

1 **Design and deployment of semiochemical traps for capturing *Anthonomus rubi* Herbst**
2 **(Coleoptera: Curculionidae) and *Lygus rugulipennis* Poppius (Heteroptera: Miridae) in**
3 **soft fruit crops**

4
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21

22

23 **Abstract**

24 Strawberry blossom weevil (SBW), *Anthonomus rubi* Herbst (Coleoptera: Curculionidae) and
25 European tarnished plant bug (ETB), *Lygus rugulipennis* Poppius (Heteroptera: Miridae), cause
26 significant damage to strawberry and raspberry crops. Using the SBW aggregation pheromone and
27 ETB sex pheromone we optimized and tested a single trap for both species. A series of field
28 experiments in crops and semi-natural habitats in five European countries tested capture of the
29 target pests and the ability to avoid captures of beneficial arthropods. A Unitrap containing a
30 trapping agent of water and detergent and with a cross vane was more efficient at capturing both
31 species compared to traps which incorporated glue as a trapping agent. Adding a green cross vane
32 deterred attraction of non-pest species such as bees, but did not compromise catches of the target
33 pests. The trap caught higher numbers of ETB and SBW if deployed at ground level and although a
34 cross vane was not important for catches of ETB it was needed for significant captures of SBW. The
35 potential for mass trapping SBW and ETB simultaneously in soft fruit crops is discussed including
36 potential improvements to make this more effective and economic to deploy.

37

38 **Key words:** Apoidea, bycatch, monitoring, mass trapping, pheromone, plant volatiles

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41 Introduction

42

43 Across Europe, strawberry blossom weevil (SBW), *Anthonomus rubi* Herbst (Coleoptera:
44 Curculionidae) and European tarnished plant bug (ETB), *Lygus rugulipennis* Poppius (Heteroptera:
45 Miridae) are serious pests in strawberry and some cane fruits causing economic loss for farmers.
46 SBW females lay eggs in flower buds and then partially sever the peduncles. Damaged buds do not
47 develop further resulting in a loss of yield (Aasen & Trandem, 2006; Jay *et al.*, 2008). ETB pierces and
48 feeds on flowers and developing fruitlets, causing fruit distortion and considerably decreasing fruit
49 quality for market, up to 80% distorted fruits (Cross *et al.*, 2011; Fitzgerald & Jay, 2011).

50 Foliar applications of insecticides are the main method of controlling these pests. The loss of
51 active compounds through the pesticides approval process, the evolution of pesticide resistance in
52 many pest populations (e.g. in SWB, Aasen & Trandem, 2006), the need for selective control
53 measures to prevent disruption of integrated pest management (IPM) practices (Hillocks, 2012;
54 2013) and high losses in organic production all require better timed and targeted control
55 applications and alternative control methods for key pest species. In addition, the incidence of
56 pesticide residues in fresh produce (European Food Safety Authority, 2015) and harm to beneficial
57 insects (e.g. Croft & Brown, 1975; Cressey, 2015) are all justifications for alternative approaches to
58 pesticide use (Hillocks, 2012, 2013).

59 In the EU, users of pesticides are required by law to monitor pests when possible, and only
60 apply pesticides when pests are present in damaging numbers and other measures have failed,
61 taking the resistance risk into account (Sustainable Use Directive 2009/128/EC). The use of
62 pheromone traps for monitoring insect pests is widespread in Europe and other main fruit growing
63 regions of the world (Walton *et al.*, 2004; Teixeira *et al.*, 2009; Haghani *et al.*, 2016). Trap design,
64 placement and attractants may all have an important role in pheromone trap effectiveness,
65 depending on pest behaviour and finding the best combination of these factors will improve trap
66 efficacy (Blackmer *et al.*, 2008; Switzer *et al.*, 2009; Singh *et al.*, 2013; Renkema *et al.*, 2014).

67 Effective monitoring traps also have the potential to control pests through mass trapping
68 (Faccoli and Stergulc, 2008; Witzgall *et al.*, 2010; Abbes *et al.*, 2012; Mwatawala *et al.*, 2015) aiming
69 to reduce pest numbers, sufficiently, to reduce fruit damage. Mass trapping has been used in the
70 long term management of many pests and has the potential to be exploited for commercial
71 strawberry production by suppressing or even eradicating low-density, isolated pest populations (El-
72 Sayed *et al.*, 2006). The combination of mass trapping and releases of the predator *Nesidiocoris*
73 *tenuis* (Reuter) resulted in a 50% reduction in tomato fruit infestation by the tomato leaf miner, *Tuta*
74 *absoluta* (Meyrick) (Lepidoptera: Gelechiidae), compared to conventional treatments (Abbes *et al.*,
75 2012). Mass trapping often reduces populations of pests in crops (e.g. Mafra-Neto & Habib, 2003),
76 but there are fewer studies demonstrating successful damage reduction. Examples of successful use
77 of mass trapping against Coleoptera include the spruce bark beetle, *Ips typographus* (L.) (Faccoli &
78 Stergulc, 2008), and the palm weevils *Rhynchophorus palmarum* (L.) (Oehschlager *et al.*, 2002) and *R.*
79 *ferrugineus* (Olivier) (Dembilio & Jaques, 2015).

80 The male-produced aggregation pheromone of SBW was identified as a blend of Grandlure I,
81 Grandlure II and lavandulol by Innocenzi *et al.* (2001), and further work was carried out to make the
82 blend more cost-effective by Innocenzi *et al.* (2001) and Cross *et al.* (2006b). In addition, the effect
83 of host plant volatiles on SBW was investigated. Bichão *et al.* (2005a,b) showed that some neurons
84 on the antenna of *A. rubi* are narrowly tuned to a few structurally related sesquiterpenes, aromatics
85 or monoterpenes. Adding these plant volatiles to the aggregation pheromone has the potential to
86 increase the attractiveness to SBW (Cross *et al.*, 2006b; Wibe *et al.*, 2011; 2014a). Currently a blend
87 of SBW aggregation pheromone and one plant volatile, 1,4-dimethoxybenzene, is widely used for
88 SBW monitoring (Wibe *et al.*, 2011; 2014a).

89 Three compounds have been identified as components of the ETB female sex pheromone
90 (Innocenzi *et al.*, 2004; Frati *et al.*, 2009) and a blend of these was further optimised and tested in
91 field trials (Innocenzi *et al.*, 2004, 2005; Fountain *et al.*, 2008, 2011; Cross *et al.*, 2011) to develop an
92 effective lure and trap for monitoring males (Fountain *et al.*, 2014). In addition, some plant volatiles

93 such as phenylacetaldehyde have been identified as attractants for female ETB (Frati *et al.*, 2009;
94 Fountain *et al.*, 2010; Koczor *et al.*, 2012).

