

**Plant volatiles and oviposition behavior in the selection of
barley cultivars by wheat stem sawfly (Hymenoptera:
Cephalidae)**

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12 **Plant volatiles and oviposition behavior in the selection of barley cultivars by wheat stem**

13 **sawfly (Hymenoptera: Cephidae)**

14

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26

27 **Abstract**

28 Wheat stem sawfly, *Cephus cinctus* Norton, females display complex behaviors for host selection
29 and oviposition. Susceptible hollow stem wheat (*Triticum aestivum* L.) cultivars release a greater
30 amount of attractive compound, (Z)-3-hexenyl acetate and receive a greater number of eggs
31 compared to resistant solid stem wheat cultivars. However, barley (*Hordeum vulgare* L.) is
32 becoming a more common host for *C. cinctus* in Montana. Therefore, one question that arises is
33 how similar are host selection and oviposition behaviors on barley cultivars compared to wheat
34 cultivars? To answer this question, we carried out greenhouse experiments using two barley
35 cultivars: 'Hockett' and 'Craft.' Between these cultivars at Zadoks scale 34 and 49, we compared
36 host selection decision using a Y-tube olfactometer, compared oviposition behaviors on stems,
37 and counted the number of eggs inside individual stems. In Y-tube bioassays, we found a
38 greater number of *C. cinctus* females were attracted to the airstream passing over 'Hockett'
39 when the choices were the airstreams passing over 'Craft' and 'Hockett.' Although the
40 frequencies of oviposition behaviors were similar between these cultivars, the number of eggs
41 was greater in 'Hockett.' Volatile profiles indicated that the amount of linalool was greater in the
42 airstream from 'Craft' than in 'Hockett' at Zadoks stage 34 while the amount of (Z)-3-hexenyl
43 acetate, was greater in airstream from 'Hockett' at both Zadoks 34 and 49. These results suggest
44 that volatiles of barley plants strongly influenced host selection behavior of ovipositing *C.*
45 *cinctus* females, while discriminating behaviors do not differ between cultivars.

46

47 **Keywords:** Y-tube olfactometer, linalool, (Z)-3-hexenyl acetate, *Cephus cinctus*, Barley

48

49 **Introduction**

50 In insects, host selection behaviors are complex and often repeated in a fixed order (Atkins
51 1980). For oviposition, these behaviors are governed by several factors, such as offspring survival
52 rate (Gripenberg et al. 2010), host plant defense mechanisms, environmental factors, plus adult
53 longevity and capacity for dispersal (Larsson and Ekbohm 1995). At first, the female insect
54 discriminates between non-host and host plants, and subsequently distinguishes the most
55 suitable plants within the range of available host plants (Schoonhoven et al. 2005). To identify a
56 suitable host, the insect first evaluates a blend of volatile compounds that perceive from the
57 host plants (Cha et al. 2011) and subsequently evaluates the plant architecture (Rudgers and
58 Whitney 2006). These behaviors are less refined in polyphagous insects compared to
59 oligophagous insects (Gripenberg et al. 2010).

60 One example of an oligophagous insect species is the wheat stem sawfly, *Cephus*
61 *cinctus* Norton (Hymenoptera: Cephidae). This species causes serious economic losses in wheat
62 (Beres et al. 2011a), and more recently, in barley (Varella et al. 2018) crops grown on the
63 Northern Great Plains of the USA and the Canadian Prairie provinces. The economic loss caused
64 by this species is due to larval feeding injuries and stem cutting by mature larvae. Once the crop
65 reaches its maturity, the larva descends to the root crown to make a hibernaculum for
66 overwintering (Criddle 1923). Before making a hibernaculum, the larva makes a circular notch
67 that encircles the stem internally and makes the stem susceptible to lodging in response to wind
68 and gravity (Ainslie 1929, Weiss and Morrill 1992). Lodged stems are not easily collected by
69 combine harvesters and cause major losses (Holmes and Peterson 1965, Beres et al. 2007,
70 Bekkerman and Weaver 2018). Approximate annual economic loss caused by this species in

71 Montana is estimated between US \$40–80 million (Fulbright et al. 2017) with annual losses in
72 North America estimated at US \$350 million (Beres et al. 2011a).

73 Several management tools are in practice to reduce *C. cinctus* losses, but the
74 effectiveness of these tools is variable and limited. For instance, insecticide applications are
75 generally ineffective (Knodel et al. 2009), while several tillage operations — more than one is
76 required to expose overwintered larvae — increase soil erosion and nutrient losses in the field
77 (Morrill et al. 1993) and reduce the survival rate of specialist parasitoids (Runyon et al. 2002). The
78 parasitoid species tend towards locally profound effects but have limited overall impact (Morrill
79 et al. 1998, Weaver et al. 2004, 2005, Portman et al. 2018, Achhami et al. 2020b). A foundation
80 for management is afforded by planting resistant solid-stem wheat cultivars (Beres et al. 2011b,
81 Varella et al. 2015), but these can vary in effectiveness in response to environmental conditions
82 (Holmes 1984, Beres et al. 2017). In a manner similar to stem solidness in wheat, some hollow
83 stem barley cultivars kill a greater number of neonates at the site where they emerge from the
84 egg (Achhami et al. 2020a, b, c, Varella et al. 2018) but specific cultivar traits that are associated
85 with killing neonates have yet to be explored. The constrained success of these approaches has
86 led to a search for alternative tools to be used in IPM (Morrill et al. 2001, Weaver et al. 2004,
87 Beres et al. 2009).

