

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

MR GORDON FITCH (Orcid ID : 0000-0002-2471-1160)

Article type : Research Article

gmfitch@umich.edu

Roads pose a significant barrier to bee movement, mediated by road size, traffic, and bee identity.

Gordon Fitch^{1*}† and Chatura Vaidya¹†

¹Department of Ecology and Evolutionary Biology

University of Michigan

Ann Arbor, MI, USA 48109

*Corresponding author

†The authors contributed equally to this paper.**Abstract**

1. Roads are a major driver of environmental stress, yet we know surprisingly little about how roads impact the movement of insect pollinators, and consequent pollination.

2. We investigated the influence of roads on pollinator movement and pollination by examining patterns of pigment transfer between focal plants of two species, *Coreopsis verticillata* and *Monarda fistulosa*. We asked whether roads reduced pigment transfer, and what characteristics of roads were important in driving this reduction. We also evaluated whether pollinator assemblage differed between species, and if this mediated the effect of roads on pigment transfer.

3. Plants across a road from a pigment-added plant received significantly less pigment than plants on the same side of the road. This effect was stronger for coreopsis than for monarda. The

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/1365-2664.13884](https://doi.org/10.1111/1365-2664.13884)

This article is protected by copyright. All rights reserved

29 mean body size of visitors to coreopsis was significantly smaller than that of visitors to monarda,
30 suggesting that smaller bees are more limited by roads.

31 4. Road width was the best predictor of pigment transfer, with a smaller effect of traffic volume;
32 further research is needed to fully disentangle the effects of different road characteristics.

33 Roadside habitat had little influence on pigment transfer, and roadside plants did not receive
34 significantly less pigment than plants in contiguous habitat.

35 *Synthesis and applications.* This study demonstrates that roads pose substantial barriers to bee
36 movement, reducing pollen flow between plants located across roadways from one another. Road
37 characteristics, particularly width and traffic volume, mediated this effect, as did bee size. Our
38 results suggest that the effects of roads on pollinators and pollination can be mitigated by many
39 of the same design strategies currently being implemented to reduce human traffic accidents,
40 offering the opportunity for win-win scenarios.

41 **Keywords: bees, road ecology, movement ecology, pollinator movement, barrier effect,**
42 **pollen movement**

43

44

45 **Introduction**

46 Road development is a ubiquitous driver of environmental stress (Van Der Ree et al.,
47 2015). Currently, the road network extends to about 32 million km across the globe (IRF, 2017),
48 and it is projected to increase by an additional 25 million km globally by 2050 (Dulac, 2013).

49 While the bulk of studies measuring the effect of roads on ecological communities have focused
50 on vertebrate animals (Bennett, 2017), there is increasing evidence that roads also impact
51 invertebrates – and particularly insects – both via mortality from vehicle collisions (Baxter-
52 Gilbert et al., 2015) and by altering movement patterns (Andersson et al., 2017; Bhattacharya et
53 al., 2003). Yet the few studies evaluating the degree to which roads represent barriers to insect
54 movement are largely anecdotal or poorly replicated (Bhattacharya et al., 2003; Franzén et al.,
55 2009; Remon et al., 2018; Zurbuchen et al., 2010), save for a small number of studies on
56 butterflies (Munguira & Thomas, 1992; Ries & Debinski, 2001). In general, we lack a rigorous
57 quantification of the extent to which roads limit insect movement.

58 Further complicating our understanding of the effects of roads on insects, in landscapes
59 otherwise dominated by agriculture, roadsides are important habitat for insects (Gardiner et al.,

60 2018; Phillips et al., 2020). This is particularly true when roadsides are managed to resemble
61 semi-natural meadow or prairie habitat (Hopwood, 2008; Phillips et al., 2019; Ries et al., 2001).
62 Fully understanding the effects of roads therefore requires disentangling the effects of the roads
63 themselves (i.e. of the bare surface and vehicle traffic) from management of roadside vegetation.
64 Moreover, all roads are not created equal. The degree to which roads represent barriers to the
65 movement of insect pollinators likely depends on the characteristics of the roads – particularly
66 their width and the speed and volume of traffic. Yet despite widespread recognition that roads
67 pose a barrier to insect pollinator movement, little research has been done to determine how
68 particular road attributes mediate this effect.

