1 **High sampling effectiveness for non-bee pollinators using**
2 **vane traps in both open and wooded habitats**

3

4 Running Title: Vane traps sample non-bee pollinators

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18

19 **Abstract**

- 20 1. Non-bee insects are important for pollination, yet few
21 studies have assessed the effectiveness of sampling
22 these taxa using low cost passive techniques, such as
23 coloured vane traps, among different habitat types.
- 24 2. This study sampled 192 sites—108 in wooded and 84 in
25 open habitats within an agricultural region of southern
26 Australia. Pairs of blue and yellow vane traps were
27 placed at each site for a period of seven days during the
28 austral spring.
- 29 3. Overall, 3114 flies (Diptera) from 19 families and
30 528 wasps (non-bee and non-formicid Hymenoptera)
31 from 16 families were collected during the study. This
32 sampling was representative of the region, with vane
33 traps equally or more likely to collect as many families
34 from both taxa as those reported on the Atlas of Living
35 Australia (ALA) database for the sampling area.
- 36 4. Blue vane taps (BVTs) had greater average richness of
37 both flies and wasps and greater abundance of
38 individuals than yellow vane traps (YVTs). BVTs were
39 particularly favoured by certain fly and wasp families
40 known to pollinate flowers (e.g. Syrphidae,
41 Bombyliidae and Scoliidae), whilst YVTs sampled
42 some less common fly families, such as Acroceridae

43 and Bibionidae that also provide additional ecosystem
44 services to pollination.

45 5. Vane traps are an effective passive sampling technique
46 for non-bee pollinators, such as flies and wasps. This
47 study supports the use of vane traps as a component of
48 the sampling protocol for ecological census and
49 population monitoring within multiple habitat types, to
50 effectively sample a more complete pollinator
51 community.

52

53 **Key words**

54 Wasp trapping, Fly trapping, Unscented colour traps, Colour
55 preference, Habitat type, BVT, YVT, Agricultural landscapes,
56 Biological repository, Online database

57 INTRODUCTION

58 Understanding the distribution of bees within agricultural
59 landscapes has received much of the research interest in
60 pollination and community ecology (Kremen *et al.*, 2002;
61 Kennedy *et al.*, 2013; Koh *et al.*, 2016). This is largely due to
62 their important role in the pollination of many native plant and
63 crop species (Heard, 1999; Morse & Calderone, 2000; Klein *et al.*, 2006). However, there is also growing evidence of the
64 importance of non-bee insect taxa in pollination, such as flies,
65 wasps, beetles and butterflies (Potts *et al.*, 2016; Rader *et al.*,
66 2016; Ollerton, 2017). Some of these non-bee taxa also play a
67 vital role in other ecosystem services, such as pest control and
68 nutrient cycling (Zhang *et al.*, 2007; McCravy, 2018), and may
69 forage more effectively than bees under different climatic
70 conditions (Inouye & Pyke, 1988; Lefebvre *et al.*, 2018). Non-
71 bee pollinators may even replace bees as the dominant flower
72 visitors in heavily modified environments (Stavert *et al.*, 2018).
73 Further, pollination may be more effectively achieved if
74 multiple species, including non-bee pollinators, visit flowers
75 (Brittain *et al.*, 2013; Alomar *et al.*, 2018; Winfree *et al.*, 2018;
76 Thomson, 2019). Yet to date, non-bee pollinators have been
77 under-represented in pollination studies and there is no
78 consensus on a passive survey method that effectively samples
79 both the bee and non-bee pollinator fauna of a given area.

81

82 Active sampling is often used for targeting particular species,
83 recording plant-pollinator interactions or obtaining density
84 estimates for a given area (Larsson & Franzen, 2008; Campbell
85 *et al.*, 2016; Taki *et al.* 2018). However, sampling in this way
86 can be time consuming, and results may be biased by the skills
87 and experience of surveyors (Westphal *et al.*, 2008; McCravy,
88 2018). Alternatively, passive sampling allows for a greater
89 range of data collection (i.e. day and night, across seasons) at a
90 relatively low cost (Saunders & Luck, 2013; McCravy, 2018).
91 Passive sampling methods are usually easy to install and do not
92 require any specialist skills from the operator (Missa *et al.*,
93 2009; Westphal *et al.*, 2008; Saunders & Luck, 2013). Many
94 different trapping methods are potentially suited to sampling
95 insect fauna, depending on the type of question being asked. A
96 simple and effective sampling technique is the use of coloured
97 components, which are known to attract many pollinator groups
98 (Kirk, 1984; Pickering & Stock, 2003; Desouhant *et al.*, 2010;
99 Vrdoljak & Samways, 2012). For example, coloured pan traps
100 are a widely used and often highly successful method for
101 sampling the pollinator community (Westphal *et al.*, 2008;
102 Saunders & Luck, 2013). Coloured sticky traps have also been
103 used for arthropod studies, however these can vary greatly in
104 their ability to collect certain groups (e.g. Hymenoptera,
105 Coleoptera, Hoback *et al.*, 1999; Pickering & Stock, 2003) and
106 make identification of small specimens difficult (i.e. <4 mm,

107 Pickering & Stock, 2003). Coloured Malaise traps are rarely
 108 used for pollinator-specific surveys and are not as efficient as
 109 pan traps (Campbell & Hanula, 2007). However, non-coloured
 110 Malaise traps are widely used and effective for general
 111 arthropod sampling (Kitching *et al.*, 2001; Campbell & Hanula,
 112 2007; Missa *et al.*, 2009). Coloured vane traps are much less
 113 studied, but have recently shown great potential, especially for
 114 bees (Gibbs *et al.*, 2017; Hall, 2018). Yet very little is known
 115 about their effectiveness in sampling the non-bee pollinator
 116 community.