88 Although all the above-mentioned management tools are a component of IPM, plant
89 resistance centered IPM could be the best option for *C. cinctus* management because of its
90 unique biology. First, the larvae cannot leave a host stem where they hatch. Second, obligate
91 larval cannibalism occurs when multiple eggs are placed within a stem (Wallace and McNeal
92 1966, Buteler et al. 2015, Achhami et al. 2020b). Cannibalism occurs because of ovipositing *C.*

93 *cinctus* females display a surprising behavior where females are not able to discriminate hosts
94 that are already oviposited in by conspecific females (Buteler et al. 2009). Third, females oviposit
95 in a wide range of host plants irrespective of their larval survival. For example, larval survival
96 rates in solid stem wheat cultivars (Varella et al. 2015, Talbert et al. 2014), downy brome (Perez-
97 Mendoza et al. 2006), and barley (Varella et al. 2018) are lower than survival rates in susceptible
98 hollow stem wheat cultivars and there is effectively no survival in oat, *Avena sativa* L. (Criddle
99 1922, Weaver et al. 2004). Finally, in wheat, ovipositing females are attracted to cultivars that
100 release a greater amount of (*Z*)-3-hexenyl acetate (Weaver et al. 2009), among other attractive
101 compounds (Piesik et al. 2008). In essence, larval survival and the size of the next generation are
102 largely dependent on the host choices made by the ovipositing females. This unique biology
103 indicates the significance of host plant resistance for this species — specifically resistance factors
104 that influence the host selection and oviposition behaviors in plant-centered IPM.

105 We selected barley as a host to study host selection and oviposition behaviors of *C.*
106 *cinctus* for several reasons. First, *C. cinctus* is native to the northern part of the Northern Great
107 Plains (Leisure et al. 2016), but this species is widening its geographic adaptation to the
108 southern part of the United States (Bekkerman and Weaver 2018). Second, barley production in
109 Montana is the third highest in the United States (US Department of Agriculture 2020) and
110 barley is becoming a more common host to *C. cinctus*. Third, in the past, barley was described as
111 a resistant crop to *C. cinctus* (Farstad and Platt 1946), but recent studies have reported that both
112 infestation rate and larval survival rate in barley are in increasing (Varella et al. 2018, Achhami et
113 al. 2020c). Therefore, the question arises does the ovipositing female display a similar host
114 selection and oviposition behaviors as is known for wheat cultivars? Consequently, we initiated a

115 study in the greenhouse using two barley cultivars: 'Craft' and 'Hockett.' 'Hockett' has a greater
116 number of eggs per infested stem and is therefore a more susceptible cultivar, whereas 'Craft'
117 has both a lower number of eggs and greater larval mortality due to antibiosis and is therefore a
118 more resistant cultivar (Achhami et al. 2020 a, b, c).

119

120 **Materials and Methods**

121 **Insect Preparation**

122 For the experiments, we acquired adult *C. cinctus* from wheat stubs that contained overwintering
123 diapause larvae. Stubs were collected from a wheat field near Amsterdam, MT, USA
124 (45°45'29.85" N, 111°22'49.32" W) and were maintained in cold storage (0–4°C) for 3–6 months
125 to allow them to complete diapause. For adult emergence, the stored stubs were transferred
126 into plastic Tupperware® boxes (Tupperware Corporation; Orlando, FL, USA) (70 × 35 × 20 cm)
127 and held at room temperature (22–27°C) for 4–5 weeks. Newly emerged adults were held in 2 L
128 Ball® Mason glass jars (Jarden Home Brands, Daleville, IN, USA) that contained moistened filter
129 paper and several wooden skewers to allow the adults to perch, rest, and climb until used for
130 experiments. We used 24–48 h old adults for the experiments.

131

132 **Plant Culture**

133 Two barley cultivars, 'Hockett' (PI 657121) and 'Craft' (PI 646158), developed by Montana State
134 University, were used in the experiment. These cultivars were grown as single plants in tapered
135 plastic cone-tainers (6.35 cm diameter top × 25.4 cm deep) in a greenhouse at the Montana
136 State University Plant Growth Center (MSU-PGC) in Bozeman, MT USA. We used MSU-PGC soil

137 mix (equal parts of sterilized Bozeman silt loam soil and washed concrete sand with Canadian
138 sphagnum peat moss incorporated) and Sunshine Mix#1 (Canadian sphagnum peat moss,
139 perlite, vermiculite, and Dolomite lime: Sun Gro Horticulture, Bellevue, WA, USA) in a 1:1 ratio as
140 the medium for plant growth. Both natural light and supplemental artificial light (GE Multi-Vapor
141 Lamps model MVR 1000/C/U, GE Lighting, General Electric Company, Cleveland, OH) were used,
142 with a photoperiod of 14:10 (L:D) h, $22 \pm 2^\circ\text{C}$ and 20–40% RH. Plants were watered daily and
143 fertilized twice a week with Peters General Purpose Fertilizer (J.R. Peters, Allentown, PA) at 100
144 ppm in aqueous solution.

145

146 **Y-tube Olfactometer Bioassay**

147 We conducted Y-tube olfactometer bioassay using a procedure similar to one first illustrated in
148 Daisy et al. (2002) with modifications as described in Piesik et al. (2008) and Varella et al. (2016).
149 The bioassay was used to evaluate whether *C. cinctus* females recognized and were attracted to
150 volatiles emanating from live plants. We tested the relative attraction of *C. cinctus* females to the
151 volatile compounds present in the airstreams passing over the two barley cultivars (Hockett and
152 Craft), or those from a single cultivar versus a pure air source that served as the control.
153 Humidified air was delivered through a charcoal filter and then split into two streams using a
154 threaded 24/410 (inner diameter 24-mm) cap with a Teflon liner coupled to a 0.64 cm Swagelok
155 union to deliver air to a pair of glass chambers (40-mm diameter and 800-mm long). Each glass
156 chamber enclosed a single barley plant in a pot or a pot without a plant that purified air passed
157 over to establish the odor sources. An enhanced spectrum LED grow light (Sunshine Systems
158 Grow UFO Light SS–Gu90W; Burton, OH) was used to approximate field light levels. We sealed

159 the base of the plant stem with a Teflon sleeve to prevent unfiltered air from entering the
160 system. Next, Teflon tubing delivered the air from the odor source chambers to each arm of the
161 Y-tube. Stimulus odors were conveyed to each arm of the Y-tube via glass tubes. For consistent
162 air flow, we used an airflow of 0.1 L/min. Tests were conducted in a 28-mm diameter × 300-mm
163 long Corning glass tubing that branched at 20 cm, had an interior angle at the "Y" of 120°, with
164 diverging arms that extended for 4 cm in each direction before becoming parallel to each other.
165 The length of parallel arms measured 10 cm. We used a fiber optic illuminator (T-Q/FOI-1,
166 TechniQuip Corp, EL Segundo, CA, USA) at the center of the Y-tube arms (Varella et al. 2016).

