1 **Responses to colour and host odour cues in three cereal pest species, in the**
2 **context of ecology and control**
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13 Running head: Odour and colour preferences in cereal pests
14

15 **Abstract**
16

17 Many insects show a greater attraction to multimodal cues, e.g. odour and colour combined,
18 than to either cue alone. Despite the potential to apply the knowledge to improve control
19 strategies, studies of multiple stimuli have not been undertaken for stored product pest insects.
20 We tested orientation towards a food odour (crushed white maize) in combination with a colour
21 cue (coloured paper with different surface spectral reflectance properties) in three storage pest
22 beetle species, using motion tracking to monitor their behaviour. While the maize weevil,
23 *Sitophilus zeamais* (Motsch.), showed attraction to both odour and colour stimuli, particularly to
24 both cues in combination, this was not observed in the bostrichid pests *Rhyzopertha dominica*
25 (F.) (lesser grain borer) or *Prostephanus truncatus* (Horn) (larger grain borer). The yellow

26 stimulus was particularly attractive to *S. zeamais*, and control experiments showed that this was
27 neither a result of the insects moving towards darker-coloured areas of the arena, nor their being
28 repelled by optical brighteners in white paper. Visual stimuli may play a role in location of host
29 material by *S. zeamais*, and can be used to inform trap design for the control or monitoring of
30 maize weevils. The lack of visual responses by the two grain borers is likely to relate to their
31 different host seeking behaviours and ecological background, which should be taken into
32 account when devising control methods.

33

34 Keywords: colour vision, insect orientation, olfaction, host odours, stored product pest,
35 olfactometer
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37
38

Introduction

Monitoring traps to aid in control of coleopteran storage pests have been developed for both commercial use in large scale industry in developed countries and for small-scale use amongst subsistence farmers in developing countries (Barak & Burkholder 1985; Collins & Chambers 2003; Ukeh, Udo & Ogban 2008; Campbell 2012). Conversely, mass-trapping of these pests has been overlooked and underutilized as a pest management tool. Effective mass-trapping requires an understanding of how insects orient towards different stimuli.

Many current trap designs for monitoring insects typically use pheromones or kairomones and rely on olfaction (Likhayo & Hodges 2000; Hodges *et al.* 2004; Torr, Mangwiro & Hall 2006). Consequently, many studies of insect agricultural pests and vectors focus on chemo-orientation behaviour. This is particularly the case in stored product pests. However, colour vision is virtually universal in insects (Briscoe & Chittka 2001) and plays a role in host location in many species. Neglecting visual responses when designing traps and control strategies presupposes that vision plays no significant part in orientation - or that the resolution of the eyes in tiny insects is too poor for visual cues to be relevant at anything other than close range. However, work on species such as aphids (Kennedy, Booth & Kershaw 1961; Döring & Chittka 2007), tsetse (Green & Cosens 1983; Green 1986; Lindh *et al.* 2012) and Colorado beetles (Otálora-Luna & Dickens 2011) shows the value of taking multiple stimuli into account.

Colour vision in some insect species has been extensively studied, especially pollinators (Lunau & Maier 1995; Chittka & Raine 2006), for whom colour is a cue for a specific flower's location, and *Drosophila melanogaster* (Meigen), where the well-characterized genome has enabled exploration of the genetic and developmental basis of colour vision (Morante & Desplan 2008). However, for most insects, particularly those of economic or agricultural significance, colour vision studies are limited to outcome-driven experiments focusing only on relative responses to (often unquantified) colours without consideration of causative physiological or behavioural mechanisms, as has been highlighted by Döring & Chittka (2007). Although this can lead to methods of control, e.g. by sticky traps, it can prove difficult to replicate or refine the effectiveness of a trap – a problem that emerged in control of tsetse, for example, when phthalogen blue dye became unfavourable for safety reasons (Lindh *et al.* 2012). Design of experiments should take into account existing knowledge of insect colour vision, as this can be used to refine control strategies (Lindh *et al.* 2012). Quantification of visual stimuli using a

73   spectrophotometer provides spectral reflectance curves for coloured surfaces, which facilitates

74   investigation of underlying mechanisms of colour-mediated behaviours (Döring *et al.* 2012).