117

118 The quality of colours used for passive trapping varies
 119 according to trap type. When sampling pollinators using pan,
 120 sticky or vane traps, the most commonly used colours are
 121 white, yellow and blue (Abrahamczyk *et al.*, 2010; Vrdoljak &
 122 Samways, 2012; McCravy, 2018). Blue and yellow coloured
 123 pan traps are often found to be the most attractive to insects,
 124 but there is no consensus on the greater attraction of either
 125 colour for different taxa or in varied habitat types (Kirk, 1984;
 126 Campbell & Hanula, 2007; Abrahamczyk *et al.*, 2010;
 127 Saunders & Luck, 2013). This is possibly due to variance in
 128 reflectance and vibrancy owing to a lack of consistent quality
 129 of colour and design (e.g. pre-painted bowls: Campbell &
 130 Hanula, 2007; Joshi *et al.*, 2015, self-painted bowls: Westphal
 131 *et al.*, 2008; Shrestha *et al.*, 2019). This makes comparing

132 different pan trap studies problematic. Some pollinating insect
 133 taxa, such as butterflies and moths (Lepidoptera), also appear to
 134 be largely underrepresented in pan trap studies (Missa *et al.*,
 135 2009; Vrdoljak & Samways, 2012) but are sometimes caught in
 136 large numbers using Malaise traps (Campbell & Hanula, 2007).
 137 However, Malaise traps do not appear to be particularly
 138 efficient at sampling non-*Apis* bees (Bartholomew & Prowell,
 139 2005; Campbell & Hanula, 2007; Missa *et al.*, 2009), thus do
 140 not provide comprehensive sampling for pollinators.
 141 Alternatively, vane traps come in only two colours (yellow and
 142 blue) and their commercial availability (and thus consistency of
 143 colour) potentially allows for greater comparison between
 144 studies (Hall, 2018).

145
 146 Vane traps are very effective at attracting bees without the need
 147 for pheromones or other liquids (Stephen & Rao, 2005; Hall,
 148 2018), and are increasingly being used to sample wild bee
 149 populations worldwide (Kimoto *et al.*, 2012; Lentini *et al.*,
 150 2012; Gibbs *et al.*, 2017). Both Stephen & Rao (2005) and Hall
 151 (2018) found blue vane traps (BVTs) to be particularly
 152 effective at sampling wild bee diversity compared to yellow
 153 vane traps (YVTs). Yet, in the Stephen & Rao (2005) study,
 154 very few non-bee pollinators were sampled, and no reports of
 155 non-bee taxa appear in other vane trap studies, other than to
 156 mention that they were removed (e.g. Joshi *et al.*, 2015).

157 However, because bees are often poorly represented using other
 158 passive methods typically used to survey arthropods (e.g. sticky
 159 traps: Pickering & Stock, 2003, Malaise traps: Missa *et al.*,
 160 2009), if we are to effectively sample both bee and non-bee
 161 communities, a more representative passive survey method is
 162 required. Vane traps are one such possibility as they are easy to
 163 install, provide consistent sampling effectiveness over large
 164 areas and are useable over longer time periods, compared with
 165 other widely used methods (Stephen & Rao, 2007; Kimoto *et al.*,
 166 2012; Lentini *et al.*, 2012). However, to truly test the
 167 effectiveness of this trapping technique, we also need to
 168 compare results to what is known for a given area. This can be
 169 achieved by utilising online biological repositories (Belbin &
 170 Williams, 2016).

171

172 This study tests the efficacy of BVTs and YVTs in sampling
 173 the non-bee fauna of both open and wooded areas within an
 174 agricultural landscape of southern Australia. To test this, the
 175 following questions were asked:

- 176 1) Do vane traps capture a representative sample of the
 177 non-bee pollinating fauna of agricultural landscapes?
- 178 2) Is there a difference in the effectiveness of vane traps
 179 when sampling flies and wasps using different colours
 180 and/or within open and wooded environments?

181 3) Is there a consistent response between the two
182 taxonomic groups and/or families within them?

183

184 **MATERIALS AND METHODS**

185 **Study area and system**

186 This study was conducted across a rural landscape of 11,550
187 km² on the riverine plains of north-central Victoria, Australia,
188 with a sampling area of ~3,600 km² (Fig. S1a). The region has
189 been transformed by human land-use, predominantly
190 agricultural production, experiencing extensive clearing of over
191 80% of native vegetation (Environmental Conservation
192 Council, 2001). Much of the wooded vegetation now occurs
193 along linear roadsides and streams (Hall *et al.*, 2018), or as
194 isolated trees within farm paddocks (Gibbons *et al.*, 2008).
195 Human land-use consists of sheep and cattle grazing along with
196 the cropping of wheat, oats, canola and legumes, often on a
197 rotational basis (Bell & Moore, 2012). Average annual rainfall
198 for the district is 492-648 mm per year, and daytime maximum
199 spring temperatures are typically 16-27°C (Bureau of
200 Meteorology, 2016).

201

202 Surveys were conducted within 24 individual farming
203 landscapes (each 1 km diameter) across the study region, each
204 containing both wooded cover and open farmland with a range
205 in tree cover from ~5-22%, which is indicative of the region.

206 All sites within farms (n=8 per farm, n=192 in total) were
207 selected such that they were similar in vegetation
208 characteristics and topography. Major roads and river systems
209 were avoided for consistency. For full details of methods, see
210 Hall (2018).