95 For both target species, initial testing assessed different trap types and colours, most
96 frequently using traps which incorporated sticky glue as the trapping agent (Innocenzi *et al.*, 2001;
97 Cross *et al.*, 2006a, 2006b; Jay *et al.*, 2008). These traps were not optimal for SBW as weevils were
98 often found around the traps, but not in or on them (Cross *et al.*, 2006a). Initial experiments for
99 attracting ETB employed various sticky trap designs and colours but this was before the pheromone
100 was widely available (Holopainen *et al.*, 2001; Blackmer *et al.*, 2008).

101 Changes in trap design leading to improved pest capture will make a monitoring trap more
102 sensitive and mass trapping more effective. Traps must be competitive with the surrounding crop,
103 ensure the pest is captured and not kill or disrupt significant numbers of natural enemies and other
104 beneficial insects, e.g. pollinators. In addition, it should not become saturated with bycatch and it
105 should be easy to use and maintain, and be cost effective.

106 To help reduce pesticide inputs, further development of the traps was necessary to a)
107 improve target pest capture, b) combine traps for two common species in strawberry and c) develop
108 a trap which was easy to maintain and economically viable for future mass trapping. Studies were
109 carried out in Denmark, Latvia, Norway, Switzerland and the UK comparing the effect of various trap
110 designs on captures of the target pests including non-target, beneficial, species.

111

112 **Materials and methods**

113 *Traps*

114 Two basic designs of trap were evaluated; delta traps (20 cm x 20 cm) with white sticky inserts and
115 green Unitraps consisting of a bucket with a funnelled entrance and green or white cross vanes
116 between the bucket and the roof (bucket 16 cm dia, 12.5 cm high with 3 cm dia opening, cross vanes
117 10 cm high, cover 16.5 cm dia). The latter trap, from hereon in, will be referred to as Unitraps. Water
118 (250 ml) and a drop of detergent was added to the Unitraps as killing agent. Traps were purchased

119 from Agrisense (Treforest, Pontypridd, UK), International Pheromone Systems Ltd. (The Wirral,
120 Merseyside, UK) or Agralan (Swindon, UK).

121

122 *Lures*

123 For trapping ETB with live females, individual mature, virgin, female ETB from a laboratory culture
124 were contained in a cage (hair roller 6 cm x 3 cm with gauze around the outside and a lid at either
125 end, holding the gauze in place). The cage contained a piece of damp paper and a section of bean as
126 food and was anchored into the top of the trap under the roof. Female ETB were replaced weekly.

127 Lures for SBW were polyethylene sachets containing 100 µl of 1:4:1 blend of Grandlure I:
128 Grandlure II: lavandulol plus 200 mg 1,4-dimethoxybenzene (Wibe *et al.*, 2014) (International
129 Pheromone Systems Ltd.). Lures for ETB were pipette tips containing 10 µg hexyl butyrate, 0.3 µg
130 (*E*)-2-hexenyl butyrate and 2 µg (*E*)-4-oxo-2-hexenal in 100 µl sunflower oil (Fountain *et al.*, 2014),
131 prepared at the Natural Resources Institute. Lures were hung from the roof of delta traps or the
132 cover of Unitraps.

133

134 *Comparison of delta traps and Unitraps for trapping ETB*

135 Two experiments were carried out in a weed field (*Chenopodium* and *Matricaria*) at NIAB EMR in the
136 UK (Lat: 51.285494 north, Long: 0.461177 east) using virgin female ETB as bait (Table 1). In
137 Experiment A (27 June – 11 July 2008), delta traps and Unitraps were compared with different
138 materials for retaining the insects. The delta traps had either the standard wet glue inserts, dry glue
139 inserts (Agrisense), wet glue inserts with additional sticker or wet glue inserts sprayed with
140 cypermethrin (0.0014 ml sticky base⁻¹, equivalent to 0.35 L ha⁻¹). The Unitraps had white cross vanes
141 or cross vanes constructed from white insect trapping cards impregnated with lambda-cyhalothrin. A
142 clear delta trap was also tested, made of clear vinyl sheets held together at the top with a paper
143 binder and with a white, wet, glue insert (Table 1).

144 In Experiment B (27 August – 1 September 2008), different coloured traps were compared.
145 These were green delta traps, clear delta traps, and green Unitraps with white, green or yellow cross
146 vanes. A sticky stake trap was also tested consisting of a wooden stake (3 x 3 x 40 cm) inserted in the
147 ground and coated in Oecotack insect trapping glue (Agralan) above ground.

148 Delta traps were suspended on two bamboo canes (50 cm above the ground) and Unitraps
149 were dug into the ground to the level of the funnel. Four replicates of each trap in each experiment
150 in a randomised block design were spaced >10 m apart and the numbers of male ETB captured
151 recorded weekly.

152

153 *Investigation of effects of grid on Unitraps for ETB to exclude capture of bees (Apoidea)*

154 The trial was set up in a weed field (*Chenopodium* and *Matricaria*) at NIAB EMR in 2009. Traps were
155 Unitraps with a green or white cross vanes baited with ETB pheromone. The latter were tested with
156 or without a black plastic grid (4 x 5 mm mesh) fitted over the hole of the funnel since white is
157 attractive to bees. Catches of ETB in these traps were compared with those in a green delta trap with
158 a white sticky insert. There were 5 replicates of each treatment in a randomised block design and
159 the traps were spaced >10 m apart. Traps were in place from 11 May - 19 June and from 11 May - 18
160 August and catches were recorded either weekly or fortnightly.

161

162 *Effect of cross vane height on catches of ETB and SBW*

163 ETB trials were in the UK on the perimeter of a strawberry crop at NIAB EMR (15 July - 12 August
164 2013). SBW trials were in a strawberry crop in the north-west area of Norway, Møre and Romsdal
165 County (Lat: 62.697778 north, Long: 7.385278 east) (2 July – 27 August 2013). Traps were baited
166 with the corresponding synthetic pheromone lures.

167 For the ETB trial, Unitraps had either no cross vane (lid attached directly to bucket), a normal
168 size cross vane (10 cm high), or the cross vane area was doubled by joining up two cross vanes with a
169 1 ml pipette tip on each corner to secure the vanes (20 cm high). For the SBW test, Unitraps had

170 either no cross vane, a standard height (10 cm) cross vane or a vane which was half the height (5
171 cm). All cross vanes were green and the traps stood on the ground and held in place with a wire
172 hoop.

173 There were 10 replicates of each treatment arranged in a randomised complete block
174 design. Plots were rows with single traps deployed spaced 10 m apart for ETB and 20 m apart for
175 SBW. Counts of SBW and male ETB were made weekly.

176

177 *Comparison of trap designs for trapping of SBW and ETB*

178 Ten different trap designs were compared to find one effective trap for both SBW and ETB.
179 Experiments were carried out in the UK and Latvia on two occasions, one to coincide with SBW
180 emergence (UK 19 March – 05 July 2012; Latvia 17 May – 19 July 2012) and the second with the ETB
181 main flight period (UK 23-31 July 2012; Latvia 30 July - 31 August 2012).