75

76   Various Coleoptera are reported to have colour vision (Briscoe & Chittka 2001), including

77   tested species in the Glaphyridae (Martínez-Harms *et al.* 2012), Scarabaeidae, Carabidae,

78   Coccinellidae and Chrysomelidae such as the pest species *Leptinotarsa decemlineata* (Say)

79   (Colorado beetle) (Döring & Skorupski 2007; Otálora-Luna & Dickens 2011). It is probable that

80   this is also the case for storage pest beetles, including in *Sitophilus zeamais* (Motsch.), the maize

81   weevil, and Bostrichidae such as *Rhyzopertha dominica* (F.) and *Prostephanus truncatus* (Horn)

82   (lesser and larger grain borers). In these cases, combining colour cues with attractive odour cues

83   could enhance the effectiveness of storage pest traps. Past studies of these Coleoptera have most

84   often indicated the presence of three or four photoreceptor types, typically with UV-, blue-,

85   green- and red-sensitivity in the four-receptor species (Briscoe & Chittka 2001).

86

87   Food odours (wheat, maize, etc.) enhance attraction to pheromones in many species, so have

88   potential as low-cost attractants in traps (Likhayo & Hodges 2000; Bashir *et al.* 2001); but while

89   this may work well for *S. zeamais* (Ukeh *et al.* 2010), the evidence for the *R. dominica* and *P.*

90   *truncatus* responses to host odours is less clear (Fadamiro, Gudrups & Hodges 1998; Ukeh &

91   Umoetok 2007; Nguyen, Hodges & Belmain 2008). It is likely that for bostrichids, food is

92   discovered by chance by a few individuals, which then release aggregation pheromones to

93   attract conspecifics. Attraction to host odours may consequently play little or no role. It is also

94   virtually unknown whether, despite their small eyes, visual attraction may be more important in

95   these insects than previously assumed, and little work has been carried out to test this (Reza &

96   Parween 2006).

97

98   In the present study, we used a four-arm olfactometer to test the orientation of three pest beetle

99   species (*S. zeamais*, *P. truncatus*, *R. dominica*) towards host odours (from crushed white maize,

100 a common staple in African countries which suffers severe losses to beetle pests) and colour

101 cues (blue, yellow and green), with options to approach the cues singly (odour or colour) or in

102 combination (odour *and* colour), in order to evaluate their role in host orientation in these

103 species.

104

105 **Methods**

106

Insect cultures

Cultures of *S. zeamais* and *P. truncatus* from Malawi and *R. dominica* from Kenya were maintained in Kilner jars on 500 g organic wheat (*S. zeamais*, *R. dominica*) or 800 g yellow maize (*P. truncatus*), on a 14:10 light/dark cycle at 25°C and 60% rh (Jayasekara *et al.* 2005). For each generation, we removed a minimum of 100 mixed-sex adults to fresh medium for reculturing. For experiments, a subsample of the host material (derived from these multiple parents) was removed from the jar and kept in a plastic container (*S. zeamais*, *R. dominica*) or screw-topped Kilner jar (*P. truncatus*), and newly-emerged adults were removed daily, ensuring that the experimental individuals were of known age (ranging from 24 hours to 6 weeks). As experiments took place over several weeks for each replicate, it is unlikely that experimental individuals were closely related. After removal, we kept insects in temporary holding containers without food for at least 3 hours before experiments began in order to ensure motivation to orient towards food. Preliminary studies indicated that the precise period of food deprivation did not affect the response to host odours significantly in weevils or bostrichids.

Before an experiment, we sexed adults of *P. truncatus* and *S. zeamais* (using Shires and McCarthy (1976) and Dobie *et al.* (1991), respectively) so that this could be included in statistics as an explanatory variable. Determination of the sex of *R. dominica* individuals non-destructively is impractical, so unsexed individuals were used. Tests took place at 26 ± 2°C and ambient humidity (typically 30-55%).

