses will not necessarily be attractive owing to complex relationship between odour perception and behavioural response in insects (Bruce *et al*., 2005; Bruce and Pickett, 2011). It is possible that the same volatiles will be attractive when tested at different concentrations, or when tested together as a blend. Although attractiveness to most of the volatiles used in this study has not previously been demonstrated for *T. castaneum,* some of the volatiles have previously been used in research involving *Tribolium* species. For example, 3-octanone has been identified as a volatile that can be found in *Tribolium* infested flour, but is absent from clean flour (AbuelInnor *et al*., 2010), and this could explain the advantage of strong attraction of both strains to this volatile. In addition, hexanal has also been shown to be attractive to *T. confusum* when used in a blend with other plant volatiles (Wenda-piesik *et al*., 2016).

Taken together, the results of our EAG and behavioural experiments have revealed previously unidentified attractive compounds for *T. castaneum*, which have the potential to be used to improve the effectiveness of commercial *Tribolium* lures. There are also several wheat germ oil and fungal derived volatiles that elicited strong antennal depolarisations that have not yet been tested behaviourally, compounds that also have the potential to be highly attractive to *T. castaneum*. We are now exploring whether there are any synergistic effects of the attractive volatiles we have identified when they are encountered together. If fungal volatiles are indicators that grains are in a condition that *T. castaneum* can feed upon, it is likely that a stronger attractive response will be elicited when wheat germ oil and fungal volatiles are encountered together. This could be an important factor in adapting these volatiles for use as a *T. castaneum* lure. *Tribolium castaneum* has

been shown to be less attracted to lures when tested in an environment without a strong airflow (Campbell, 2012). We are therefore also doing behavioural experiments in environments closer to those encountered in a warehouse, which should provide better information about how attractive these compounds are to *T. castaneum* under real world conditions.

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