167 For bioassays, we used either a pair of 'Hockett' and 'pure air,' or 'Craft' and 'pure air,' or
168 'Hockett' and 'Craft,' as test stimuli. We used plants that were at the Zadoks 34 growth stage
169 (Zadoks et al. 1974). This point in plant development is early in stem elongation, which is
170 synchronized with the initiation of *C. cinctus* oviposition in the field.

171 We released individual females at the basal unbranched section of Y-tube and placed a wire
172 (about 10 cm long) from the release point to the bifurcation to facilitate female movement
173 upwind toward the junction of Y-tube. Each released female was observed for 5 min or until it
174 entered one of the two arms of the Y-tube olfactometer. We repeated this process for 10 trials
175 of 'Hockett' versus 'Craft'; five trials of 'Hockett' versus 'pure air' and seven trials of 'Craft' versus
176 'pure air.' For each trial, we tested 13–20 females, using a new Y-tube for each female.

177

178 **Volatile Organic Compounds Collection and Quantification**

179 We collected and quantified volatile organic compounds (VOCs) from the barley plants as first
180 described in Piesik et al. (2006). Briefly, plant volatiles were collected by pulling air through a

181 volatile collection trap for 6-h between 1000 and 1600 h. Plants of each cultivar were enclosed in
182 a 40-mm diameter and 800-mm length glass volatile collection chamber. The glass chamber was
183 attached to a volatile collection port and was open on the other end to enclose the plant. Glass
184 volatile collection traps (6.35-mm OD x 76-mm long; Sigma Scientific, Micanopy, FL) containing
185 30 mg of HayeSeq-Q adsorbent (Alltech Associates, Deerfield, IL) were inserted into each volatile
186 collector port. In each glass chamber, purified and humidified air was supplied at a rate of 1.0
187 L/min. Airflow was maintained by a regulated vacuum pump at the same flow rate. We used a
188 Teflon guillotine to encircle the base of the plant stems; it was taped to the glass chamber to
189 prevent outside air from entering the system.

190 To elute the traps, we used 200 μ l dichloromethane and collected the aliquot in a glass
191 insert within a 1.5 ml screw-top glass vial using gentle pressure from purified nitrogen gas.
192 Thereafter, we added 10 μ l of the internal standard, nonyl acetate (Sigma-Aldrich, Milwaukee,
193 WI), in a dichloromethane solution. The collected samples were analyzed by gas
194 chromatography-mass spectrometry (GC-MS: Agilent 6890 instrument; Agilent Technologies,
195 Santa Clara, CA, USA) fitted with a fused silica-column (30 m \times 0.25-mm) with a 0.25 μ m DB-5
196 stationary phase. The oven was held at 50°C for 4 min after injection into a 250°C port and
197 increased at 5°C per min to 160°C, followed by a 25°C per min ramp to 280°C for 3 min, with the
198 carrier gas maintained at a flow rate of 1.2 L/min. The samples were injected onto the column in
199 pulsed-splitless mode that had an initial pressure of 0.84 kg/cm for 1 min. A constant
200 temperature of 300°C was maintained on the transfer line to the mass selective detector (MSD).
201 Compound detection in the eluted samples was performed by mass selective detector (MSD,

202 Agilent 5973: Agilent Technologies) in electron-impact ionization mode scanning masses 10–
203 300.

204 We identified the VOCs by comparing the mass spectrum in the NIST mass spectra library
205 (Rev D. 02.00) and retention times of authentic standards. We reported and compared
206 compounds with a matching score >900 in the NIST library. We quantified the compounds in
207 reference to above-ground plant biomass (g) as ng/g/h.

208 On each day of volatile collection, a set of six plants of each cultivar were randomly
209 arranged within the 12-chamber volatile collection system. We repeated the collections four or
210 five times for plants at Zadoks 34 and 49 in 2018 and five times for both growth stages in 2020.

211

212 **Oviposition Behaviors**

213 We recorded *C. cinctus* oviposition behaviors as described by Buteler et al. (2009) and Varella et
214 al. (2017). For oviposition behaviors, two plants (one 'Hockett' and one 'Craft') were placed in a
215 46.5 × 46.5 × 91 cm cage with 530-µm mesh openings (BioQuip products; Rancho Dominguez,
216 California, USA). In each cage with a designated pair of plants, we released 10 females and 5
217 males of *C. cinctus* and allowed them to mate and then oviposit. The behavior experiment was
218 conducted for three hours (1000 to 1300 h) in the greenhouse. After releasing *C. cinctus* inside
219 the cage, we recorded the frequencies and duration of each behavior for the females on both
220 plants. As described by Buteler et al. (2012), we also summarized these behaviors into four
221 categories: 1) resting (did not move but sometimes rubbed antennae), 2) walking on the leaves
222 and stems, 3) abdominal tapping (several light strikes of the ovipositor on the stem), and 4)
223 ovipositor insertion. We compared both frequency and duration of each behavior between the

224 two cultivars within a similar developmental stage (Zadoks stage 34 or 49). We used 10 pairs of
225 plants that were at Zadoks 34 and 6 pairs of plants that were at Zadoks 49.