Olfactometry

Insects were tested between 09:00 and 18:30 in a separate room to the insect culture room, using a four-arm olfactometer according to a similar paradigm to Arnold, Stevenson and Belmain (2012) (Fig. 1). A four-arm olfactometer allows simultaneous presentation of one or more odours, alongside areas without these odours, but also permits presentation of coloured stimuli to an insect by placing coloured surfaces on sections of the floor of the olfactometer arena. The setup also permits motion tracking via a camera, so insects' behaviour over a period of time can be monitored rather than merely recording their first decision. In this apparatus, each arm (quadrant) was attached to a gas-washing bottle; air was drawn out of the olfactometer and consequently through each arm, with each airstream being filtered over a charcoal filter (Agilent Technologies, Wokingham, Berks, UK) to remove environmental odours, and then drawn through a gas-washing bottle (empty or containing an odour stimulus) before it entered the arena. Components were connected with 0.6 mm Ø tubing (Tygon, Sigma-Aldrich, St Louis,

141   MO, USA). The arena was continuously video-recorded during the 10-minute monitoring period

142   for each insect, using a monochromatic camera connected to a desktop computer running

143   EthoVision 3.1 (Noldus, Spink & Tegelenbosch 2001). This is a motion-tracking software

144   application which can automatically calculate the percentage of time a beetle spends in each

145   quadrant of the arena. Lighting for experiments was provided by high-lux plant growth lamps

146   (irradiance in centre of room: 25.0 µmol m^{-2} s^{-1}; directly beneath camera: 6.5 µmol m^{-2} s^{-1}).

147

148   We presented both odour and colour stimuli simultaneously. In each of two of the gas washing

149   bottles there was 50 g of roughly crushed white maize bought commercially in Malawi (created

150   by placing whole grains in a clean plastic bag and then crushing using a hammer for three

151   minutes per 100 g sample). Maize was found to be the most attractive grain to *S. zeamais*

152   according to Trematerra *et al.* (2013), regardless of rearing medium, and therefore we tested

153   how responses to this might be modified in the presence of a different mode of stimulus. The

154   other two bottles in our experiment remained empty. Glassware items were washed with 70%

155   ethanol and allowed to dry if their contents were changed. Ukeh *et al.* (2012) identified that the

156   major odour components of maize eliciting positive responses from *S. zeamais* are hexanal, (E)-

157   2-heptenal, and octanal, and that when presented in a 3-odour blend, this combination is

158   attractive to this species. In all experiments, one arm of the arena had both colour and odour

159   cues present, one had colour alone, one odour alone, and one had neither the colour cue nor the

160   maize odour cue present. Air was drawn through the olfactometer at 800 ml/min and arms were

161   calibrated to ensure that airflow through each of the four arms was equal.

162

163   The colour stimuli were created by placing coloured paper on the floor of the arena, to cover

164   half the total arena surface (two adjacent quadrants); the other half was covered by plain white

165   paper (which may also have a distinct colouration to insect eyes), and both paper types were

166   then covered by a layer of UV-transparent clear plastic, ensuring that the walking surface was

167   consistent in texture and odour, regardless of the colour. Interior surfaces of the olfactometer,

168   including the floor, were cleaned down with industrial methylated spirits (IMS) after each insect

169   was tested, to minimize contamination of surfaces with insect odours.

170

171   We tested three different colours, appearing yellow, blue and green to human perception

172   (datasets involving each colour are henceforth referred to as "yellow trial", "blue trial", etc.).