69 Understanding the effects of roads on pollinating insects is particularly crucial. Since
70 insect pollination is essential to reproduction in many plant species, road impacts on pollinators
71 are likely to have cascading effects on pollination and plant populations. Indeed, studies have
72 documented reduced pollination (Cunningham, 2000; Dargas et al., 2016) and seed set
73 (Cunningham, 2000) in insect-pollinated plants growing alongside roads (but see Grobler &
74 Campbell, 2020). As barriers to pollinator (and therefore pollen) movement, roads may also
75 genetically isolate plant populations (Nobarinezhad et al., 2019). While urbanization, which
76 includes but is not limited to increasing road density, has been shown to result in high rates of
77 local extinction and increased selfing (Cheptou & Avendaño V, 2006; Dornier & Cheptou,
78 2012), the role of roads *per se* in driving pollen limitation and population fragmentation in plants
79 has been scarcely evaluated.

80 Here, we begin to fill these research gaps by reporting the results from an investigation
81 into how roads of varying characteristics affect pollen movement in two plant species. We
82 examined patterns of transfer of fluorescent pigment (an analogue for pollen) between focal
83 plants of two native, insect-pollinated species, wild bergamot (*Monarda fistulosa*; Lamiaceae;
84 hereafter “monarda”) and threadleaf coreopsis (*Coreopsis verticillata* ‘Zagreb’; Asteraceae;
85 hereafter “coreopsis”), at 47 road- or pathside sites in Ann Arbor, Michigan, USA. Using these
86 experimental plants, we asked 1) if patterns of pigment transfer differ depending on whether a
87 plant is located across the road versus along the road from a pigment-added plant, and 2) if this
88 effect is mediated by pollinator assemblage. Additionally, we asked 3) which road characteristics
89 (lane number, traffic volume, traffic speed, roadside habitat) determine the degree to which roads
90 serve as barriers to pollinator movement. Finally, we asked 4) whether proximity to a road or

91 path influenced the magnitude of pigment transfer, relative to pigment transfer through
92 contiguous habitat not adjacent to a road.

93 We hypothesized that plants across a road from the pigment-added plant would receive
94 less pigment than plants alongside the road. We expected the magnitude of the barrier posed by
95 roads to differ based on pollinator body size, which in bees is strongly correlated with flight
96 ability (Greenleaf et al., 2007). Since we expected the pollinator assemblage of coreopsis to be
97 comprised of smaller-bodied insects than that of monarda, we hypothesized that coreopsis would
98 see greater declines in pollen transfer. We further hypothesized that road width, traffic volume
99 and speed would mediate the degree to which roads impede pollinator movement. Finally, we
100 hypothesized that roadsides would support fewer pollinators than contiguous habitats away from
101 a road, and those pollinators present would experience greater disruption to foraging, so pigment
102 transfer along roads would be reduced relative to contiguous sites.

103

104 Materials and Methods

105 *Study system*

106 This study was conducted in Ann Arbor, Michigan, USA, a small city (population 130,000),
107 from July 6 to August 10 2020. We used two species of flowering plants native to the region:
108 monarda and coreopsis. Both species are insect-pollinated; monarda is capable of selfing but
109 seed set increases dramatically with insect pollination (Cruden et al. 1984). Coreopsis is likely
110 self-incompatible, given what is known about the breeding system of congeners (Banovetz &
111 Scheiner, 1994; Smith & Deng, 2012). Differences in flower morphology between the species
112 suggest that they are visited by distinct assemblages of pollinators (see insets in Figure 1). We
113 purchased plants from Bluestone Perennials, Inc. (Madison, Ohio, USA) and maintained them
114 individually in 5.7L plastic pots until flowering began.

115

116 *Data collection*

117 We selected 47 sites adjacent to a road or path (hereafter road/path site), representing a
118 wide spectrum of road sizes and traffic volumes and speeds (see Supporting information, Table
119 S1 for site characteristics, and Figure S1 for representative images of sites). Sites were separated
120 from one another by at least 500m, with the exception of paved sidewalks, which were located
121 within 50 m of a road site (but plants were set out on a different day). Monarda and coreopsis

122 were each present in low densities at fewer than five sites. At these sites, we situated
123 experimental plants such that there were no intervening conspecifics.