226

227 **Oviposition Choice**

228 Oviposition choice tests were performed to study *C. cinctus* oviposition preferences between
229 two cultivars. For this study in the greenhouse, we used screen cages (46.5 × 46.5 × 91 cm) with
230 530 μm mesh openings (BioQuip Products; Rancho Dominguez, CA). Each cage contained one
231 plant of each cultivar at a similar developmental stage at Zadoks 34 or 49. Ten females and 5
232 males were released into each cage and we allowed them to mate and to oviposit for three days.
233 Thereafter, we removed the plants from the cage, dissected the stems, and counted the number
234 of eggs. In total, we used 25 pairs of plants at Zadoks 34 and 16 pairs of plants at Zadoks 49.

235

236 **Data Analyses**

237 The results from Y-tube olfactometer assays were analyzed using a Chi-square test for small
238 sample sizes (Sokal and Rohlf 1995). For the oviposition choice-test trials, the number of eggs
239 per plant for each cultivar at each developmental stage were analyzed using linear and nonlinear
240 mixed effects models (nlme) (Pinheiro et al. 2019). Replicate was used as a random factor, and
241 stem height and stem diameters were used as covariates in the model. The response variable
242 "eggs per plant" was transformed using $\log(x + 2)$ before analysis to achieve normality of the
243 residuals, but untransformed data are presented. Further, mean stem height, and stem diameter
244 between two cultivars and between two developmental stages were analyzed with a t-test and a
245 Wilcoxon rank sum test respectively.

246 We used a factorial multivariate analysis of variance (MANOVA) to determine differences
247 among the VOCs emitted by cultivars at two developmental stages. Wilks' lambda test statistic
248 was used to determine a significant main effect in the MANOVA. One-way ANOVAs were
249 conducted to further analyze the differences between the cultivars within plant stage for each
250 compound. Amounts of volatiles were transformed using $\log(x+2)$ to better achieve normality,
251 but untransformed data are presented.

252 For oviposition behaviors, we removed the resting behavior from our analysis because half
253 of the females ($n = 7$) remained inactive (only displayed resting behavior) during the entire
254 observation period. Durations of specific oviposition behaviors (i.e., walking, abdominal tapping,
255 and ovipositor insertion) were analyzed using linear and nonlinear mixed effects models (nlme)
256 (Pinheiro et al. 2019). Fixed effects included cultivars and plant stage, while day of experiment
257 was a random factor. The duration data were transformed using a Box-Cox transformation (Box
258 and Cox 1964), but untransformed data are presented. The frequencies of each oviposition
259 behavior were subjected to a Chi-square test to distinguish between the cultivars and between
260 the two plant stages. All analyses were conducted in the computing interface R (R Core Team,
261 2019).

262

263 **Results**

264 **Host Selection Bioassay in Y-tube Olfactometer**

265 In Y-tube olfactometer bioassays, a greater number of females were attracted to the airstream
266 passing over 'Hockett' when the choices were 'Hockett and 'Pure air' ($\chi^2 = 5.70$, $df = 1$, $P = 0.02$);
267 and were also attracted to the airstream passing over 'Craft' when the choices were 'Craft' and

268 'Pure air' ($\chi^2 = 4.67$, $df = 1$, $P = 0.03$) (Fig 1). In comparisons between the two cultivars, females
269 were more attracted to 'Hockett' when stimuli choices were the airstream passing over either
270 'Craft' or 'Hockett' ($\chi^2 = 5.23$, $df = 1$, $P = 0.02$) (Fig. 1). Females took a similar interval (in
271 seconds) to select a preferred stimulus between the Y-tube arms for 'Hockett' (mean \pm SE:
272 120.42 ± 8.66) and 'Craft' (139.83 ± 13.42) (Kruskal-Wallis $\chi^2 = 1.47$, $df = 1$, $P = 0.23$).

273

274 **Barley Plant Biomass and Volatile Organic Compounds**

275 By year and cultivar, the above ground plant biomass was similar between two barley cultivars
276 within a similar developmental stage (Zadoks 34: cultivar*year: $F = 0.77$; $df = 1$, 110; $P = 0.37$,
277 cultivar: $F = 0.07$; $df = 1$, 101, $P = 0.77$; Zadoks 49: cultivar*year: $F = 0.87$; $df = 1$; 80, $P = 0.35$,
278 cultivar: $F = 0.91$; $df = 1$, 80; $P = 0.34$) (Fig. 2).

279 At Zadoks 34, the amounts of the compounds β -phellandrene: $F = 8.86$; $df = 1$, 101; $P =$
280 0.003 ; linalool: $F = 19.46$; $df = 1$, 101; $P < 0.001$ were different by year (Fig. 2). At Zadoks 49, the
281 compounds β -phellandrene: $F = 5.56$; $df = 1$, 80; $P = 0.02$; (*Z*)-3-hexenyl acetate: $F = 7.5$; $df = 1$,
282 80; $P = 0.007$; D-limonene: $F = 4.51$; $df = 1$, 80; $P = 0.03$; α -terpinene, $F = 6.29$; $df = 1$, 80; $P =$
283 0.01 ; β -caryophyllene: $F = 3.77$; $df = 1$, 80; $P = 0.05$ were also different by year (Fig 2).