173   The blue stimulus, with highest reflectance in the blue (400-500 nm) region generally and a

174   reflectance maximum at 490 nm represents a short-wavelength dominated stimulus of low

175 importance to the insects' ecology (and therefore is predicted to be of low attractiveness). The

176 green stimulus, with a maximum at 530 nm, corresponds to medium-wavelength dominance and

177 could be considered representative of fresh vegetation. Finally, the yellow stimulus, peaking at

178 590 nm, is a long-wavelength dominated stimulus and corresponds broadly to the colour of

179 cereals such as ripe wheat and maize and therefore is most ecologically-relevant. Spectral

180 reflectance curves for the coloured papers used are shown in Fig 2. We measured these using a

181 procedure as in Chittka & Kevan (2005) using an Avantes AvaSpec-2048 spectrophotometer

182 and an AvaLight-DH-S-BAL Deuterium-Halogen light source, calibrated relative to a $BaSO_4$

183 white standard (Avantes WS-2), using a fine probe (FCR-7UV200-2-1.5 x 100) at 45° to the

184 stimulus surface. The overall irradiance of the three colour stimuli was similar (relative to

185 $BaSO_4$ white standard: blue 94%, green 75%, yellow 95%), so a response to irradiance alone

186 should result in similar observed behaviour of the insects in the presence of all three colours, but

187 particularly blue and yellow. HSV (hue, saturation, brightness value) figures are provided in

188 Table 1 for the three colour paper types, indicative of their appearance to human eyes.

189

190 We food-deprived insects by keeping them in a container with no food present for between 3

191 and 48 hours (*S. zeamais*) or between 3 and 24 hours (*R. dominica*, *P. truncatus*, as these insects

192 show rapid decreases in motility if starved for more than 24 hours (Nguyen 2008)) to ensure that

193 they were motivated to seek food. We only tested insects that were active and showing no

194 difficulty walking. We tested 30 adults of *S. zeamais* in the yellow trial, 34 in the blue trial and

195 30 in the green trial. We tested 75 adults of *P. truncatus* and 75 of *R. dominica* in total, 25 per

196 species in each colour trial. We recorded each insect only once, and pseudo-randomized the

197 arrangement of odour and colour arms for each insect so that the odours and colours were never

198 consistently associated with any particular quadrant of the arena (one arm always had both cues,

199 one always had neither, and the other two had either odour *or* colour).

200

201 Because beetles may prefer darker areas of the arena rather than specifically the colour, or may

202 find the optical brighteners in white paper to be deterrent, we controlled for these possibilities.

203 We tested 15 insects of *S. zeamais* with a choice between white paper and grey paper (the same

204 white paper, but printed in uniform grey using a standard desktop printer) (spectrum in Fig. 2b),

205 in which the chromaticity of the paper was not altered but the paper reflected less light overall

206 (peak reflectance only 36.6% of the white paper peak). Secondly, we tested 15 further *S.*

207 *zeamais* with the white paper replaced by cream paper (spectrum in Fig. 2a) that still reflected

208 all wavelengths to some extent but lacked optical brighteners and did not fluoresce, versus

209   yellow paper as previously, to see whether the preference for yellow paper persisted or if it

210   ceased when the other stimulus lacked optical brighteners.

211

212   **Data analysis**

213   We performed Friedman, Wilcoxon and Generalized Linear Model (GLM) tests in SPSS (SPSS

214   Statistics 19, SPSS Inc., IBM, Chicago, IL, USA). These were performed to investigate both

215   whether insects showed a preference for the coloured *or* the maize-odour areas of the arena

216   (50% of the total arena in each case), but also to see whether there was maximal preference for

217   the multimodal quadrant of the arena containing both colour *and* maize odour. The effect of age

218   and/or sex on preferences was also tested by including them as variables (age in hours, sex as a

219   categorical variable) in a GLM analysis, which was performed using a quasibinomial

220   distribution with a logit link to analyse decisions about quadrants chosen.

221

222   **Results**

223

224   *Sitophilus zeamais*

225   Overall, beetles preferred the quadrants with the odour of maize present (55.7% of time spent

226   there; Wilcoxon test, odour $Z = 1.958$, $p = 0.050$) to quadrants without odour. They also tended

227   to prefer the coloured quadrants to white quadrants, spending 56.1% of their time in the two

228   coloured areas (Wilcoxon test, colour: $Z = 1.938$, $p = 0.053$). There were significant differences

229   between the time spent in the four arena quadrants, and beetles preferred the "multimodal"

230   quadrant containing both an odour and a colour cue over the other three quadrants (Friedman

231   test, $\chi^2 = 7.910$, p $= 0.048$) (Fig. 3).