182 In the UK, organic strawberry plantations in Hereford (Lat: 52.050051 north, Long:-2.491226
183 west) were utilised for the SBW experiment and a strawberry crop at NIAB EMR for the ETB test. In
184 Latvia the SBW trial was set up in organic strawberry plantations (Lat: 57.113804 north, Long:
185 24.530401 east; Lat: 56.807688 north, Long: 24.271681 east; Lat: 56.630664 north, Long: 23.344844
186 east; Lat: 56.595720 north, Long: 23.272959 east; Lat: 56.921632 north, Long: 23.211209 east) and
187 the ETB trial in a sowing of *Medicago sativa* L. near Vecauce (Lat: 56.595720 north, Long: 23.27295
188 east).

189 Trap designs tested are shown in Table 2 and were baited with the synthetic SBW or ETB
190 lures according to the target pest. Traps included Unitraps with different coloured cross vanes, with
191 and without excluders. The effect of attaching the lure inside the bucket rather than to the top of
192 the cross vanes was tested. Traps made of a vertical cylinder of card (25 cm x 10 cm dia) coated with
193 dry glue in different colours were tested as these are supplied commercially for ETB (Agralan), as
194 were the Xlure-RTU sticky trap (12 cm x 3 cm) from Russell IPM. Two simple, homemade traps were

195 tested: the sticky stake trap and a white plastic strip (45 x 150 x 3 mm) in 45 mm pot containing
196 water + 0.1% Triton X100.

197 The two experiments (one in the UK and one in Latvia) were set up as a randomised
198 complete block design. There were five replicates of each treatment in each experiment. Because of
199 the area needed for each replicate, experiments were conducted across several plantations, with
200 each considered a replicate with all 10 treatments. Traps were deployed 15-20 m apart along the
201 edges of the fields. The traps were stood on the ground, with foliage around them removed. Sticky
202 traps were set with their bottom edge touching the ground. Unitraps were held in place with a wire
203 hoop and contained water plus a drop of detergent.

204 Total counts of SBW and male ETB in each trap were made every 2 weeks. Bycatch of other
205 notable insects were recorded into broad taxa and included honeybees, bumblebees, solitary bees
206 and Diptera (>2 mm).

207

208 *Effect of trap height and habitat on catches of ETB and SBW*

209 The ETB trial was set up in a strawberry plantation (cv. 'Finesse') at NIAB EMR (14 August - 26
210 September 2011). Treatments were green cross vane Unitraps with water and detergent as a
211 trapping agent. Traps were wired in position at ground level (0 m) or onto the bracing bar of the
212 tunnel (1.25 m from the ground) or onto the centre top ridge pole of tunnel (4 m from the ground).
213 Traps were baited with the synthetic ETB sex pheromone lure. The trial had a randomised complete
214 block design with 5 replicates and the traps were arranged around the edge of the plantation >10 m
215 apart. Trap catches were recorded weekly.

216 The SBW trials were carried out in raspberry in 2012 at three locations in Denmark, Norway
217 and Switzerland (Table 3). Three habitat types were tested; the crop (raspberry), the boundary of
218 the crop and 50 m into the adjacent forest. Traps were green Unitraps with a white cross vane with a
219 synthetic SBW lure, a pollinator exclusion grid across the funnel opening, and water with detergent
220 as the trapping agent in the bucket. Traps were mounted on poles at three heights above ground

221 level (0 m, 0.5 m and 1.5 m). There were 9 replicates in Denmark and Norway and 12 in Switzerland.
222 Traps in each habitat were >3 m apart and catches recorded every 7-14 days.

223

224 *Data analysis*

225 All analyses were carried out using Genstat v 14 (VSN International, 2011). Mean total catches
226 during the experiments were square root transformed, to stabilise the variances, and subjected to
227 analysis of variance (ANOVA). Where significant differences between means were indicated, these
228 were tested for significance by a Least Significant Difference (LSD) test using $P < 0.05$ to indicate
229 significant differences.

230 For the UK experiments fixed effects were the treatments (e.g. trap design, cross vane
231 height, trap height, habitat etc.) and random effects were the blocks in the randomised experiment
232 designs.

233 The Latvia and UK data for the trap type experiment to capture SBW or ETB were combined
234 and analysed using ANOVA. The fixed effects were trap type and country and random effects were
235 blocks nested within country and treatments nested within blocks. Data from this experiment was
236 also analysed using a Generalised Linear Mixed Model (GLMM) (Breslow, N.E. & Clayton, D.G., 1993)
237 with the Poisson distribution and a log-link. This was analysed with the same fixed effects and the
238 same random effect model as the ANOVA, above. Where the ANOVA gave significant trap type x
239 country interactions for all variables, this was not the case in the GLMM analyses for any of the
240 variables. This lack of interaction in the GLMM with its log-link implies that the trap type means
241 differed between the two countries by a fixed multiplier which corresponded to the relative
242 abundances in the two countries. To preserve conformity with other analyses the overall means of
243 trap type were reported from the ANOVA analyses.

244 When analysing the effect of trap height on catches of SBW catches, mean counts over the
245 trapping season for countries (Norway, Denmark, Switzerland) were analysed using ANOVA with trap

246 height, habitat and country as fixed effects and replicates within country, and height within blocks as
247 random effects. A GLMM analysis was also used but in this case gave similar results to the ANOVA.

248

249 **Results**

250 *Comparison of delta traps and Unitraps for trapping ETB*

251 In Experiment A, catches of ETB were low and there were no significant differences between the
252 trap types tested ($F_{6,18} = 1.22$, $P=0.343$; data not shown). The addition of lambda-cyhalothrin or
253 cypermethrin to the traps did not significantly improve ETB catches.

254 In Experiment B, there were significantly more male ETB captured in the green cross vane
255 Unitrap compared to the green or clear delta traps ($F_{6,18} = 2.16$, $P=0.096$, $l_{sd}=2.768$, Figure 1).
256 Unitraps with white or yellow cross vanes had intermediate trap catches.

257

258 *Investigation of effects of a grid on Unitraps for ETB to exclude capture of bees (Apoidea)*

259 Using a plastic grid over the hole of the funnel in the white cross vane Unitraps significantly reduced
260 the numbers of captured bumblebees ($F_{4,16} = 6.25$, $P=0.003$, $l_{sd}=0.837$, Figure 2) and honeybees
261 ($F_{4,16}=60.44$, $P<0.001$, $l_{sd}=0.756$, Figure 2). Although the white cross vane Unitraps had fewer ETB if a
262 grid was placed over the funnel hole, this was not significant. The main differences were that the
263 green cross vane Unitrap without a grid captured significantly more male ETB than the green Unitrap
264 with a grid or a green delta trap with a sticky glue insert ($F_{4,16}=7.24$, $P=0.002$, $l_{sd}=1.248$, Figure 2).
265 These results suggested that a grid is not needed if a green cross vane is used in the Unitraps. Green
266 cross vanes reduced the bycatch whilst maintaining a significant catch of male ETB.