284 At Zadoks 34, the amounts of the compounds β -phellandrene ($F = 11.58$, $P < 0.001$), (*Z*)-
285 3-hexenyl acetate ($F = 6.64$; $df = 1$, 101; $P = 0.01$), D-limonene ($F = 4.52$; $df = 1$, 101; $P = 0.03$),
286 and linalool ($F = 20.99$; $df = 1, 101$; $P < 0.001$) were different between cultivars; however other
287 compounds such as nonanal ($F = 0.28$; $df = 1$, 101; $P = 0.59$), α -terpinene $F = 1.19$; $df = 1$, 101; P
288 $= 0.27$), α -terpineol ($F = 2.62$; $df = 1$, 101; $P = 0.10$), decanal ($F = 0.62$; $df = 1$, 101; $P = 0.43$), α -
289 copaene ($F = 0.14$; $df = 1$, 101; $P = 0.69$), α -elemene ($F = 0.09$; $df = 1$, 101; $P = 0.76$), β -

290 caryophyllene ($F = 0.24$; $df = 1, 101$; $P = 0.62$), and α -caryophyllene ($F = 1.36$; $df = 1, 101$; $P =$
291 0.24) were similar between the two cultivars (Fig 2).

292 At Zadoks 49, the amounts of the compounds β -phellandrene ($F = 9.27$; $df = 1, 80$; $P =$
293 0.003), (*Z*)-3-hexenyl acetate ($F = 9.06$; $df = 1, 80$; $P = 0.003$), D-limonene ($F = 6.02$; $df = 1, 80$; P
294 $= 0.01$), α -terpineol ($F = 4.85$; $df = 1, 80$; $P = 0.03$), α -copaene ($F = 8.83$; $df = 1, 80$; $P = 0.03$),
295 decanal ($F = 4.96$; $df = 1, 80$; $P = 0.02$), and β -caryophyllene ($F = 4.87$; $df = 1, 80$; $P = 0.03$) were
296 different between two cultivars; however, the amounts of linalool ($F = 3.40$; $df = 1, 80$; $P = 0.07$),
297 nonanal ($F = 95$; $df = 1, 80$; $P = 0.33$), α -elemene ($F = 2.31$; $df = 1, 80$; $P = 0.13$), and α -
298 caryophyllene ($F = 1.41$; $df = 1, 80$; $P = 0.23$) were similar between these two cultivars (Fig. 2).

299

300 **Oviposition Behaviors and Host Preference**

301 The frequencies of the remaining three oviposition behaviors (walking, abdominal tapping, and
302 ovipositor insertion) were similar between the two cultivars at Zadoks 34 (Table 1). However, the
303 walking and abdominal tapping frequencies were significantly greater on 'Hockett' stems than
304 on 'Craft' stems at Zadoks 49 (Table 1).

305 The duration of abdominal tapping events was not significantly different between the two
306 cultivars ($F = 1.21$; $df = 1, 34$; $P = 0.27$) at Zadoks 34, but was significantly longer on the plants
307 that were at Zadoks 49 than on plants that were at Zadoks 34 ($F = 4.19$; $df = 1, 34$; $P = 0.04$)
308 (Table 2). Finally, the duration of both walking and ovipositor insertion events were similar
309 different two cultivars (walking: $F = 0.34$; $df = 1, 34$; $P = 0.46$; ovipositor insertion: $F = 0.38$; $df =$
310 $1, 34$; $P = 0.53$) (Table 2).

311

312 **Host Plant Preference for Oviposition**

313 The number of eggs per stem was significantly greater in 'Hockett' than in 'Craft' at both growth
314 stages (Zadoks 34: $F = 10.13$; $df = 1, 92$; $P = 0.002$; Zadoks 49: $F = 6.50$; $df = 1, 113$; $P = 0.01$)
315 (Table 3). Mean stem height was similar in both cultivars within each growth stage (Zadoks 34:
316 Wilcoxon rank sum value = 1102, $P = 0.24$; Zadoks 49: Wilcoxon rank sum value = 1998.5 $P =$
317 0.27) (Table 3). But mean stem diameter was greater in 'Craft' than in 'Hockett' within each
318 growth stage (Zadoks 34: Wilcoxon rank sum value = 2005.5 $P < 0.001$; Zadoks 49: Wilcoxon rank
319 sum value = 2755.5 $P < 0.001$) (Table 3). The number of tillers were not different by cultivar
320 within each growth stage (Zadoks 34: $t = 0.67$; $df = 20$; $P = 0.51$; Zadoks 49: $t = 0.65$; $df = 18$; $P =$
321 0.52).

322

323 **Discussion**

324 Volatile compounds from barley influenced host selection and oviposition behaviors of *C.*
325 *cinctus*. We found 'Hockett' released a greater amount of (*Z*)-3-hexenyl acetate compared to
326 'Craft' (Fig. 2); subsequently, a greater number of ovipositing females were attracted to airstream
327 passing over 'Hockett' than the airstream passing over 'Craft' in the Y-tube olfactometer
328 bioassay (Fig. 1), and recorded a greater number of eggs per stem in 'Hockett' than in 'Craft'
329 (Table 3). Most of our results are aligned with previously reported results for wheat crops where
330 host selection and oviposition behaviors are influenced by host plant phenological traits—stem
331 diameter, volatile compound profiles, and plant growth stage (Buteler et al 2012, Weaver et al.
332 2009). Additionally, 'Hockett' was the preferred cultivar over 'Craft' which is a similar result to
333 that reported in barley field experiments in which 'Hockett' also received a greater number of
334 eggs than 'Craft' (Achhami et al. 2020b, c).