232

233   Focusing on the individual colours tested, in the yellow trial insects significantly preferred the

234   odour of maize (66.0% of time spent in either maize alone or yellow + maize quadrants,

235   Wilcoxon test, $Z = 3.211$, $p = 0.001$). There were significant differences between the time spent

236   in the four arena quadrants, and the quadrant with both the yellow colour cue and the maize

237   odour cue was most favoured (36.6% of time spent there, Friedman test, $\chi^2 = 16.861$, $p = 0.001$).

238

239   Although insects in the blue trial spent more time in the presence of colour (56.7% of time in the

240   two coloured quadrants) and maize odour (56.4% of time) than away from the two types of

241   stimuli, these preferences were not significant (Wilcoxon test, colour: $Z = 1.188$, $p = 0.235$;

242   odour: $Z = 1.222$, $p = 0.222$). During the blue trial, *S. zeamais* individuals spent most time in the

243 multimodal quadrant containing both blue colour and maize odour, but the preference for this

244 quadrant was not significant (Friedman test, $\chi^2 = 1.735$, $p = 0.629$).

245

246 In the green trial, insects spent 60.0% of their time overall in one of the green-coloured

247 quadrants (Wilcoxon test, $Z = 1.643$, $p = 0.110$), but they did not differentiate between green

248 colour with an odour cue and green without. The green + maize quadrant was not significantly

249 more attractive than any of the others (Friedman test, $\chi^2 = 3.075$, $p = 0.380$). It appears that the

250 presence of both odour and colour in an area is, in general, attractive to *S. zeamais.* Yellow

251 colour, in particular, interacted with food odours to enhance attractiveness of an area to this

252 species.

253

254 There was a significant interactive effect of colour and sex on both preference for colour overall

255 and preference for the multimodal quadrant, with females showing a stronger preference for

256 green and yellow, especially the yellow multimodal quadrant, while males preferred blue more

257 strongly overall and the multimodal quadrant when it was blue (Generalized Linear Model

258 (GLM), colour: $F_2 = 4.555$, $p = 0.013$, multimodal: $F_2 = 3.395$, $p = 0.038$). Age did not affect

259 attraction to colour in general (GLM, $F_1 = 0.210$, $p = 0.648$), to the multimodal quadrant ($F_1 =$

260 0.411, $p = 0.523$) or to maize odours ($F_1 = 0.094$, $p = 0.760$).

261

262 ***Rhyzopertha dominica***

263 *R. dominica* adults did not prefer quadrants with maize odour over those without (blue: $Z =$

264 0.441, $p = 0.659$, yellow: $Z = 1.546$, $p = 0.122$, green: $Z = 0.405$, $p = 0.685$). They also did not

265 exhibit a preference for any of the colours tested (blue: $Z = 0.087$, $p = 0.931$, yellow: $Z = 1.180$,

266 $p = 0.238$, green: $Z = 0.789$, $p = 0.430$) (Fig. 4). Furthermore, the multimodal quadrant of the

267 olfactometer, containing both visual and odour cues, was not favoured: beetles spent only

268 23.6%, 20.0% and 26.2% of their time in the colour + maize quadrant in the blue, yellow and

269 green tests respectively (Friedman test, blue: $\chi^2 = 0.182$, $p = 0.980$, yellow: $\chi^2 = 3.248$, $p =$

270 0.355, green: $\chi^2 = 0.217$, $p = 0.975$). Beetles of differing ages did not respond differently to the

271 presence of colour in a quadrant (GLM, $t_{56} = 1.373$, $p = 0.184$), odour presence (GLM, $t_{47} =$

272 1.297, $p = 0.201$) or to the multimodal quadrant (GLM, $t_{57} = 1.313$, $p = 0.195$).