211

212 **Sampling**

213 *Field sampling*

214 Non-bee pollinator insects (flies and wasps) were sampled
215 using vane traps (SpringStarTM LLC, www.springstar.net),
216 comprising a 64 oz (1892 ml) plastic collecting jar, screw top
217 funnel and two interconnecting ultraviolet semitransparent
218 polypropylene vanes, coloured either blue or yellow (Figs. S1b
219 and S1c). These are known to be highly reflective when
220 exposed to 365 nm filtered (UV-A), and midrange 302 nm
221 filtered light (UV-B) (Stephen & Rao, 2005). Two pairs of
222 traps (one blue and one yellow) were hung from a tree branch
223 or pole at a height of approximately 2 m within the typical
224 features present in the landscape. Thus, 2 pairs were deployed
225 in roadsides with or without tree cover, 2 in creeks with or
226 without tree cover, 2 in scattered trees within farmland and 2 in
227 open farmland: n=108 wooded sites, n=84 open sites (see Hall
228 *et al.*, 2019 for description of landscape-scale design, and Hall,
229 2018 for full methods). Traps remained in place for seven days
230 during the southern hemisphere spring months, from October to

231 November 2015. No pheromones, liquids or killing agents were
232 used in traps. Samples were collected after one week and stored
233 in 70% ethanol then pinned for identification. Identification of
234 flies and wasps to family-level was conducted using available
235 keys (Marshall, 2017; CSIRO, 2018). Other insect taxa
236 sampled were removed due to low sampling (e.g. Lepidoptera)
237 or because they (e.g. Coleoptera, Formicidae) were more likely
238 caught in traps during mass flight events related to breeding
239 (Sullivan, 1981; McHugh & Liebherr, 2009).

240

241 *Database search*

242 To determine if vane traps were effective at sampling a
243 representative fly and wasp community within agriculturally
244 dominated landscapes containing both wooded and open
245 habitats, we compared our sampled fauna to families known to
246 occur within the study region. To do this, we generated an area
247 report using the *tools* dropdown menu in the Atlas of Living
248 Australia (ALA) spatial portal (Atlas of living Australia, 2019;
249 <https://spatial.ala.org.au/>), to incorporate a comparable area that
250 encapsulated our sampling area (~3,600 km²). We used only
251 spatially valid records (i.e. those where geographic location
252 was provided). We then compared families recorded on the
253 ALA to those sampled within our study. Note that data
254 provided in an area report could conceivably cover a timespan
255 from 1600 - 2018 AD, however no survey date was provided

256 for records with the report, so we must assume that most
 257 records span a considerable time period (likely 1900 – present),
 258 much longer than our two months of sampling conducted in a
 259 single season. To investigate if non-bee pollinators in our
 260 sampling area were also indicative of the whole region, we
 261 conducted a wider search on the ALA (11,500 km²) which also
 262 comprised forested and mountainous areas that were not
 263 sampled in our study (Table S2, Fig. S2). Further, species
 264 utilise resources at different times of the year, leading to
 265 substantial temporal turnover in insect communities within an
 266 area (Lambkin *et al.*, 2011; Thomsen *et al.*, 2016; Winfree *et*
 267 *al.*, 2018). Thus, to help interpret differences in peak activity
 268 periods for each fly and wasp family (and thus likelihood of
 269 capture during our sampling period), records were taken from
 270 the ALA database for the state of Victoria, grouped by month
 271 in which individuals were reported on the database (Fig. S3).

272

273 **Statistical analyses**

274 We conducted three separate analyses corresponding to our
 275 three main questions. First, to determine if vane traps were
 276 effective at sampling the non-bee fauna compared with
 277 historical records for these groups, we ran generalised linear
 278 models in package *nlme* (Pinheiro *et al.*, 2018), assuming a
 279 binomial distribution for presence/absence data. We compared
 280 the number of fly and wasp families separately to those

281 recorded on the ALA database. We ran separate models
 282 comparing presence of families (pooled richness) in different
 283 coloured traps (blue and yellow), and within different habitat
 284 types (open and wooded). Sites containing tree cover (roads
 285 and creeks with tree cover and scattered tree sites) were
 286 classified as ‘wooded’, whilst sites that lacked tree cover (roads
 287 and creeks without tree cover and open farmland) were
 288 classified as ‘open’. Overdispersion was checked using Pearson
 289 residuals (Zuur *et al.*, 2013) and none was detected in any
 290 model. For one model (wasp-colour preference), we fitted a
 291 Firth’s bias-reduced logistic regression in package *logistf*
 292 (Heinze *et al.*, 2018) due to BVTs containing all wasp families
 293 sampled (separation problem, Heinze & Schemper, 2002). To
 294 determine the level of overlap in families between the ALA,
 295 BVTs and YVTs, we calculated the Morista-Horn index in
 296 package *divo* (Pietrzak *et al.*, 2016). This quantifies the level of
 297 overlap in families between sampling methods, giving a value
 298 range between zero (no overlap) and one (perfect overlap).

299

300 Second, to determine if there was a difference in the
 301 effectiveness of vane traps when sampling flies and wasps
 302 using different colours and/or within open and wooded
 303 environments, we ran generalised linear mixed models in
 304 package *nlme* (Pinheiro *et al.*, 2018). We ran separate models
 305 to test for differences in the richness and abundance of ‘all

306 individuals', 'all fly families' and 'all wasp families'. We
 307 assumed a Poisson distribution for richness measures and a
 308 negative binomial distribution for abundance data. Due to
 309 clustering of sites within 1 km diameter landscapes and pairing
 310 of different colour traps at each survey point, *landscape* and the
 311 survey location (*pair*) were used as random effects in the
 312 models.

313

314 Last, to test if the response of different fly and wasp families
 315 were consistent, we tested differences in the abundance of each
 316 family with >30 individuals sampled (see Table S2) between
 317 trap colours and habitat types, using generalised linear mixed
 318 models in the *nlme* package (Pinheiro *et al.*, 2018). We
 319 assumed a Poisson distribution for all models, except for where
 320 overdispersion was detected (Syrphidae, Tachinidae), where we
 321 assigned a negative binomial distribution (Zuur *et al.*, 2013),
 322 using the *MASS* package (Venables & Ripley, 2002). Again,
 323 due to clustering of sites, *landscape* and the survey location
 324 (*pair*) were used as random effects in the models. All statistical
 325 analyses were conducted in R (v.3.5.1, R Core Team, 2018).

326

327 **RESULTS**

328 Overall, 3114 flies (Diptera) and 528 wasps (non-bee and non-
 329 formicid Hymenoptera) were collected during the study,
 330 comprising 19 families of flies and 16 families of wasps (Table

331 S1). Syrphidae was the most abundant family sampled with a
332 total of 1396 individuals caught (45 % of the total abundance),
333 including 87 % in BVTs.