267

268 *Effect of cross vane height on catches of ETB and SBW*

269 In the UK trial, the height of the green cross vane had no effect on the numbers of male ETB trapped
270 in the Unitraps. There was also no effect on bumblebee ($F_{2,18}=1.19$, $P=0.328$, $l_{sd}=0.380$), carabid
271 ($F_{2,18}=0.08$, $P=0.924$, $l_{sd}=0.876$), spider ($F_{2,18}=0.78$, $P=0.474$, $l_{sd}=0.716$), earwig ($F_{2,18}=1.47$, $P=0.257$,

272 lsd=0.689), large Diptera ($F_{2,18}=0.92$, $P=0.417$, lsd=0.845) or ant numbers ($F_{2,18}=0.10$, $P=0.908$,
273 lsd=0.695). However, in general, captures of some notable groups of invertebrates did increase as
274 the vane height was increased, including the mirid *Calocoris norvegicus* (Gmelin) ($F_{2,18}=11.12$,
275 $P<0.001$, lsd=0.729), slugs ($F_{2,18}=3.58$, $P=0.049$, lsd=1.010), Coccinellidae ($F_{2,18}=5.68$, $P=0.012$,
276 lsd=0.801) and Opilione ($F_{2,18}=9.04$, $P=0.002$, lsd=0.718, Figure 3).

277 In the trial in Norway, SBW catches were significantly lower in the half height (5 cm) cross
278 vane Unitraps compared to the full height cross vane traps ($F_{2,18}=5.21$, $P=0.016$, lsd=0.681, Figure 4).
279 When the cross vane was removed altogether, intermediate numbers of SBW were captured.

280

281 *Comparison of trap designs for trapping of SBW and ETB*

282 For the SBW and ETB experiments there was no evidence of a trap type x country interaction in the
283 GLMM analysis for SBW counts, ETB counts, or any of the by-catches (not reported), but there were
284 significant differences between the trap types. Numbers of SBW captured in the Unitraps with white
285 or green cross vanes, even with the 4 x 5 mm excluder grid, ($F_{9,72}=13.42$, $P<0.001$, lsd=0.889, Figure
286 5A) were significantly higher than in other trap types. The lure placed inside the bucket of the
287 Unitrap, instead of under the lid, captured fewer SBW and was comparable in captures to the sticky
288 stake, the pot trap and the yellow sticky card traps (Figure 5A).

289 By-catch was also affected by trap design. Higher numbers of bumblebees, in particular,
290 were captured in the Unitraps with the white cross vanes and no, 4 x 5 mm, excluder grid compared
291 to most other traps ($F_{9,72}=10.74$, $P<0.001$, lsd=0.418, Figure 5B). This was also true for honeybees
292 ($F_{9,72}=2.83$, $P=0.007$, lsd=0.583, Figure 5C) and solitary bees ($F_{9,72}=5.15$, $P<0.001$, lsd=0.632, Figure
293 5D). However significant numbers of solitary bees were also captured in the pot trap and blue and
294 yellow dry sticky glue traps.

295 Large Diptera (>2 mm) were more abundant on traps which had glue as the trapping agent
296 in comparison to liquid based traps and the Xlure R.T.U floor trap ($F_{9,72}=52.12$, $P<0.001$, lsd=1.986,
297 Figure 5E).

298 Later in the season, in the ETB trial, more ETB were captured in green or white cross vane
299 Unitraps without the excluder grid compared to other trap types, although small numbers were
300 captured in the pot trap and in the Unitrap with the excluder grid ($F_{9,72}=33.76$, $P<0.001$, $l_{sd}=1.414$,
301 Figure 6A). Placing the pheromone lure inside the bucket of the Unitrap did not increase catches of
302 ETB (Figure 6A).

303 By-catch, later in the season, included honeybees and large Diptera. Honeybees were more
304 likely to be captured in the Unitraps with the white cross vane and without the 4 x 5 mm excluder
305 grid; small but significant numbers of honeybees were also captured on the blue sticky card
306 ($F_{9,72}=3.57$, $P=0.001$, $l_{sd}=0.598$, Figure 6B). As with the previous study, earlier in the season, by-
307 catches of large Diptera (>2 mm) in the ETB experiment were higher on traps which comprised sticky
308 glue, including the yellow wet and dry cards, the blue card and the sticky stake trap, in comparison
309 to traps which used a liquid as the method of capture ($F_{9,72}=50.09$, $P<0.001$, $l_{sd}=1.333$, Figure 6C).

310

311 *Effect of trap height and habitat on catches of ETB and SBW*

312 In the UK experiment there were significantly more male ETB caught in traps placed on the ground
313 within the strawberry row (mean 22.2) than in the traps wired to the tunnel bracing bar 1.25 m
314 above ground (mean 1.0) or the ridge pole 4 m above ground (mean 0.2) ($F_{2,8}=29.13$, $P<0.001$,
315 $l_{sd}=1.558$, data not shown).

316 There were significant interactions between country and habitat ($F_{4,14} = 10.61$, $P<0.001$, $l_{sd} =$
317 0.55), and habitat and trap height ($F_{4,42}=3.13$, $P=0.024$, $l_{sd}=0.257$). There were no other significant
318 interactions. The numbers of SBW captured in Denmark and Switzerland were lower than those in
319 Norway, particularly in raspberry crops and the crop boundary compared to the forest (Figure 7A). In
320 the boundary and the crop, SBW was more likely to be trapped at 0 m compared to 0.5 or 1.5 m
321 (Figure 7B).

322

323 **Discussion**

324 In this study we developed and tested traps for SBW and ETB monitoring. The best trap,
325 effectively capturing both species with minimal bycatch, was the green cross vane Unitrap with no
326 excluder grid over the hole to the funnel (Figure 8). This trap gave best capture of the two pest
327 species if deployed on the ground. The white cross vanes previously used to trap SBW (Cross *et al.*,
328 2006a, b; Wibe *et al.*, 2014) did not improve capture compared to green cross vanes and more bees
329 were attracted. Bees could be excluded by use of a plastic mesh grid over the Unitrap funnel
330 entrance, but this impeded the capture of ETB. Water and detergent were better for trapping ETB
331 than glue, and ETB and SBW have been observed to free themselves from glue traps (pers. obs.).
332 Overall the sticky traps were unsuitable for trapping SBW and ETB because they became
333 contaminated and potentially saturated with other arthropods.

334 The height of the Unitrap cross vanes did not affect the capture of ETB, but the 10 cm cross
335 vanes were most effective for capture of SBW. However, an increase in cross vane height did
336 increase the capture of some beneficial arthropods including Coccinellidae and Opilione. Trap
337 contaminants, in particular slugs, seemed to increase with vane height and hence the management
338 of slugs through irrigation control and crop management is needed. In addition, the height which the
339 trap is placed affected trapping efficacy. The ETB traps placed near to the ground at crop height
340 captured more males than traps placed higher in the tunnel structure. Likewise, SBW was more
341 likely, in Norway, to be captured on the ground in the forest. This may be because the wild fruits are
342 closer to the ground compared to commercially grown fruits which are tied vertically into post and
343 wire systems with the fruit higher than would be natural.