335 Briefly, the host selection process involves a series of steps: searching, recognizing,
336 selecting, and accepting or ovipositing are just a few names (Schoonhoven et al. 2005). Olfactory
337 cues that perceive from a host plant play a key role in discriminating between host plants and
338 non-host plants (Cardé and Willis 2008). Our Y-tube olfactometer bioassay proved that *C. cinctus*
339 females also exploited blends of volatile compounds to distinguish between ambient air and
340 host plants as well as between the two host plants (Fig. 1). In this instance, both host plants
341 released (*Z*)-3-hexenyl acetate, an attractive compound (Piesik et al. 2008) to ovipositing *C.*
342 *cinctus* females (Weaver et al. 2009). The amount the compound released by each host plant was
343 sufficient to readily distinguish it from pure air (non-host) in Y-tube arm airstream. Furthermore,
344 a greater number of females were attracted to the airstream passing over 'Hockett' when the
345 choices were 'Hockett' and 'Craft' (Fig 1). Our results were similar to the results from wheat crops
346 in which ovipositing females are attracted towards a cultivar that emits a greater amount of (*Z*)-
347 3-hexenyl acetate (Weaver et al. 2009). Overall, the result supports the hypothesis that the *C.*
348 *cinctus* female exploits the olfactory cues not only to discriminate between non-host and host
349 plants but also to scrutinize volatile blends between available, suitable host plants.

350 After landing on a host plant, *C. cinctus* females display a series of oviposition behaviors:
351 walking, abdominal tapping, and ovipositor insertion (Buteler et al. 2009). In our study, the
352 females showed a similar pattern for these behaviors in terms of duration between 'Hockett' and
353 'Craft' within each developmental stage (Zadoks 34 and 49) (Table 2). Similarly, we found similar
354 frequencies of these behaviors on smaller plants for both cultivars (Zadoks 34), but not on the
355 larger plants at Zadoks 49 (Table 2). For plants at Zadoks 34, only the main stem contains
356 internodes suitable for oviposition. A minimal number of suitable tillers with internodes available

357 for oviposition could result in a similar pattern for these behaviors in both durations and
358 frequencies between two cultivars. Alternatively, it could be possible that both cultivars released
359 a threshold amount of the bouquet of volatiles needed to engage ovipositing females.

360 After judging a host (by walking) and oviposition site (by abdominal tapping and inserting
361 ovipositor), laying an egg at a suitable place is the next step. We found a greater number of
362 eggs per stem in 'Hockett' than in 'Craft' at both developmental stages (Table 3). Several
363 previous studies have reported that 'Hockett' is preferred over 'Craft' by the number of eggs per
364 stem (Varella et al. 2018, Achhami et al. 2020 b, c). Our results align with previously reported
365 results that female prefers volatiles from susceptible cultivars and thus lays more eggs in wheat
366 (Weaver et al. 2009, Varella et al. 2016) and now, in barley cultivars (Achhami et al. 2020 a, b, c).

367 *Cephus cinctus* females exploit visual and tactile cues (thicker and taller stems) (Buteler et
368 al. 2009) and olfactory cues for oviposition in wheat (Buteler et al. 2009, Buteler and Weaver
369 2012). We found a significantly greater stem diameter in 'Craft' than in 'Hockett' at both
370 developmental stages. The seemingly contradictory result — not selecting the host that has
371 thicker stems — from Buteler et al. (2009) could be due to the condition for choosing thicker
372 stems by females was only measured within the same cultivar and is not applicable across
373 cultivars. In addition to host traits, other factors could influence the host selection and
374 oviposition. These characteristics are innate memory of the host plant at adult emergence and
375 the duration of female lifespan. Females may accept any available plant as a host regardless of
376 subsequent offspring survival rate if host encounters are limited. For example, comparatively low
377 larval survival rates occur in solid stem wheat (Varella et al. 2015, Talbert et al. 2014), downy
378 brome (Perez-Mendoza et al. 2006), barley (Varella et al. 2018), and there is no survival in oat

379 species (Criddle 1922, Weaver et al. 2004). Discrepancies in offspring survival rate and
380 oviposition preference in other insects such as the fall armyworm, *Spodoptera frugiperda* J.E.
381 Smith, have been reported. Armyworm females laid more eggs on Balsas teosinte (*Zea*
382 *mays* ssp. *parviglumis* Iltis & Doebley) than in maize (*Zea mays* L.) even though larval mortality is
383 higher in teosinte than in maize (Bernal et al. 2015). Similarly, female *Spodoptera*
384 *littoralis* Boisduval lays more eggs on a host where larval parasitism rate is lower (Sadek et al.
385 2010). Selecting a less preferable host is the strategy used by females to reduce the parasitism
386 rates in offspring. Additionally, females may choose to lay eggs in any available hosts rather
387 than die without laying eggs if preferred hosts are not available, particularly the species like *C.*
388 *cinctus* with short adult lifespans, < 7 days. Further study is needed to decipher the role of plant
389 structure (visual cues) and surface chemical cues for host selection and oviposition behaviors.

390 Conversely, we measured a greater amount of linalool in 'Craft' than in 'Hockett,' thus the
391 concentration of this monoterpene may play a role in orienting *C. cinctus* females away from
392 'Craft' at close range. This is because linalool is well known as a defense compound in plants by
393 either direct toxicity (Weaver et al. 1991, Davoudi et al. 2011) or repellency (Muller et al. 2009,
394 Dekker et al. 2011) as well as an attractant for natural enemies of pests (Kessler and Baldwin
395 2001, Du et al. 1998). The release of greater amounts of linalool from wheat and barley plants
396 induced by *C. cinctus* larval feeding, after pathogen infection or after mechanical injury are well
397 documented (Piesik et al. 2006a, 2006b, 2009, 2011, 2013). A greater understanding is needed of
398 the role of odor and contact chemoreceptors (Gress et al. 2013, Robertson et al. 2018) used by
399 *C. cinctus* populations (Lesieur et al. 2016) to assess and adapt to new host plants. In addition,
400 the role of the compound β -caryophyllene that is activated after infection of barley by soil-

401 borne microbes (Piesik et al. 2011) and of the other detected compounds in the host selection
402 and oviposition behaviors of *C. cinctus* is yet to be studied.

403 Our current results provide evidence to support the attractiveness of (Z)-3-hexenyl acetate
404 and its role to attract ovipositing *C. cinctus* females to a specific cultivar of barley. Consequently,
405 our study provides a foundation to manipulate cultivar traits that change insect preference that
406 can be exploited for pest management.