334

335 For both orders, BVTs caught more insects with 67.5 % of the
336 total abundance (68 % for flies and 63 % for wasps, Table S1).

337 Four fly families were found only in YVTs (Acroceridae,
338 Bibionidae, Conopidae, Scatopsidae) whereas six fly or wasp
339 families were captured only in BVTs (Diptera: Chironomidae,
340 Sarcophagidae; Hymenoptera: Crabronidae, Gasteruptiidae,
341 Mutillidae, Vespidae). Almost all of these families (only found
342 in one colour) were sampled by a single individual (except
343 Scatopsidae n=2, Mutillidae n=2, Vespidae n=6; Table S1).

344

345 In total, 1935 insects were caught in open areas, which
346 represented 53% of the total abundance. Six families were only
347 sampled in open habitat (Table S1). These open-associated
348 families often only had one or two individuals (singletons or
349 doubletons), however two families were found in greater
350 numbers—Sphecidae (n=29) and Vespidae (n=6). Similarly, 14
351 families were only found in wooded habitats, with only five
352 families sampled having more than one or two individuals—
353 Lauxaniidae (n=3), Mycetophilidae (n=7), Chrysididae (n=7),
354 Pompilidae (n=5) and Scelionidae (n=4) (Table S1).

355

356 *Effectiveness of sampling using vane traps*

357 A total of 19 fly and 9 wasp families were recorded on the
358 ALA within our sampling area (Fig. 1). Combined BVTs and
359 YVTs sampled 19 families of flies, 12 of which were not
360 reported on the ALA (one was caught only in BVTs, four only
361 in YVTs and seven in both BVTs and YVTs). Twelve fly
362 families reported on the ALA were not caught in vane traps
363 during the study (Fig. 1). Overall, there was no difference in
364 the number of fly families sampled in our study in both BVTs
365 (Est = -0.52, SE = 0.51, P=0.31) and YVTs (Est = -0.26, SE =
366 0.52, P=0.61), or within wooded habitats (Est = -0.26, SE =
367 0.52, P=0.61; Figs. 1a, 1b). In contrast, fewer fly families were
368 collected in open habitats compared with historical ALA
369 records for the sample area (Est = -1.06, SE = 0.53, P=0.04;
370 Fig.1b). There was some overlap in families recorded on the
371 ALA and those sampled using BVTs and YVTs (flies: 30-40%,
372 wasps: ~55%, Figs. 1a, 1c). There was greater overlap based on
373 habitat for wasps (~66%) than flies (20-35%) (Figs. 1b, 1d).

374
375 BVTs had a higher probability of sampling wasp families
376 compared with all available records on the ALA for the
377 sampling area (Est = 3.26, SE = 1.56, P<0.01), however there
378 was no difference in the likelihood for other variables tested
379 (YVTs: Est = 0.78, SE = 0.76, P=0.28; Open: Est = 0.26, SE =
380 0.72, P=0.72; Wooded: Est = 0.85, SE = 0.77, P=0.27, Figs. 1c,

1d). Every family of wasp reported on the ALA (n=9) was also caught in BVTs, with seven additional families sampled using this colour that were not recorded on the ALA (Fig. 1c). Of the 16 families of wasps sampled, YVTs caught 12 families (Fig. 1c). Ten wasp families were sampled within open habitat types, including one unique family (Gasteruptionidae), while twelve families were collected in wooded habitats, including four unique families (Chrysididae, Evaniidae, Mutillidae, Scelionidae, Fig. 1d).

At the regional level encompassing our sampling area (11,500 km²), we found 39 fly and 13 wasp families recorded on the ALA (Fig. S2). Twenty fly families reported for the entire region on the ALA were not caught in vane traps during the study. Overall, fewer fly families were sampled in our study in both BVTs (Est = -1.99, SE = 0.53, P<0.01) and YVTs (Est = -1.78, SE = 0.53, P<0.01), along with both open (Est = -2.45, SE = 0.55, P<0.01) and wooded (Est = -1.78, SE = 0.53, P<0.01) habitat types compared with the wider region encompassing various habitat types and altitudinal gradients (Fig. S2). There was no difference in the number of wasp families recorded at the regional level on the ALA and those sampled in this study (BVTs: Est = 2.15, SE = 1.60, P=0.09; YVTs: Est = -0.33, SE = 0.84, P=0.69; Open: Est = -0.95, SE = 0.82, P=0.24; Wooded: Est = -0.37, SE = 0.86, P=0.67, Fig. S2). Every

406 family of wasp reported on the ALA (13 families) at this scale
 407 was also caught in BVTs, with an additional family
 408 (Mutillidae) found that was not recorded on the ALA. Both
 409 BVTs and YVTs also sampled Bethylidae and Evaniidae,
 410 which were not recorded on the ALA (Fig. S2).

411

412 The majority of fly families sampled using all methods showed
 413 a peak activity period matching our sampling period of
 414 October-November (Fig. S3a), however six families in
 415 particular were either too rarely sampled to determine trends or
 416 showed different peak activity periods (Anthomyidae,
 417 Mycetophylidae, Platystomatidae, Sarcophagidae, Scatopsidae
 418 and Sciaridae, Fig. S3a). Around half of all wasp families
 419 recorded showed a similar activity peak to our sampling period,
 420 while most others were more active during the summer months
 421 (December-February) and Bethylidae had only low numbers
 422 recorded on the ALA, all within the first six months of the year
 423 (Fig. S3b).

424

425 *Trap colour*

426 BVTs sampled greater average richness of all wasp and fly
 427 families combined, and greater abundance of all individuals
 428 across the study (Table 1, Figs. 2a, 2d). When modelled
 429 separately, all fly families also had greater average richness and
 430 abundance of individuals in BVTs (Table 1, Figs. 2b, 2e).