124 A set of three conspecific plants (either alone or together with a set of plants from the
125 other species) were deployed at each road/path site. We collected data from coreopsis at a total
126 of 41 sites (27 roads, 5 bike paths, 9 pedestrian paths), and from monarda at 25 sites (22 roads, 3
127 bike paths). On one plant of the three, we applied luminous pigment (BioQuip, Rancho
128 Dominguez, California, USA) to all inflorescences (red for coreopsis, blue for monarda; Figure
129 S2). This plant was placed alongside the road/path. We placed a second plant ('across' plant)
130 across the road/path from the pigment-added plant, and measured the distance between these two
131 plants (distance between plants ranged from 1.4-41.1 m). The third plant ('along' plant) was
132 placed on the same side and at the same distance from the pigment-added plant as the 'across'
133 plant (Figure S3 illustrates the experimental setup). The distance between experimental plants
134 and the road or path edge varied somewhat depending on site conditions, but was always <3.5 m.
135 Plant set-up occurred between 0800-1030 h on warm, sunny days during the work week
136 (Monday-Friday) and collection between 1630-1900 h on the same day.

137 Upon collection, we took the 'across' and 'along' plants from each site into a dark
138 location and used a UV flashlight to detect pigment deposited on flowers. On each plant, we
139 counted the total number of inflorescences (and, for monarda, individual flowers) that held
140 pigment on reproductive structures, as well as the total number of open inflorescences (and
141 flowers for monarda). Since we observed pigment being moved by both wind and non-
142 pollinating insects, we only counted inflorescences where the pigment was found on
143 reproductive structures alone, i.e, not on petals. To avoid potential bias, we obscured plant
144 position (i.e. across or along) and site until after checking for pigment. After all flowers were
145 tallied, we removed inflorescences (for coreopsis) or flowers (for monarda) that held pigment, so
146 the plants could be used for data collection again on subsequent days. We also counted the
147 number of open inflorescences and flowers with pigment on the pigment-added plants; these
148 were not removed, though fresh pigment was added each sampling day. Thus pigment-added
149 plants were always pigment-added, while across and along plants were randomly assigned to
150 location from one day to the next.

151 At each road/path site, we recorded: 1) GPS coordinates for the center of the arranged plants, 2)
152 the distance between plants, 3) the number of striped lanes for vehicular traffic, 4) the posted

153 speed limit (measure of traffic speed), 5) traffic volume and 6) four binary attributes of roadside
154 vegetation that we hypothesized might influence pigment transfer [presence of 1) unmowed
155 weedy vegetation, 2) semi-natural meadow, 3) ornamental flower beds, and 4) lawn]. We
156 additionally combined attributes 1-3 to assess the effect of the presence of significant floral
157 resources, since each of these habitats supported high floral densities.

158 For ‘along’ plants at road sites, we also recorded whether their orientation relative to the
159 pigment-added plant was with or against the flow of traffic in the adjacent lane. Traffic volume
160 was determined by recording the number of vehicles passing by on the road for 5 min, either at
161 the time of set-up or collection which corresponded to high-traffic commuting hours. For car-free
162 roads, traffic surveys separately tallied the number of bicycles and pedestrians passing the plants.
163 The study occurred during the COVID-19 pandemic, when traffic volume was substantially
164 reduced.

165 To see whether proximity to a road or path influenced pigment transfer, we selected six
166 sites $\geq 100\text{m}$ from a road with contiguous, unbroken habitat; either mowed lawn [three sites] or
167 semi-natural meadow [three sites]. At each site, we set up an identical array of five plants, with a
168 pigment-added plant at the center, and four plants situated perpendicular to one another at
169 distances of 5 m, 15 m, 25 m, and 35 m from the pigment-added plant in each cardinal direction
170 (Figure S3). This range of distances nearly spans the range of distances at which our
171 road/pathside plants were placed (1.4-41.1 m). Due to its shorter flowering period, monarda was
172 included at only three of the six sites (two lawns and one meadow). Protocols for quantifying
173 pigment transfer were identical to those used at road/path sites.

174 We also conducted pollinator observations at 46 sites. where we observed one plant for
175 five minutes, and recorded all floral visitors. We identified visitors to morphospecies on the
176 wing, and recorded the number of visits by each morphospecies. Any contact with the
177 reproductive parts of the floral unit constituted a visit. Visits by the same individual to multiple
178 floral units were recorded separately. For coreopsis, we recorded visits to inflorescences, while
179 for monarda we recorded visits to flowers, including multiple flowers within the same
180 inflorescence. We also recorded the number of open floral units at the time of the observation.
181 Pollinator observations were used to determine the composition of the pollinator assemblage for
182 each species.

183

184 *Data Analysis*

185 We performed all analyses using R v.4.0.2 (R Core Team, 2020). All analyses were
186 conducted separately for the two plant species.