344 Although this trapping system is cost effective for monitoring, further improvements and
345 reduction in cost need to be made for mass trapping. An attempt was made to find a low cost, small,
346 trap that could be deployed in large numbers for mass trapping. However, none of the traps,
347 including prototypes, were as affective as the Unitrap with green cross vanes (Figure 8).

348 An obvious flaw in the trap system tested here is the lack of an attractant for female ETB.
349 However, phenylacetaldehyde and/or (*E*)-cinnamaldehyde (Koczor *et al.*, 2012) could be added to

350 the sex pheromone to increase catches of female ETB. An alternative method to the drowning
351 solution, which needs to be emptied and topped up on a monthly basis, would make the trap easier
352 to maintain. There is the potential to incorporate an insecticide (Navarro-Llopis *et al.*, 2014) or
353 biological control agent onto the inside of the trap. This would be a lure and kill system where the
354 insect would enter and then die either inside the trap or after leaving.

355 In addition, the lures also need maintenance. SBW lures last at least two months under field
356 conditions (Cross *et al.*, 2006a), but the longevity of the ETB lures is approximately four weeks
357 (Fountain *et al.*, 2014). It would be beneficial to increase the longevity of these lures and/or improve
358 the deployment and replacement of lures in the traps. Finally, the traps were often placed in the leg
359 row of the strawberry crop to avoid disturbance by spray machinery and fruit pickers, but this made
360 them difficult to access on a regular basis (see Figure 8).

361 In this study we did not test lures for both species in the trap at the same time. Further
362 studies are required to ensure that the components of the two lures remain attractive to the pest
363 species when placed together. It is considered unlikely there would be any interaction in view of the
364 very different chemical structures of the pheromone components. A combined trap would save time
365 in monitoring and push the cost-benefit ratio of mass trapping in the right direction. There is a real
366 potential to mass trap (Fountain *et al.*, 2015) or lure and kill ETB and SBW in strawberry crops.
367 Future research should concentrate on trap optimization, ease of use and economics of deploying
368 and maintaining these systems for reducing fruit damage in strawberry crops.

369

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376

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Table 1 Comparison of traps for capturing male ETB using virgin female ETB as bait in UK.

Trap	Capture device
<i>Experiment A (27 June – 11 July 2008)</i>	
Green delta	Wet glue sticky insert
Green delta	Dry glue sticky insert
Green delta	Wet glue sticky insert + cypermethrin
Green delta	Wet glue sticky insert + Oecotack
Unitrap (white cross vane)	Correx card + water with detergent
Unitrap (lambda-cyhalothrin white cross vane)	Water + detergent
Clear delta	Wet glue sticky insert
<i>Experiment B (27 August – 1 September 2008)</i>	
Green delta	Wet glue sticky insert + Oecotack
Clear delta	Wet glue sticky insert + Oecotack
Agrisense vane funneled (white cross vanes)	Water + detergent
Unitrap (green cross vanes)	Water + detergent
Unitrap (white cross vanes)	Water + detergent
Unitrap (yellow cross vanes)	Water + detergent
Sticky stake trap	Oecotack

495

496

Table 2 Trap designs tested for trapping SBW and ETB in the UK and Latvia. Traps were baited with synthetic SBW or ETB lures.

Trap design	Killing agent	Position of lure	Source of trap
Unitrap: White cross vane with excluder-grid (4x5 mm)	Water + 0.1% Triton X100	Top of cross vane	Agralan
Unitrap: White cross vane with excluder-grid (4x5 mm)	Water + 0.1% Triton X100	Inside bucket attached to funnel	Agralan
Unitrap: White cross vane without excluder-grid	Water + 0.1% Triton X100	Top of cross vane	Agralan
Unitrap: Green cross vane without excluder-grid	Water + 0.1% Triton X100	Top of cross vane	Agralan
Sticky stake trap (2.5 x 2.5 x 50 cm)	Oecotack wet glue	Top of stake	NIAB EMR
Yellow card cylinder (25 x 10 cm)	Coated in wet glue	Top of cylinder	Agralan
Blue card cylinder (25 x 10 cm)	Coated in dry glue	Top of cylinder	Agralan
Yellow card cylinder (25 x 10 cm)	Coated in dry glue	Top of cylinder	Agralan
White plastic strip (45 x 150 x 3 mm) in 45 mm pot	Water + 0.1% Triton X100	Top of strip	NIAB EMR
Xlure R.T.U floor trap	Oecotack sticky insert inside	Inside, in middle on base	Russell

Table 3 Location and age of raspberry plantations used for testing the effect of trap height on catches of SBW (CH Switzerland, NO Norway, DK Denmark).

Country	Location	Coordinates		Production type	Plantation year
		Latitude	Longitude east		
CH	Bruson, Valais	46.0037,	7.3191	Open	2005
CH	Nendaz, Valais	46.1834,	7.2942	Open	2005
CH	St Sébastien, Valais	46.198,	7.31306	Open	2004
NO	Skåla, Molde	62.6953,	07.3769	Protected	2010
NO	Skjønshøyen	60.8278,	10.7970	Open	2008
NO	Torp	59.6677,	10.6912	Open	2010
DK	Gyrstinge	55.4770,	11.6830	Open	2010
DK	Eggeslevmagle	55.2843,	11.3300	Protected	2010
DK	Kildebrønde	55.6033,	12.2650	Protected	2010

Figure 8 Green cross vane Unitrap anchored into place on the ground in a strawberry crop. The pheromone dispensers are deployed in the cage inserted into the lid.