431 Wasp families were sampled equally in both colours (Table 1,
432 Figs. 2c, 2f).

433

434 Of the nine families sampled with greater than 30 individuals,
435 three were sampled more often in BVTs than YVTs (Diptera:
436 Bombyliidae $p<0.01$, Syrphidae $p<0.01$, Hymenoptera:
437 Scoliidae $p<0.01$; Table 2). Four families were sampled more
438 often in YVTs (Diptera: Muscidae $p<0.01$, Tachinidae $p=0.03$,
439 Therevidae $p=0.02$, Hymenoptera: Bethyridae $p<0.01$). There
440 was no difference between colours for Braconidae or Tiphidae
441 (Hymenoptera) (Table 2).

442

443 *Habitat*

444 Average richness of all fly and wasp families did not differ
445 between habitat type, even when modelled separately (Table 1,
446 Figs. 3a-3c). However, the abundance of all flies and wasps
447 was greater in open habitats (Table 1, Fig. 3d). This trend
448 occurred when also modelling fly abundance separately (Table
449 1, Fig. 3e). Wasp abundance was sampled equally in both
450 habitat types (Table 1, Fig. 3f).

451

452 Of the nine families sampled with greater than 30 individuals,
453 three families were found more often in open areas
454 (Bombyliidae $p<0.01$, Syrphidae $p<0.01$ and Scoliidae $p<0.01$),
455 whilst three families were more often sampled in wooded areas

456 (Muscidae $p=0.03$, Bethylidae $p<0.01$, Braconidae $p<0.01$;
457 Table 2). Three families showed no preference for either
458 habitat type (Tachinidae, Therevidae, Tiphidae; Table 2).

459

460 **DISCUSSION**

461 This study demonstrates that vane traps can be used to
462 representatively sample the non-bee pollinator fauna among
463 different habitat types within agricultural landscapes. At the
464 scale of the sampling area, we collected and identified an equal
465 number of families within a short sampling period to that
466 recorded on an historical database. When broadening the scale
467 to encompass the region, we found our sampling to be equal for
468 wasps, but less representative for the fly fauna, potentially due
469 to the inclusion of larger forest patches, waterbodies and
470 altitudinal gradients at this scale. Large numbers of individuals
471 were collected from multiple fly and wasp families across the
472 survey period, with certain families known to be pollinators
473 (e.g. Syrphidae: Armstrong, 1979; Bombyliidae: Larson *et al.*,
474 2001; Scoliidae: Vithanage & Ironside, 1986) displaying a
475 marked colour preference for BVTs. In contrast, family
476 richness and abundance of insects was lower in YVTs, but this
477 colour sampled some less common families important to the
478 provision of multiple ecosystem services, including pollination
479 (e.g. Acroceridae: Winterton, 2012; Bibionidae: D'Arcy-Burt &
480 Blackshaw, 1991). These results, combined with similar

findings for the bee fauna within this region (Hall, 2018),
indicate that vane traps are an important survey tool to
understand the demography and distribution of pollinator
communities.

Sampling effectiveness

Whilst many studies have compared different trapping methods
and tested colour attraction in pollinating insects (Kirk, 1984;
Campbell & Hanula, 2007; Saunders & Luck, 2013), few have
directly compared results to the known insect fauna of the
study region in question. Here, we not only compare two
colours of vane traps (blue and yellow), but also measure the
effectiveness of this sampling method compared to a baseline
of insect diversity within the same study area. The ALA is a
comprehensive repository of environmental and biological
information for Australia (Atlas of Living Australia, 2019). It
has been used to inform plant suitability under climate-change
(Booth *et al.*, 2012; Belbin & Williams, 2016), but such
databases are still rarely used as a reference point for insect
communities within a geographic area (but see Godefroid *et al.*,
2015; De Palma *et al.*, 2017; Young *et al.*, 2017 for examples
of effective use of historical data obtained through databases
and museum specimens). If we are to understand the
distribution and dynamics of pollinator communities, surveys
should be conducted across large spatial scales and compared

506 with existing databases (Bartomeus *et al.*, 2018). Vane traps
507 appear well suited for such comparison studies.

508

509 At the scale of the sampling area, vane traps sampled an
510 equivalent diversity of fly and wasp families to the ALA
511 database. In fact, the diversity of wasp families captured was
512 greater than that recorded for the study area. The number of fly
513 families sampled was lower than, but comparable to, the ALA
514 and sampled some families not recorded on it. The lower
515 number of fly families sampled in our study is likely due to the
516 short sampling period. We sampled during the austral spring
517 which may not align with the peak activity of certain families,
518 such as Anthomyiidae and Platystomatidae, which may more
519 likely be recorded in early summer or autumn (Fig. S3a).

520

521 When expanded to the regional scale, the number of wasp
522 families remained the same, however a number of additional
523 fly families were recorded on the ALA that were not sampled
524 in vane traps. There are a number of possible reasons for this.
525 First, as discussed above, the main activity period of those
526 additional fly families is different from our sampling period
527 (e.g. Chloropidae, Drosophilidae), or they have been rarely
528 recorded (e.g. only one record on the ALA for Pediciidae, Fig.
529 S3a). Second, such families may be more often found within
530 mountainous habitats, intact forest or waterbodies (e.g.

531 Chloropidae, Drosophilidae, Empididae: Lambkin *et al.*, 2011;
 532 Ephydriidae: Keiper *et al.*, 2002). These habitats were present
 533 within the greater regional area but not our sampling area, thus
 534 these families would be less likely to be found in more open,
 535 low altitude plains that comprise the less diverse habitats of our
 536 modified farm landscapes. Finally, families containing small-
 537 sized insects seem under-represented in our sampling. Such
 538 specimens were potentially missed when sorting traps due to
 539 their small size. However, these tiny insects are also likely to
 540 be missed in active surveys, such as sweep netting, and
 541 potentially unidentifiable when liquids or sticking agents are
 542 used with traps (Pickering & Stock, 2003).