187

188 *Do pollinator assemblages between the two plant species differ by body size?*

189 To test whether the pollinator assemblages of the two plant species differed by body size,
190 we used intertegular distance (ITD), a close correlate of body size and flight ability (Greenleaf et
191 al., 2007; Ricketts et al., 2008), as our measure of body size. Most visits to both species were
192 made by bees (97%), with additional visits from Coleoptera, small Diptera, a wasp species and a
193 large butterfly species. Because they accounted for so few visits, non-bees were removed from
194 the dataset prior to analysis. We calculated a community-weighted mean ITD per observation by
195 multiplying the number of visits from each species by its ITD, summing these values, and
196 dividing by the total number of visits. We then performed linear regression of community-
197 weighted mean ITD against plant species.

198

199 *Do roads and paths influence pigment transfer?*

200 We calculated the proportion of inflorescences with pigment by dividing the number of
201 inflorescences with pigment by the number with open flowers. For monarda, we additionally
202 calculated the proportion of flowers that received pigment. Results were qualitatively similar for
203 flowers and inflorescences. Since inflorescence data are more easily compared with data from
204 coreopsis, we only report inflorescence-level data here (see Table S2 for flower-level results) To
205 determine whether the proportion of inflorescences with pigment differed between ‘across’ and
206 ‘along’ plants, we used a paired t-test, both with data from all sites combined and separately
207 considering road, bike path, and pedestrian path sites. We compared overall levels of pigment
208 transfer between the two species using a t-test.

209

210 *Which road characteristics influence pigment transfer?*

211 We evaluated the effect of four road characteristics (interplant distance, number of lanes
212 of vehicular traffic, speed limit, and traffic volume) on the proportion of flowers with pigment
213 using binomial GLMs. We counted bike paths as 0.5 lanes, and pedestrian paths as 0 lanes.
214 Because of the high degree of collinearity among the four road characteristics (Table S3),

215 including more than one variable in a model resulted in variance inflation factors >4 . Therefore,
216 we constructed four separate models, one for each road characteristic. All models had the
217 proportion of flowers with pigment as the dependent variable, and plant position ('along' or
218 'across') and the relevant road variable as predictors. Analysis of model residuals indicated
219 significant heteroscedasticity in all models for coreopsis. Thus, we ran updated models with
220 observations weighted by the reciprocal of the absolute value of the residuals from the
221 unweighted model (Strutz, 2016). After weighting, all models conformed to assumptions of
222 normality and heteroscedasticity. To evaluate the relative importance of the four road
223 characteristics in determining patterns of pigment transfer, we compared pseudo- R^2 and AIC_C
224 values for the four models. To determine whether the effect of the road characteristics depended
225 on plant position, we ran another set of models that included a plant position \times road
226 characteristic interaction term and checked for significance of the interaction term and overall
227 model fit.

228 Because the distance between plants, while related to road width, was experimentally
229 imposed, we examined the effect of the other three road variables on pigment deposition while
230 controlling for the effect of interplant distance. To do so, we ran linear models with the residuals
231 from the binomial GLM for interplant distance as the response variable, and one of the remaining
232 three road variables (number of lanes, traffic volume, and speed limit) as the predictor. Because
233 lane number and traffic volume had similar relationships with pigment deposition (see Results),
234 we did the same procedure with these two variables (i.e., test the effect of one using the residuals
235 from a model of the other) to identify which was a more important determinant of pigment
236 movement patterns.

237 To test for the effect of road/pathside vegetation attributes, we updated the best model (as
238 selected by the procedure outlined above) to include the habitat attributes. We compared this
239 model to the model without habitat attributes using AIC_C , and conducted stepwise model
240 simplification, removing the habitat attribute with the smallest effect on pigment transfer, until
241 the best model was found.

242 To test whether traffic-induced airflow impacted pigment transfer, we only used data
243 from 'along' plants at road sites. Using the best model for pigment transfer on this subsetted
244 dataset, we added a binary variable indicating orientation to the pigment-added plant relative to
245 flow of traffic ('with' or 'against' traffic), and checked for significance.

246

247 *Does pigment transfer differ between road/path sites and contiguous sites?*

248 To test whether being adjacent to a road affected pigment transfer, we used the data from
249 contiguous sites to regress the proportion of flowers with pigment against distance from the
250 pigment-added plant, again using weighted binomial GLMs. We then compared the predicted
251 relationship to that predicted using data drawn from the ‘along’ plants.

252

253 **Results**

254 *Do pollinator assemblages between the two plant species differ by body size?*

255 We observed 65 insect visits to coreopsis and 356 to monarda. The overwhelming
256 majority of visits to both species were from bees (97%). The ITD of observed bees ranged from
257 1.2 to 3.3 mm (Table S4); this represents nearly the full range of body sizes for locally-occurring
258 bees (Fitch, Glaum, et al., 2019).