407

408

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410

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417

418 **References**

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669 **Table 1:** Summary statistics for *Cephus cinctus* oviposition behavior frequencies on two barley
670 cultivars

	Cultivar	Zadoks stage	Behaviors							
			Walking		Abdominal tapping		Ovipositor insertion			
			Chi- Observed square	P- value	Chi- Observed square	P- value	Chi- Observed square	P- value		
Craft	34	121	1.53	0.22	19	0.22	0.64	13	0.53	0.47
Hockett	34	141			22			17		
Craft	49	153	19.26	<0.001	58	24.53	<0.001	21	1.92	0.17
Hockett	49	240			125			31		

671 Bold value indicates difference between cultivars within the Zadoks stage (*P* at the 0.05 level of
672 significance).

673

674 **Table 2:** Mean (\pm SE) duration in minutes for *Cephus cinctus* oviposition behaviors on two barley
675 cultivars

Cultivar	Zadoks stage	Walking	Abdominal tapping	Ovipositor insertion
Craft	34	11.62 \pm 5.23a	0.72 \pm 0.46a	1.006 \pm 0.09a
Hockett	34	11.53 \pm 6.23a	1.001 \pm 0.58a	1.06 \pm 0.51a
Craft	49	14.83 \pm 7.03a	2.28 \pm 1.47a	1.49 \pm 0.78a
Hockett	49	23.47 \pm 9.98a	4.96 \pm 2.28a	2.51 \pm 1.15a

676 Comparisons were made between the cultivars within Zadoks stage categories. Rows with
677 different letters within a column with corresponding Zadoks stage indicate a significant
678 difference (*P* < 0.05 level of significance).

679

680 **Table 3:** Stem height (mean \pm SE), stem diameter (mean \pm SE), and number of *Cephus cinctus*
681 eggs per stem (mean \pm SE) in oviposition choice tests using two barley cultivars at two
682 developmental stages.

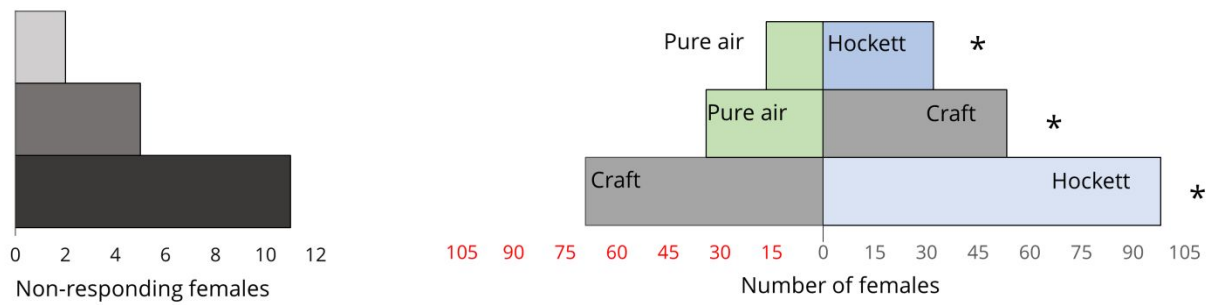
Cultivar	Zadoks stage	Height (cm)	Diameter (mm)	Number of tillers	Eggs
Craft	34	20.64 \pm 1.17a	2.64 \pm 0.07a	4.54 \pm 0.28a	1.00 \pm 0.25a
Hockett	34	22.90 \pm 1.27a	2.14 \pm 0.04b	4.81 \pm 0.29a	1.98 \pm 0.36b
Craft	49	37.35 \pm 1.88a	2.64 \pm 0.07a	5.7 \pm 0.67a	0.16 \pm 0.09a
Hockett	49	34.26 \pm 1.84a	2.18 \pm 0.04b	6.3 \pm 0.63a	0.48 \pm 0.11b

683 Comparisons are made between rows of parameters within each Zadoks stage. Rows with
684 different letters within a column of the corresponding Zadoks stage indicate significant
685 difference (*P* < 0.05).