543

544 **Colour preference**

545 We found greater richness and abundance of non-bee
 546 pollinators in BVTs than in YVTs, indicating a strong colour
 547 preference to BVTs for most insect taxa sampled. This is
 548 consistent with other vane trap studies targeting bees (Stephen
 549 & Rao, 2005; Joshi *et al.*, 2015; Hall, 2018) and is the first
 550 study comparing the effectiveness of these traps for sampling
 551 many of the non-bee fauna. Studies using other survey methods
 552 show some inconsistency in their ability to detect colour
 553 preference. For instance, Saunders & Luck (2013) found that
 554 yellow pan traps were preferred by pollinators (Hymenoptera
 555 and Diptera) across various habitats, but catches in each colour

556 trap varied with habitat type. Similarly, Abrahamczyk *et al.*
 557 (2010) found that in tropical and subtropical forests, yellow
 558 traps sampled more non-formicid Hymenoptera than blue,
 559 mainly due to a few families—Crabronidae, Ichneumonidae,
 560 Nyssonidae and Pompilidae. However, Campbell & Hanula
 561 (2007) found that blue pan traps were more effective than
 562 yellow pan, standard Malaise or coloured Malaise traps in
 563 sampling flower visiting insects in forested ecosystems. Such
 564 inconsistencies may be attributable to differences in reflectance
 565 of colours under different environmental conditions.

566

567 The UV-reflectance of traps possibly influences colour
 568 preference by pollinating insects. Bees and hoverflies are
 569 known to respond to the UV reflectance of flowers (Koski &
 570 Ashman, 2014), and other vane trap studies have tested the
 571 influence of reflectance on colour choice by bees (Stephen &
 572 Rao, 2005; Joshi *et al.*, 2015). Studies using other trapping
 573 methods have also tested UV reflectance, with varying results.
 574 For instance, Sircom *et al.* (2018) found different coloured pan
 575 traps used for bee surveys reflected to various degrees across
 576 wavelengths, with yellow traps generally having higher
 577 reflectance, while Shrestha *et al.* (2019) found hymenopterans
 578 showed no preference based on reflectance, but dipterans were
 579 generally more attracted to non-fluorescent than fluorescent
 580 traps. The preference for BVTs has been recorded in bees

581 despite differing reports on the reflectance of each colour
 582 (Stephen & Rao, 2005; Joshi *et al.*, 2015; Hall, 2018). In the
 583 present study, multiple non-bee families similarly preferred
 584 BVTs over YVTs, aligning with studies of bee communities.
 585 However, Koski & Ashman (2014) show that fluorescent
 586 patterning may be as important as colour, a feature present in
 587 many flowers but not on any common trapping method. We
 588 believe reflectance is an important quality in traps, and given
 589 the consistent colour of vane traps, they are well suited to
 590 sampling non-bee pollinators, particularly those that respond to
 591 UV reflectance. Incorporating fluorescent pattern into trap
 592 design could further improve their efficiency.

593

594 The ecological requirements of insects also likely affect
 595 attraction to different coloured traps (Kirk, 1984; Saunders &
 596 Luck, 2013). For instance, Syrphidae (hoverflies), which are
 597 known to mimic bees (particularly *Apis mellifera* L.) in their
 598 foraging behaviours (Knutson & Murphy, 1990; Golding &
 599 Edmunds, 2000, Campos-Jiménez *et al.*, 2014), were sampled
 600 more often in blue-coloured traps compared to yellow by
 601 Campbell & Hanula (2007). This was supported by our finding
 602 of a seven-fold increase in abundance of this family in BVTs.
 603 In addition, Joshi *et al.* (2015) and Hall (2018) found that bees
 604 were mostly attracted by BVTs, suggesting that, having similar
 605 foraging behaviour to bees, syrphids may respond to the same

606 visual cues (Kevan & Baker, 1983; Laubertie *et al.*, 2006).
 607 Similarly, Bombyliidae (bee flies) are also known to forage on
 608 flowers (Kastinger & Weber, 2001; Larson *et al.*, 2001) and
 609 have some behavioural similarities to both syrphids and bees
 610 (Armstrong, 1979; Knutson & Murphy, 1990). Bombyliids also
 611 show greater preference for blue flowers over yellow
 612 (Kastinger & Weber, 2001) and have been more successfully
 613 trapped in blue pan traps than in yellow in forested ecosystems
 614 of south-eastern United States (Campbell & Hanula, 2007).
 615 One wasp family, Scoliidae (flower wasps), known to pollinate
 616 Macadamia and Orchids (Vithanage & Ironside 1986; Ciotek *et*
 617 *al.*, 2006), was also associated with BVTs in our study,
 618 presumably responding similarly to colour as the above taxa
 619 (Vuts *et al.*, 2012).

620
 621 In contrast, four families were found more often in YVTs.
 622 Muscidae and Tachinidae are quite large, generalist groups of
 623 flies, containing numerous species with different feeding
 624 requirements, including predatory, blood-sucking, parasitic and
 625 anthophilous behaviours (Larson *et al.*, 2001; Skevington &
 626 Dang, 2002). Despite their apparent attraction to flowers,
 627 especially nectar, very little is known about their interactions
 628 with flowers (Larson *et al.*, 2001). Therevidae contains
 629 numerous predatory flies (especially of coleopteran larvae),
 630 which mainly avoid flowers, although a few species appear to

631 feed on nectar and insect secretions (Irwin & Lyneborg, 1989;
632 Irwin 2001; Skevington & Dang, 2002; van Herk *et al.*, 2015).
633 Bethyridae are known as beneficial insects and parasitoids of
634 Lepidoptera and Coleoptera (Danthanarayana, 1980; Berry,
635 1998). Smith *et al.* (2015) sampled them on yellow sticky traps,
636 but other coloured traps were not used in this study. Little is
637 known about any possible colour attraction for these two
638 families.

639

640 Similarly, BVTs were particularly efficient at catching wasp
641 families. The four families (Crabronidae, Gasteruptiidae,
642 Mutillidae and Vespidae) only caught in BVTs comprise
643 mostly predatory or parasitic species, but are also potential
644 flower visitors, sometimes even foraging at night (Portman *et*
645 *al.*, 2010; Wilson *et al.*, 2010). These may be missed when
646 surveying only in daylight hours. A benefit of vane traps is that
647 they can be left *in situ* night and day throughout the sampling
648 period, maximizing the chances of sampling species with
649 different foraging behaviours.