259 Visitor community-weighted mean ITD was significantly larger for monarda (mean±S.E:
260 3.1±0.34 mm) than coreopsis (mean±S.E: 1.75±0.22 mm; $R^2 = 0.34$, $F_{1,16} = 9.8$, $p = 0.006$).
261 Coreopsis was visited mostly by sweat bees (60% of visits), while corbiculate bees dominated
262 visitation to monarda (*Bombus* spp. [56% of visits] and *Apis mellifera* [35% of visits]).

263

264 *Do roads and paths influence pigment transfer?*

265 Plants located across a road from a pigment-added plant received less pigment than plants
266 located alongside the road (Figure 1A,B). The magnitude of this difference was greater for
267 coreopsis than for monarda, with a 50% reduction in the number of inflorescences receiving
268 pigment for coreopsis (paired t-test: $t = -3.89$, d.f. = 26, $p < 0.001$), compared to a 34% reduction
269 for monarda (paired t-test: $t = -2.22$, d.f. = 21, $p = 0.04$). For coreopsis, the effect of bike paths
270 on pigment transfer appeared similar to the effect of roads, while for monarda plant location
271 relative to the bike path appeared to have no effect on pigment transfer, though sample size was
272 small in both cases ($N = 5$ for coreopsis, $N = 3$ for monarda; Figure 1B,E). Position relative to
273 pedestrian paths, whether unpaved or paved, appeared to have no effect on pigment transfer in
274 coreopsis (Figure 1C; we did not deploy monarda at any pedestrian path sites). Across all sites,
275 the proportion of inflorescences receiving pigment was significantly lower for monarda
276 (0.25 ± 0.04) than for coreopsis (0.46 ± 0.04 ; t-test: $t = 3.59$, d.f. = 126.5, $p < 0.001$).

277

278 *Which road characteristics influence pigment transfer?*

279 For coreopsis, all four measured road characteristics (interplant distance, number of
280 lanes, traffic volume, traffic speed) had a significant negative relationship with the proportion of
281 inflorescences receiving pigment (Figure 2A-D; Figure 3). However, once the effect of interplant
282 distance was controlled for, traffic speed no longer had a significant effect on pigment transfer
283 (Figure 3). Similarly, when the effect of lane number was controlled for, traffic volume no longer
284 affected pigment transfer, while lane number still had a significant negative effect on pigment
285 transfer even after controlling for traffic volume (Figure 3). For all models, while plant position
286 had a significant effect, there was no effect of a plant position \times road characteristic interaction
287 (Table S2).

288 For monarda, only interplant distance had a significant effect on the proportion of
289 inflorescences receiving pigment (Figure 2E-H; Figure 3) Neither plant position nor the plant
290 position \times road characteristic interaction term significantly influenced pigment transfer in any
291 model (Table S2). This was true when all sites were considered together and when road sites
292 were considered separately from path sites (Table S2).

293 While the magnitude of the difference in levels of pigment transfer between ‘across’ and
294 ‘along’ plants was greatest for smaller roadways, ‘across’ plants on smaller roads nevertheless
295 received substantial pigment in both species. On larger roads (≥ 3 lanes), by contrast, few ‘across’
296 plants received any pigment: for the 14 roads with ≥ 3 lanes, 71% of ‘across’ coreopsis plants
297 received no pigment, compared to 19% of plants on the 26 roads/paths with ≤ 2 lanes; for
298 monarda, the numbers were 75% and 1%, respectively.

299 None of the vegetation attributes of road/path verges (regular mowing, presence of semi-
300 natural habitat, presence of significant floral resources) affected pigment transfer in coreopsis (p
301 > 0.2 in all cases). For monarda, regular mowing had a marginally significant positive effect on
302 pigment transfer ($\beta = 3.7 \pm 2.0$, $z = 1.81$, $p = 0.07$); including this habitat attribute as a predictor of
303 pigment transfer improved model fit over the model including only interplant distance ($\Delta AICc =$
304 5.3).

305 For coreopsis, the orientation of the ‘along’ plant relative to the flow of traffic had a
306 significant effect on pigment transfer, with plants oriented with the flow of traffic relative to the
307 pigment-added plant having a higher proportion of inflorescences with pigment (mean \pm s.e. =

308 0.52±0.13, N = 9) than those oriented against traffic (0.40±0.08, N = 18; $z = 2.50$, $p = 0.01$).
309 There was no equivalent effect on pigment transfer for monarda (with traffic: 0.17±0.14, N = 6;
310 against traffic: 0.31±0.06, N = 15; $z = 0.21$, $p = 0.8$).