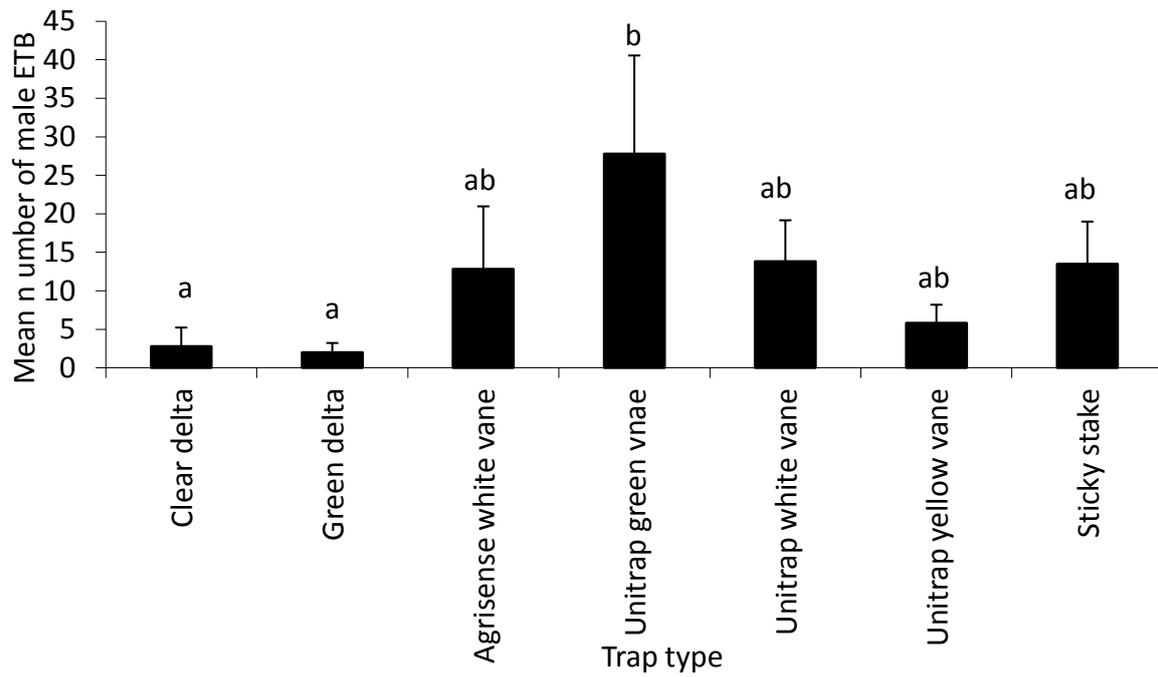


Figure 1 Mean numbers (\pm S.E.) of male ETB captured in different traps (Table 1) baited with live female ETB in the UK (27 Aug – 1 Sept 2008; means with the same letter are not significantly different, $P > 0.05$).

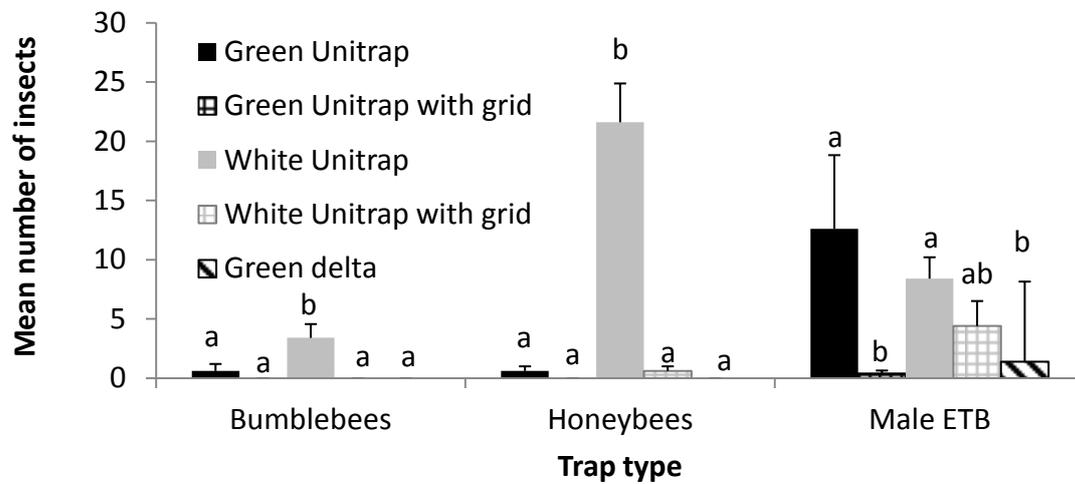


Figure 2 Mean catches (\pm S.E.) of bumblebees, honeybees and male ETB in green Unitraps with white or green coloured cross vanes, with and without a grid at the entrance to the funnel in comparison to a green Delta trap (UK, 11 May - 19 June and 11 May - 18 August; means with different letters for each species are significantly different, $P < 0.05$).

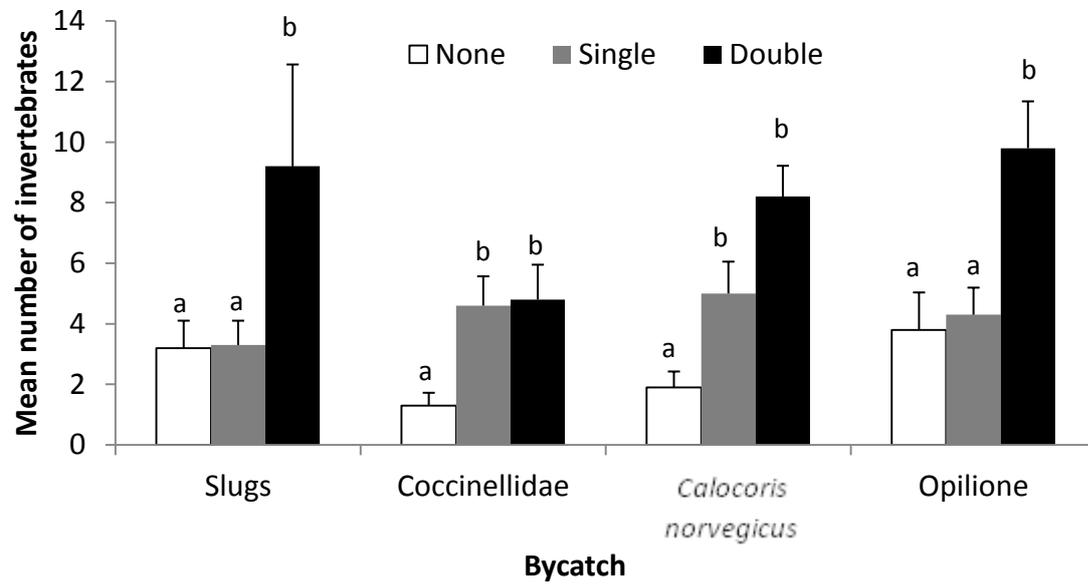


Figure 3 Mean numbers (\pm S.E.) of invertebrate bycatch captured in green Unitraps with either no cross vane, a single height (10 cm) or double height (20 cm) green cross vane (UK, 15 July - 12 August 2013; means with different letters for each invertebrate group are significantly different, $P < 0.05$).