650

651 YVTs were effective at catching less common fly families,
652 indicating both BVTs and YVTs might be required to
653 comprehensively sample dipteran pollinators. Many of these
654 species appear to require floral resources at least temporarily.
655 For example, nectar collection by adult Chironomidae might

enhance their mating success (Larson *et al.*, 2001; Høye *et al.*, 2013). Many others are important pollinators as well as providing several additional ecosystem services, such as soil turnover, parasitism and scavenging (D'Arcy-Burt & Blackshaw, 1991; Larson *et al.*, 2001; Santos *et al.*, 2008; Winterton, 2012). These services may be particularly important, or indeed drive the response of taxa, in different habitat types.

Habitat association

Colour preference of pollinators has been compared in different habitat types across numerous studies. These include comparisons within different forested ecosystems (Campbell & Hanula, 2007; Abrahamczyk *et al.*, 2010), native bush and crop (Saunders & Luck, 2013) or sunny versus shaded (vegetated) sites (Hoback *et al.*, 1999). Such studies differ slightly from ours, in that wooded habitats here consisted of linear strips or scattered trees with an open understorey of coarse woody debris, while open habitats lacked trees and much of the woody litter.

In studies comparing open to closed habitats (forest, shade), microclimatic conditions, such as light, wind or humidity (Barradas & Fanjul, 1986), or reflectance and visibility of the coloured elements likely differ between habitat type (Abrahamczyk *et al.*, 2010). In contrast, due to the relatively

681 sparse nature of wooded habitats within farmland, we believe
 682 differences between open and wooded habitats in our study are
 683 likely driven by provision of different resources (floral, nesting
 684 and prey) within each habitat type rather than microclimate or
 685 trap visibility.

686

687 Many studies stress the importance of taking into account
 688 habitat when choosing different trapping methods. Comparing
 689 coloured pan traps in native bush and almond orchard,
 690 Saunders & Luck (2013) highlighted the need to consider
 691 habitat due to no individual colour adequately trapping target
 692 insects across all habitats. Similarly, with sticky traps, Hoback
 693 *et al.* (1999) found diversity measures were not affected by trap
 694 colour but determined by position of traps (shaded or exposed).
 695 In the present study, family richness in open or wooded habitats
 696 did not differ, but abundance of non-bee pollinators was greater
 697 in open habitat, likely due to the three most abundant flower-
 698 visiting families preferring open areas.

699

700 For families present only in open habitats, several ecological
 701 traits could explain their presence. Some families (Diptera:
 702 Conopidae, Stratiomyidae; Hymenoptera: Crabronidae) are
 703 known flower visitors and would therefore be present where the
 704 flowering resources are most abundant (Conopidae: Armstrong,
 705 1979; Stratiomyidae: Kevan & Baker, 1983; Crabronidae:

706 Portman *et al.*, 2010). The three families that were associated
707 most with BVTs (Bombyliidae, Syrphidae and Scoliidae) were
708 also sampled more often in open habitats. Just as Hall *et al.*
709 (2019) attributed the higher presence of bees in open habitats to
710 greater floral resources, the presence of these families in open
711 habitats could indicate behavioural similarities with bees.

712

713 Families more often sampled in wooded habitats were either
714 associated with YVTs (Muscidae, Bethyridae) or displayed no
715 colour preference (Braconidae). Overall, we found more unique
716 families in wooded habitat which we attribute to a greater
717 diversity of nest and perch sites, litter and food (e.g. insect and
718 arachnid prey). For example, Lauxaniidae feed on leaf litter and
719 are more likely found in the presence of shrubs, trees and
720 leaves than in grasslands (Merz, 2004). Other families predate
721 or parasitise caterpillars, orthopterans or spiders (Pompilidae:
722 Rayor, 1996; Acroceridae: Winterton, 2012). Several families
723 are known to parasitise solitary bee nests, wasp nests or
724 cockroach ootheca (Sacrophagidae: Skevington & Dang, 2002;
725 Evaniidae: Deyrup & Atkinson, 1993; Chrysididae:
726 Rosenheim, 1987). Others make their nests on wood or fungi
727 (Mycetophilidae: Madwar, 1937). These results further
728 highlight the need to sample across multiple habitat types, as
729 this might affect capture of certain taxa more than colour
730 preference.

731

732 Vane traps are effective at sampling a diverse fauna present in
733 habitats associated with modified agricultural landscapes.
734 However, other habitat types such as wetlands, forests or
735 mountains were present within the wider region and appear to
736 greatly increase diversity of fly families in particular (Fig S2).
737 It is therefore important to conduct sampling across various
738 habitat types if wanting to ensure a representative insect census
739 of an entire region. Due to consistency and quality of colour
740 and ease of installation, vane traps seem an ideal method for
741 comparisons between habitats.

742

743 **Conclusions and recommendations**

744 As with any one trapping technique, there are pros and cons to
745 using vane traps for sampling insect fauna. One positive is that
746 due to placement height and the absence of detergents, vane
747 traps used here were less likely to be disturbed, drunk, or
748 spilled by animals (wild or cattle), which may be a hazard
749 when using other methods, such as pan traps. However, vane
750 traps have at times been suspected of oversampling the bee
751 community (Gibbs *et al.*, 2017). It is true that the number of
752 individual bees captured in this region was relatively large for
753 two months of sampling (Hall, 2018) and we had high
754 abundance of certain families in this study, but not to the extent
755 we would be concerned with oversampling. Regardless, caution

756 is needed to limit the impact that trapping will have on the
 757 pollinator community. One possible solution would be to
 758 sample for a shorter period but to increase the number of
 759 sampling periods to capture seasonal changes (turnover) in the
 760 pollinator community (Thomsen *et al.*, 2016; Winfree *et al.*,
 761 2018). This would further increase the effectiveness of this
 762 trapping method in sampling a representative pollinator insect
 763 community.