273

274 ***Prostephanus truncatus***

275 *P. truncatus* adults similarly showed no preference for the areas of maize odour (Wilcoxon test,

276 blue: $Z = 0.637$, $p = 0.524$, yellow: $Z = 0.629$, $p = 0.530$, green: $Z = 0.698$, $p = 0.485$). They also

277 showed no preference for coloured quadrants over uncoloured ones (Wilcoxon test: blue: $Z =$
278 0.968, $p = 0.333$, yellow: $Z = 0.448$, $p = 0.654$, green: $Z = 0.542$, $p = 0.588$) (Fig. 5). The
279 multimodal quadrant of the olfactometer with both odour and colour cues was not favoured over
280 any of the other three quadrants, with beetles spending only 11.4%, 33.8% and 30.7% of their
281 time in this quadrant in the blue, yellow and green trials respectively (Friedman test, blue: $\chi^2 =$
282 3.028, $p = 0.387$, yellow: $\chi^2 = 2.350$, $p = 0.503$ green: $\chi^2 = 0.167$, $p = 0.983$).

284 No sex or age differences were observed: females and males responded similarly to the colour
285 and odour quadrants compared to quadrants lacking stimuli (GLM, multimodal: $t_{64} = 1.034$, $p =$
286 0.305; colour: $t_{60}=1.961$, $p = 0.055$; odour: $t_{57}= 0.277$, $p = 0.783$). Likewise, individuals of
287 different ages did not show different responses to the presence of colour in a quadrant (GLM, t_{60}
288 $= 1.177$, $p = 0.244$), host odours (GLM, $t_{57} = 0.567$, $p = 0.573$) or to the multimodal quadrant
289 with maize and a colour cue presented together (GLM, $t_{64} = 0.763$, $p = 0.449$).

291 *Controls*
292 Results for the control tests are shown in Fig. 6. *S. zeamais* adults tested ($N = 27$) using the
293 control paradigm were not significantly different in their preference for coloured versus
294 "uncoloured" (white/cream) quadrants compared with those in the original yellow test (GLM, F_1
295 $= 2.534$, $p = 0.357$). This indicates that the yellow preference could not be accounted for by
296 simple repellent effects of optical brighteners as behaviour did not change when they were
297 removed from the experimental setup. In the test of preference for white against grey paper, they
298 showed no significant preference for either condition (Wilcoxon test, $Z = 0.369$, $p = 0.712$),
299 indicating that the insects do not simply orient towards the darker area of the arena.

301 **Discussion and Conclusions**

303 The majority of insects are believed to have some degree of functional colour vision (Briscoe &
304 Chittka 2001). In beetles, this is often found to be tri- or even tetrachromatic (based on three or
305 four different photoreceptor types), leading to good colour discrimination from UV to
306 orange/red parts of the spectrum. This is especially important for pollinating beetles such as
307 *Pygopleurus israelitus* (Muche) and South African monkey beetles that recognize brightly
308 coloured flowers by sight (Johnson & Midgley 2001; Van Kleunen *et al.* 2007; Martínez-Harms
309 *et al.* 2012), but the ability to discriminate visual cues using colour information is also relevant
310 for many phytophagous insects such as the Colorado beetle *Leptinotarsa decemlineata* (Döring

311 & Skorupski 2007) and pollen beetle (Döring *et al.* 2012). While storage pest beetles are often
312 crepuscular or nocturnal and spend significant periods in low or no light conditions, host
313 location is still required for population dispersal. Characterizing the importance of cues used in
314 host location is essential to devising informed control strategies.