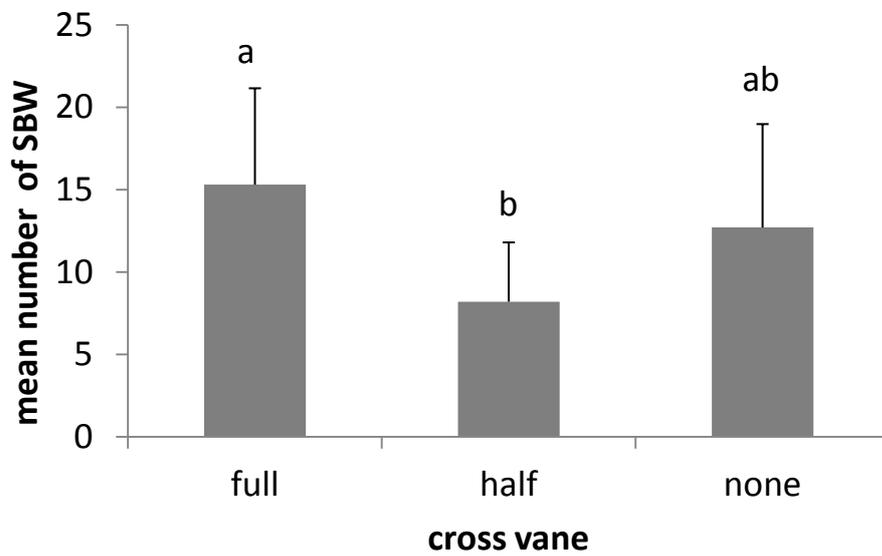


Figure 4 Mean numbers (\pm S.E.) of SBW captured in green Unitraps with either no cross vane, a full height (10 cm) or half height (5 cm) white cross vane (Norway, 16 July – 27 August 2013; means with different letters are significantly different, $P < 0.05$).

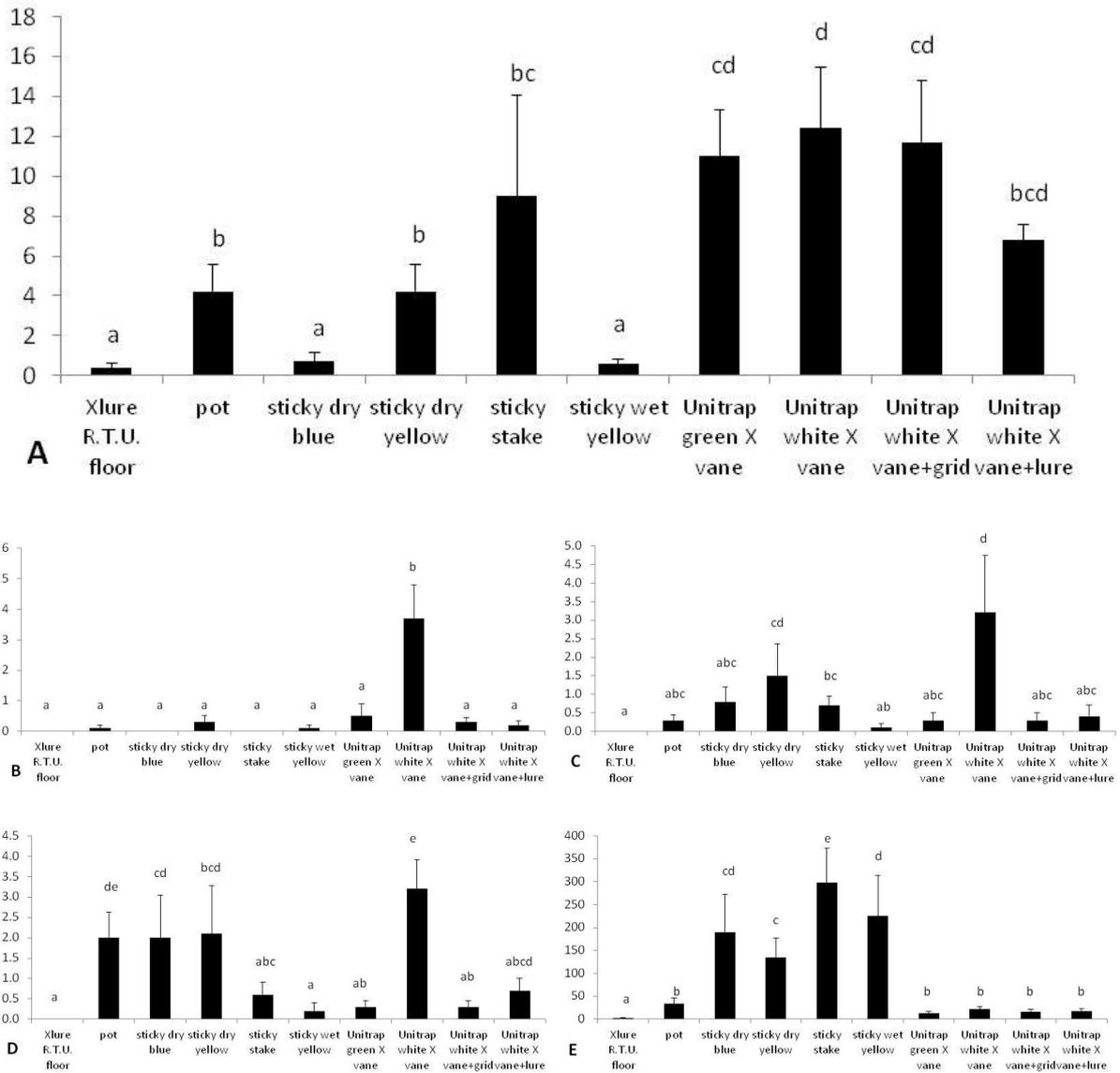


Figure 5 Mean numbers (\pm S.E.) of A) SBW, B) bumblebees, C) honeybees D) solitary bees and E) large Diptera in ten trap designs (Table 2). Data from the locations pooled by LSD test after ANOVA on square root transformed data. Means with different letters are significantly different. The arithmetic means are presented here, whereas the means of the square-root transformed counts are grouped, hence there may be apparent discrepancies in the groupings.