764

765 A further limitation is the general dearth of knowledge of insect
 766 pollinators, which makes it difficult to draw meaningful
 767 ecological conclusions, given even basic distributional data is
 768 often lacking for Australia. This highlights the importance of
 769 using historical datasets as a baseline comparison to determine
 770 how representative sampling is for an area (Bartomeus *et al.*,
 771 2018). Taxonomic keys to the region are also limiting in that
 772 they only go as far as family-level, and expertise on pollinators
 773 is hard to come by (Batley & Hogendoorn, 2009). Although
 774 identification at the species-level often allows for the greatest
 775 precision (Lenat & Resh, 2001), family-level identification
 776 provides better functional and behavioural indications than
 777 order, which is sometimes used for such studies. Indeed, clear
 778 trends appeared for three families of non-bee pollinators:
 779 Bombyliidae, Syrphidae (Diptera) and Scoliidae
 780 (Hymenoptera), which preferred BVTs here. However, it is

781 difficult to establish colour preferences for more generalist
782 families within Diptera, which may include species with very
783 different feeding behaviours (e.g. flesh flies, flower flies,
784 predatory flies). In such cases, it would be ideal to use species-
785 level classification where sufficient data exists.

786

787 Vane traps are an effective sampling method to capture a
788 representative non-bee pollinator community, including flies
789 and wasps. Whereas bees displayed clear preference for BVTs,
790 results of this study suggest that in order to sample the
791 complete pollinator community, a range of colours may be
792 more suitable. Additionally, to capture greater numbers of other
793 flower visiting insects such as butterflies or beetles, it might be
794 necessary to combine this with other trapping techniques such
795 as Malaise traps. Active techniques, such as sweep netting
796 could be employed if wanting to answer more specific
797 questions about the function of communities, but for pollinator
798 census, vane traps are a cheap and efficient method that can be
799 used to good effect across multiple habitats.

800

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TABLES AND FIGURES

Table 1: Differences by trap colour and habitat type of the richness of all fly (Diptera) and wasp (Hymenoptera) families and abundance of individuals, as well as the richness and abundance of each order when modelled separately. Bold values indicate a significant response. Reference categories used were *blue* for colour and *open* for habitat.

		Estimate	Std. Error	z value	Pr(> z)
<i>Richness</i>	Colour	-0.22	0.06	-3.38	<0.01
	Habitat	-0.01	0.07	-0.20	0.84
<i>Abundance</i>	Colour	-0.79	0.10	-7.80	<0.01
	Habitat	-0.35	0.11	-3.06	<0.01
<i>Fly richness</i>	Colour	-0.34	0.08	-4.29	<0.01

	Habitat	-0.10	0.08	-1.28	0.20
<i>Fly abundance</i>	Colour	-0.87	0.11	-8.23	<0.01
	Habitat	-0.43	0.12	-3.48	<0.01
<i>Wasp richness</i>	Colour	-0.07	0.13	-0.57	0.57
	Habitat	0.13	0.14	0.92	0.36
<i>Wasp abundance</i>	Colour	-0.27	0.18	-1.52	0.13
	Habitat	-0.02	0.19	-0.09	0.93

Table 2: Differences by trap colour and habitat type of the abundance of individuals from fly (Diptera) and wasp (Hymenoptera) families sampled with >30 individuals. Bold values indicate a significant response. Reference categories used were *blue* for colour and *open* for habitat.

Order	Family		Estimate	Std. Error	z value	Pr(> z)
<i>Diptera</i>	<i>Bombyliidae</i>	Colour	-2.79	0.24	-11.47	<0.01
		Habitat	-1.21	0.31	-3.88	<0.01
	<i>Muscidae</i>	Colour	1.17	0.31	3.82	<0.01
		Habitat	1.24	0.58	2.15	0.03
	<i>Syrphidae</i>	Colour	-2.04	0.14	-14.63	<0.01
		Habitat	-0.72	0.16	-4.45	<0.01
	<i>Tachinidae</i>	Colour	0.26	0.12	2.10	0.03
		Habitat	-0.20	0.16	-1.22	0.22

<i>Hymenoptera</i>	<i>Therevidae</i>	Colour	0.73	0.31	2.31	0.02
		Habitat	0.83	0.52	1.62	0.11
	<i>Bethylidae</i>	Colour	1.02	0.39	2.63	<0.01
		Habitat	1.93	0.72	2.67	<0.01
	<i>Braconidae</i>	Colour	0.20	0.20	0.99	0.32
		Habitat	2.26	0.64	3.52	<0.01
	<i>Scoliidae</i>	Colour	-2.81	0.34	-8.20	<0.01
		Habitat	-3.58	0.50	-7.20	<0.01
	<i>Tiphiidae</i>	Colour	-0.11	0.16	-0.71	0.48
		Habitat	0.63	0.35	1.80	0.07

Figure 1: Comparison of the number and percentage of fly and wasp families sampled in our study using two different coloured traps - blue vane trap (BVT) and yellow vane trap (YVT) and within two different levels of wooded cover (habitat type) - Open and wooded, compared with those recorded on the Atlas of Living Australia (ALA) database for the sampling area (~3, 600 km²). Records shown for fly families by (a) trap colour and (b) habitat type, and for wasps by (c) trap colour and (d) habitat type. Letters beside names indicate where families were sampled - B=blue, Y=yellow, A=ALA, O=open, W=wooded. Tables below circles provide upper quantile values for the level of overlap between the ALA, BVTs and YVTs, calculated using the Morista-Horn index. A value of zero indicates no overlap, whilst a value of 1 indicates complete overlap.

Figure 2: Average richness of (a) all insect families (flies and wasps), (b) only fly families and (c) only wasp families, trapped per site in blue versus yellow traps. Abundance of individuals for each of these groups shown in (d-f).

Figure 3: Average richness of (a) all insect families (flies and wasps), (b) only fly families and (c) only wasp families, trapped per site in open versus wooded habitats. Abundance of individuals for each of these groups shown in (d-f).