315

316 Previous work has drawn equivocal conclusions about how *P. truncatus* and *R. dominica* orient
317 towards food. Whilst some studies have reported positive attraction in these beetle species
318 towards host (cereal) odours (Bashir *et al.* 2001; Edde & Phillips 2006), others have not
319 (Crombie 1941; Dowdy *et al.* 1993; Fadamiro, Gudrups & Hodges 1998; Nguyen, Hodges &
320 Belmain 2008). However, Edde & Phillips (2006) recorded the attraction across very small
321 distances (less than 5 cm) (Edde & Phillips 2006), and the experiment took place in an enclosed
322 petri dish in still air. This has the potential to saturate the environment with host odours and any
323 orientation might occur only at close range. As other studies such as Nguyen *et al.* (2008) and
324 Fadamiro *et al.* (1998) do not provide any evidence of this orientation in these species
325 (Fadamiro, Gudrups & Hodges 1998; Nguyen, Hodges & Belmain 2008), it is instead possible
326 that the insects only responded to high levels of volatiles, higher than what might be
327 encountered in nature. As we were unable to find evidence of any orientation to cereal odours in
328 these two bostrichids, our work supports the view that *R. dominica* and *P. truncatus* will most
329 likely encounter host food entirely by chance rather than depend upon host odour mediated
330 orientation. Recruitment via aggregation pheromones (Dowdy *et al.* 1993; Fadamiro, Gudrups &
331 Hodges 1998; Bashir *et al.* 2001) as the major method of host location rather than orientation to
332 host odours is also typical behaviour in some other wood boring beetles (Schlyter *et al.* 1987;
333 Raffa, Phillips & Salom 1993); however, the situation can grow more complex in some species
334 where there are interactive effects with sex pheromones, heterospecific odour signals and host
335 kairomones, whereas other bark beetle species rely primarily on visual cues for initial
336 approaches to host material (Raffa, Phillips & Salom 1993). Conversely, we were able to detect
337 preference for maize odours in *S. zeamais*, which is in agreement with other studies (Ukeh *et al.*
338 2010; Ukeh *et al.* 2012); thus, our experimental protocol was able to detect an orientation effect
339 when present. This provides a useful comparative view, showing that *S. zeamais* uses host cues
340 to aid in food location whilst the bostrichids *R. dominica* and *P. truncatus* do not. Unlike
341 Trematerra *et al.* (2013), we did not find a difference between males and females in their
342 responses to maize odours but this could be attributed to a difference in the strain of insect, or
343 precise volatile composition of the maize varieties used. Further research to understand this
344 better is indicated.

345

346   We add to the findings on odour mediated behaviours the novel result that *P. truncatus* and *R.*

347   *dominica* are also not chromotactic: we found no evidence of colour-mediated host-finding in

348   these species, further supporting the above hypothesis that *P. truncatus* and *R. dominica* locate

349   food by chance and then attract conspecifics with aggregation pheromone. We argue that it is

350   precisely this evolutionary heritage of random host finding and reliance on aggregation

351   pheromone, that best explains why *P. truncatus* and also *R. dominica* are seemingly unaffected

352   by any host odours or colour cues.

353

354   We show that the response of *S. zeamais* to host odour was enhanced by the presence of some

355   colour stimuli. *S. zeamais* orientated towards quadrants containing any colour stimuli in

356   preference to white paper. These results could not be accounted for either by a negative

357   phototactic response causing orientation towards darker areas of the arena, or a repellency effect

358   of optical brighteners found in commercially-available printer paper, suggesting that the

359   chromatic composition of the stimuli were the cause of the observed results. The weevils were

360   most responsive to yellow (in this case, yellow including UV reflectance (Fig. 2)). This may

361   resemble the colour of ripe grain (wheat, maize) and, therefore, have adaptive significance, or

362   could be a function of the neurophysiology, as is the case with aphids' probing responses when

363   moving between differently-coloured areas (Döring & Chittka 2007). Pollen beetles exhibit a

364   similar preference for yellow stimuli as a result of their green-blue colour opponency (Döring *et*

365   *al.* 2012). Whether or not this preference extends to UV-absorbing yellow shades, which can be

366   easily discriminated from UV-reflecting yellow in other insects previously tested (Menzel *et al.*

367   1988; Menzel *et al.* 1989; Dyer & Chittka 2004; Döring & Skorupski 2007; Döring *et al.* 2012)

368   needs to be investigated.