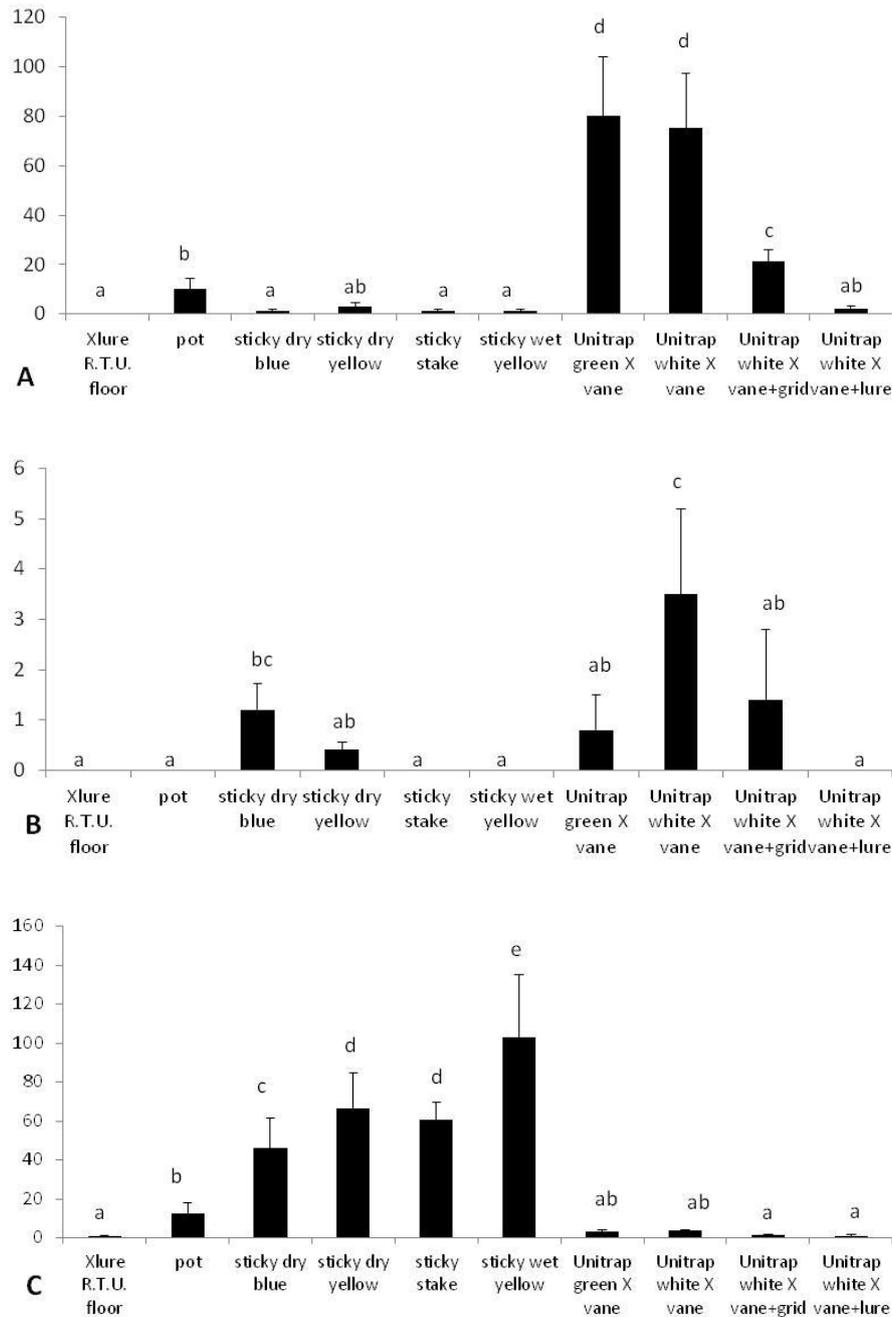


Figure 6 Mean numbers (\pm S.E.) of A) ETB, B) honeybees and C) large Diptera in ten trap designs (Table 2). Data from the locations pooled by LSD test after ANOVA on square root transformed data. Means with different letters are significantly different. There were no significant differences in the numbers of honeybees in traps in the UK. The arithmetic means are presented here, whereas the means of the square-root transformed counts are grouped hence there may be some discrepancies in the groupings.

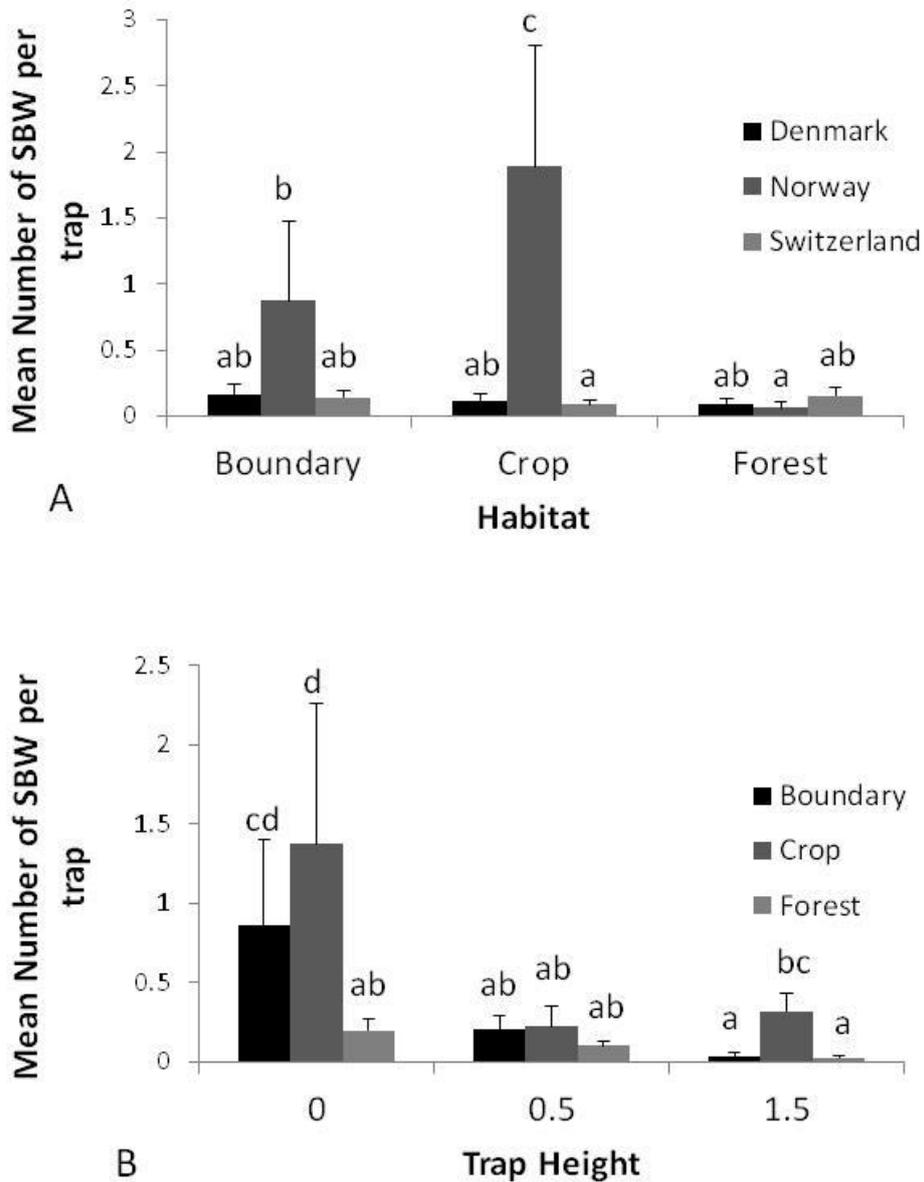


Figure 7 Mean number (\pm S.E.) of SBW per trap in A) the boundary, crop (raspberry) or forest in the 3 countries (Denmark, Norway and Switzerland) and B) at different heights (0, 0.5 and 1.5 m) in the 3 habitat types (boundary, crop and forest). Data from 9 (Denmark and Norway) or 12 locations (Switzerland) locations in 3 countries pooled by LSD test after ANOVA on square root transformed data. Means with different letters are significantly different.

Figure(s)8
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