311

312 *Does pigment transfer differ between road/path sites and contiguous sites?*

313 For coreopsis, the proportion of inflorescences with pigment was, unexpectedly, lower on
314 average in contiguous sites than in road or path sites (mean±s.e., contiguous sites: 0.13±0.05;
315 road/path sites: 0.46±0.04; Mann-Whitney U-test: $W = 593.5$, $p < 0.001$). This was driven
316 primarily by low levels of pigment transfer to nearby plants (5 m from pigment-added plant;
317 Figure S4). Plants in lawn contiguous sites received substantially less pigment than those in
318 meadow contiguous sites (lawn: 0.04±0.02; meadow: 0.26±0.11), though due to the small sample
319 size this difference was not significant (Mann-Whitney U-test: $W = 70$, $p = 0.2$). When the two
320 contiguous site habitats were considered separately, the estimated relationship between distance
321 and pigment transfer for meadow sites was indistinguishable from that for road/path sites, while
322 that for lawn sites had a significantly reduced intercept (Figure S4).

323 For monarda, there was only a slight, non-significant difference in the proportion of
324 inflorescences with pigment between contiguous sites and road sites (contiguous sites:
325 0.17±0.03; road/path sites: 0.25±0.04; Mann-Whitney U-test: $W = 297$, $p = 0.9$; Figure S4).

326

327 **Discussion**

328 Our study shows that roads and paths pose a significant barrier to bee movement, and
329 therefore substantially reduce pollen transfer. Separation by a road reduced pigment transfer (a
330 proxy for pollen transfer) between coreopsis individuals by half and between monarda
331 individuals by one third. Surprisingly, the negative effect on pigment transfer persisted even on
332 narrow and quiet roads and paths, though this effect differed between plant species. For
333 monarda, we detected an effect of roads but not of dedicated cycling paths (though the small
334 number of path sites sampled may have obscured any effect of such structures). For coreopsis,
335 even narrow cycling paths impeded pigment transfer, though pedestrian-only paths and
336 sidewalks appeared not to (Figure 1).

337 Differences between coreopsis and monarda in the effect of roads and paths on pigment
338 transfer may be due to differences in the assemblage of bees visiting each species. Mean body

339 size of bees visiting coreopsis was significantly smaller than that of bees visiting monarda. In
340 bees, foraging distance is correlated with body size (Greenleaf et al., 2007). Thus, a road that is
341 devoid of floral resources may pose more of a barrier to smaller bees. It may also be that smaller
342 bees are more vulnerable to vehicle collisions, since evidence suggests that they cross roads at
343 lower heights (Munguira & Thomas, 1992; Ries et al., 2001; Saarinen et al., 2005). Additionally,
344 smaller bees may be more vulnerable to traffic-induced air turbulence, both within and alongside
345 roads. The fact that 'along' coreopsis plants oriented downstream from pigment-added plants
346 (i.e. in the direction of vehicle travel) received more pigment than plants located upstream
347 suggests that traffic-induced airflow may influence small bee movement alongside roads. Our
348 results are consistent with findings from Franzén et al. (2009) that only 10% of marked
349 individuals of a solitary bee, *Andrena hattorfiona*, crossed a road < 10 m wide, even though it
350 was unpaved.

351 We hypothesized that traffic characteristics (volume and speed) would be the road
352 characteristic that best explained pigment transfer patterns, that is, impeding pollinator
353 movement primarily by acting as conduits for fast-moving vehicles. Instead, we found that road
354 width, not traffic volume or speed, was the best predictor of pigment transfer in coreopsis, and
355 that in monarda only interplant distance was a significant predictor. This suggests that the
356 physical presence of the road, rather than the vehicles that travel along it, is the primary
357 impediment to bee movement. However, this result should be interpreted with caution. Traffic
358 volume and road width were highly correlated ($R^2 = 0.56$). Moreover, we measured traffic
359 volume over only a 5-minute interval during peak traffic, which may not accurately capture
360 traffic volume over the course of the day at all sites (e.g., our traffic survey of a site close to a
361 hospital, with a shift schedule that differs from the typical 0900-1700 h, may have
362 underestimated total traffic volume). Similarly, our measure of traffic speed was the posted legal
363 speed limit; the degree to which traffic obeyed these limits, and thus the accuracy of this
364 measure, varied across sites (authors' personal observation). Thus, while we cannot entirely
365 disentangle the effects of the physical presence of roads from the effects of traffic, our results
366 suggest that both play a role. However, more controlled experiments (e.g. comparing pollinator
367 or pigment movement between high- and low- traffic days) are needed to disentangle the relative
368 importance of physical infrastructure and traffic.