686

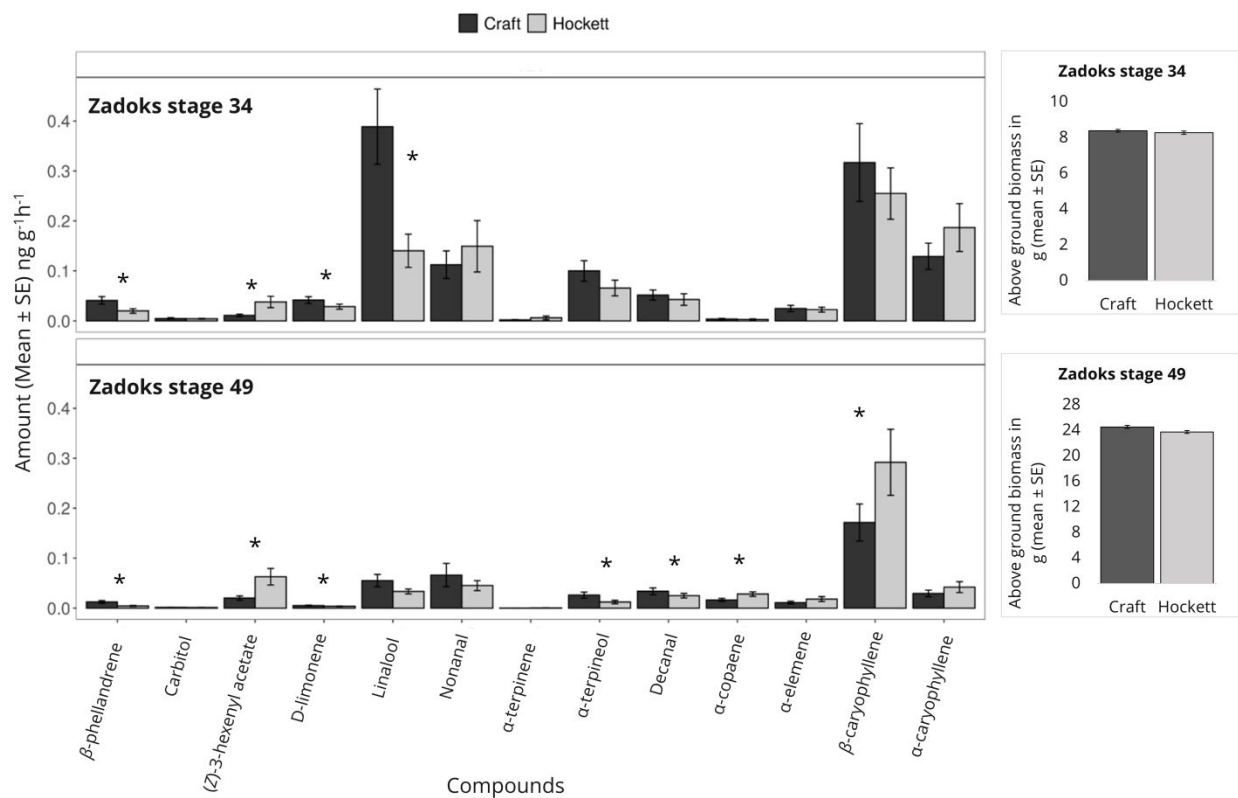
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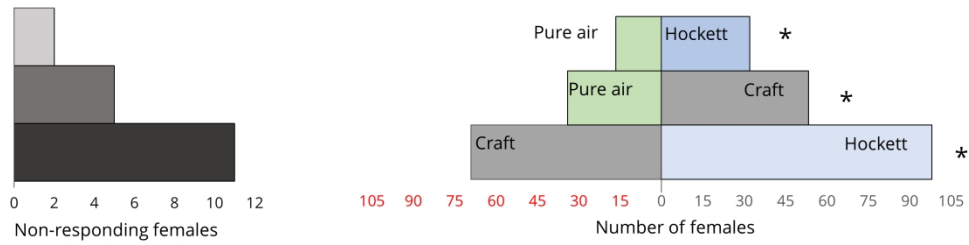
689

690 **Figure 1:** Female *Cephus cinctus* preferences in Y-tube olfactometer bioassay between two
 691 barley cultivars serving as sources of volatile blends. *Indicates a significant difference between
 692 the number of females preferring a volatile blend at $p < 0.05$. The bar graph on the left side
 693 shows number of non-responding females in addition to the corresponding choices of volatile
 694 blends.



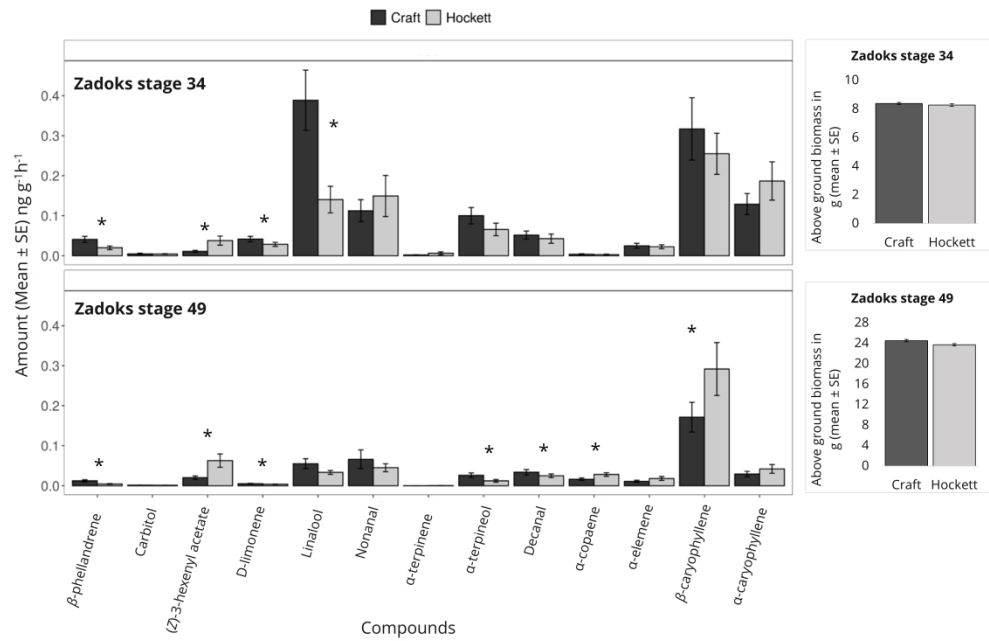
695

696 **Figure 2:** Left panels: Volatile organic compounds of two cultivars of barley plants at Zadoks
 697 stage 34 (top left) and Zadoks stage 49 (bottom left). *Indicates significant difference between
 698 two cultivars within similar developmental stages at $p < 0.05$. Right panels: Mean above ground
 699 biomass in grams.



Female *Cephus cinctus* preferences in Y-tube olfactometer bioassay between two barley cultivars serving as sources of volatile blends. *Indicates a significant difference between the number of females preferring a volatile blend at $p < 0.05$. The bar graph on the left side shows the number of non-responding females in addition to the corresponding choices of volatile blends.

279x88mm (300 x 300 DPI)



Left panels: Volatile organic compounds of two cultivars of barley plants at Zadoks stage 34 (top left) and Zadoks stage 49 (bottom left). *Indicates significant difference between two cultivars within similar developmental stages at $p < 0.05$. Right panels: Mean above-ground biomass in grams.

381x254mm (300 x 300 DPI)