369

370   *S. zeamais* showed differences between males and females in the strength of colour preferences,

371   unlike the two bostrichid species. It is not possible to determine from the current data whether

372   this is a difference in physiological capability (e.g. males having different photoreceptors) or

373   processing (females and males perceiving the same cues but responding differently to them). It

374   may be that females are better-adapted for locating food, favouring responses to yellow and

375   green cues, while males are better-adapted for finding mates and a blue preference aids

376   dispersal.

377

The results of this study show that colour cues are detected by *S. zeamais* and influence their behaviour. This has significant implications for the development of novel control or, particularly, monitoring strategies and also for experimental procedures on storage pests. If visual stimuli can affect the responses of insects, this must be controlled for in all experiments investigating other factors. Equally, when investigating control strategies such as insecticide-treated bags or pheromone lures, the effect of colour should be considered.

The use of yellow material may serve to enhance the effectiveness of mass-traps for monitoring (or perhaps, but less importantly, direct control) of *S. zeamais* in cereal stores. There is still considerable scope to refine and develop monitoring traps for specific storage pests, and this species' responses to odour and colour cues makes it particularly amenable to such methods. Odour and colour cues could potentially be incorporated in low-cost traps for deployment in poor rural areas in developing countries. Similarly, avoiding attractive colours of materials for grain storage facilities and equipment, e.g. promoting blue or white bags over yellow ones, may offer additional protection for stores. Focus now should be on optimizing the combination of colour and odour, possibly also including pheromone lures, to obtain the most attractive assemblage of cues.

Acknowledgements

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530

531 **Figure legends**

532

533 **Fig. 1 | Olfactometer, showing the stimulus administration via airflow through one of the**

534 **four arms.** The pump draws air from the central chamber, and thus through each of the arms

535 with an odour stimulus attached. Flow rate was checked regularly, ensuring that it was 200

536 ml/min through each arm (800 ml/min through the central hole). A gas-washing bottle

537 connected to a charcoal filter was attached to each of the four arms of the olfactometer.

538

539 **Fig. 2 | Spectral reflectance curves for (a) the colour stimuli for the main experiment and**

540 **the control cream stimulus and (b) the stimuli containing optical brighteners: white and**

541 **grey, and (c) photographs of samples of the stimuli.** Spectral reflectance values were

542 measured using an Avantes AvaSpec-2048 spectrophotometer with an AvaLight-DH-D-BAL

543   Deuterium-Halogen light source, relative to a BaSO$_4$ white standard. B = blue, G = green, Y =

544   yellow, Wh = white, Gy = grey, C = cream.

545

546   **Fig. 3 | Colour and odour preferences of *Sitophilus zeamais* when presented with maize**

547   **odours and green, blue or yellow coloured areas on the arena floor.** Yellow was

548   significantly attractive in combination with maize odour. Bars show mean ± SEM. *N* = blue: 34,

549   yellow: 30, green: 30.

550

551   **Fig. 4 | Colour and odour preferences of *Rhyzopertha dominica* when presented with maize**

552   **odours and green, blue or yellow coloured areas on the arena floor.** No significant odour or

553   colour preferences were observed. Bars show mean ± SEM. *N* = blue: 25, yellow: 25, green: 25.

554

555   **Fig. 5 | Colour and odour preferences of *Prostephanus truncatus* when presented with**

556   **maize odours and green, blue or yellow coloured areas on the arena floor.** No significant

557   odour or colour preferences were observed. Bars show mean ± SEM. *N* = blue: 25, yellow: 25,

558   green: 25.

559

560   **Fig. 6 | Control results (mean ± SEM) for *S. zeamais* presented with (a) yellow versus**

561   **cream floor covers and (b) white versus grey floor covers.** Yellow remains significantly

562   attractive, whilst the insects showed no preference for white or grey, indicating that

563   chromaticity, rather than overall reflectance, is responsible for the behaviour. *N* = yellow/cream:

564   15, white/grey: 15.

565

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