369 We had expected that higher quality habitats, characterized by the presence of substantial
370 floral resources and areas of semi-natural habitat, would support higher pollinator activity and
371 thus promote pigment transfer. Instead, we found no effect of vegetation attributes on pigment
372 transfer in coreopsis, and a weak but counterintuitively positive effect of the presence of lawn on
373 pigment transfer in monarda. This study occurred in an urban area, while most other studies of
374 the effects of roadside vegetation on pollinator communities have been conducted in rural
375 landscapes (but see Baldock et al., 2019). Perhaps, aspects of the urban environment, such as
376 ecological filtering (Fitch, Glaum, et al., 2019; Fitch, Wilson, et al., 2019) or impediments to
377 dispersal (Jha & Kremen, 2013) reduce the effects of roadside management and vegetation
378 characteristics documented by studies in rural landscapes (Hopwood, 2008; Phillips et al., 2019).

379 Finally, we hypothesized that plants alongside a road would experience reduced pigment
380 transfer in comparison to plants in contiguous habitat away from a road. We did not find that to
381 be the case for either species (Figure S4). For monarda, there was no difference in pigment
382 transfer between contiguous and road/path sites. Surprisingly, for coreopsis pigment transfer was
383 significantly lower at contiguous sites than at road/path sites. Our contiguous sites comprised
384 two habitats, lawn and semi-natural meadow. Our finding of low pigment transfer at contiguous
385 sites was largely driven by lawn sites; levels of pigment transfer at meadow sites were
386 indistinguishable from road/path sites. It is not surprising that isolated plants within lawns – a
387 habitat that typically has few flowers and thus attracts and supports few pollinators – would be
388 visited less than plants in higher-quality meadow habitats. It is puzzling, however, that road/path
389 sites, many of which also included lawns, did not show parallel habitat-based differences in
390 pigment transfer or overall lower levels of pigment transfer. It may be that road/path verges,
391 even those maintained as lawns, are less intensively managed than equivalent lawns in city parks,
392 and therefore provide better habitat for pollinators than the contiguous site lawns. The idea that
393 road verges provide good habitat for pollinators is supported by several studies showing that the
394 density and species richness of both flowers and pollinators are generally higher in road verges
395 as compared to agricultural (Hanley & Wilkins, 2015; Osgathorpe et al., 2012; Phillips et al.,
396 2019) and forest and woodland habitats (Riva et al., 2018). Moreover, Baldock and colleagues
397 found that the density and species richness of flowers and pollinators in road verges in three
398 cities in the UK were similar to several other urban habitats (Baldock et al., 2019). In addition,
399 bees may use road and path edges as navigational aids (Menzel et al., 2019), and, as our research

400 suggests, traffic-induced patterns of airflow may channel bees' flight along roadsides. Such
401 channeling would help explain increased pigment transfer to 'along' plants over plants in
402 contiguous sites where pollinator flight direction is not constrained. This latter explanation is
403 supported by our finding that pigment transfer to coreopsis in contiguous sites was much lower
404 than expected for plants close to the pigment-added plant, but at greater distances from the
405 pigment-added plant was equivalent to levels seen at road/path sites.

406 Taken together, the results of this study indicate that although roadsides may attract and
407 support sizable bee populations, roads pose barriers to bee movement, and consequently pollen
408 vectoring. While our findings suggest that even small roads and bicycle paths are barriers to bee
409 movement, we nevertheless observed substantial pigment transfer across these roads, suggesting
410 that this barrier does not preclude dispersal of bees and insect-vectored pollen. However,
411 pigment transfer across roads was rare in either species for roads with three or more lanes of
412 traffic. While we surveyed roads only up to five lanes wide, many roads often exceed this width.
413 Moreover, our estimate of the effect of roads is likely conservative, since the study took place
414 during the COVID-19 pandemic, when traffic was substantially reduced from pre-pandemic
415 levels. Therefore, we suggest that medium-sized and large roads may impede the movement of
416 bees sufficiently to impact foraging and pollination. We predict that this is particularly likely in
417 plants that rely on insect-vectored cross-pollination for successful reproduction (particularly
418 those pollinated primarily by small bees), and in urban areas where habitat fragments are
419 commonly surrounded on all sides by busy roads. Further research on the genetic structure of
420 populations of pollinating insects (particularly smaller species) and, insect-pollinated plants, with
421 explicit reference to roads as potential barriers, is needed to evaluate this claim.

422 In light of our findings, we recommend the evaluation and implementation of strategies to
423 make roads less of a barrier to pollinators. Habitat corridors have been effective in reducing the
424 impact of roads for many vertebrate taxa (Gilbert-Norton et al., 2010), and this concept can be
425 adapted for pollinators. With any such measure that encourages pollinators to cross the road, it
426 will be important to evaluate whether it results in elevated mortality due to increased vehicle
427 collisions, and if so whether this outweighs the benefits of increased population connectivity
428 (Keilsohn et al., 2018). Importantly, these interventions have the potential to dovetail with efforts
429 to promote alternative modes of transportation and reduce traffic accidents via so-called "road
430 diets", which reduce the area of a road dedicated to vehicular traffic (Ewing, 2008; Huang et al.,

431 2002), and consequently reduce the barrier to pollinator movement. These and related efforts
432 have the potential to reduce the environmental stress roads exert on all of us, human and non-
433 human alike.

434

435 **Data Availability Statement:** Data and code available via the Open Science Framework

436 <https://osf.io/az6qv> (Fitch & Vaidya 2021).

437

438 **Authors' contributions:** G.F. and C.V. are both first authors and listed alphabetically. G.F. and
439 C.V. conceived and designed the study, collected field data, carried out data processing and
440 analysis, and wrote and revised the manuscript.

441

442 **Acknowledgements:** We thank the homeowners who allowed us to use their yards for this study.

443 Thanks to M.D. Palmer for assistance with selecting and procuring plants, and to J.H.

444 Vandermeer, K. Sanchez, Z. Hajian-Forooshani, I. Rivera Salinas, J.R. Morris, N. Medina, and

445 two anonymous reviewers for helpful comments on earlier versions of the manuscript.

446

447 **References**

448 Andersson, P., Koffman, A., Sjödin, N. E., & Johansson, V. (2017). Roads may act as barriers to

449 flying insects: Species composition of bees and wasps differs on two sides of a large
450 highway. *Nature Conservation*, 18, 47–59.

451 <https://doi.org/10.3897/natureconservation.18.12314>

452 Baldock, K. C. R., Goddard, M. A., Hicks, D. M., Kunin, W. E., Mitschunas, N., Morse, H.,

453 Osgathorpe, L. M., Potts, S. G., Robertson, K. M., Scott, A. V., Staniczenko, P. P. A.,

454 Stone, G. N., Vaughan, I. P., & Memmott, J. (2019). A systems approach reveals urban

455 pollinator hotspots and conservation opportunities. *Nature Ecology & Evolution*, 3(3),

456 363–373. <https://doi.org/10.1038/s41559-018-0769-y>

457 Banovetz, S. J., & Scheiner, S. M. (1994). The Effects of Seed Mass on the Seed Ecology of

458 *Coreopsis lanceolata*. *The American Midland Naturalist*, 131(1), 65–74.

459 <https://doi.org/10.2307/2426609>

460 Baxter-Gilbert, J. H., Riley, J. L., Neufeld, C. J. H., Litzgus, J. D., & Lesbarrères, D. (2015).
461 Road mortality potentially responsible for billions of pollinating insect deaths annually.
462 *Journal of Insect Conservation*, 19(5), 1029–1035. [https://doi.org/10.1007/s10841-015-](https://doi.org/10.1007/s10841-015-9808-z)
463 9808-z

464 Bennett, V. J. (2017). Effects of Road Density and Pattern on the Conservation of Species and
465 Biodiversity. *Current Landscape Ecology Reports*, 2(1), 1–11.
466 <https://doi.org/10.1007/s40823-017-0020-6>

467 Bhattacharya, M., Primack, R. B., & Gerwein, J. (2003). Are roads and railroads barriers to
468 bumblebee movement in a temperate suburban conservation area? *Biological*
469 *Conservation*, 109(1), 37–45. [https://doi.org/10.1016/S0006-3207\(02\)00130-1](https://doi.org/10.1016/S0006-3207(02)00130-1)

470 Cheptou, P. O., & Avendaño V, L. G. (2006). Pollination processes and the Allee effect in highly
471 fragmented populations: Consequences for the mating system in urban environments.
472 *New Phytologist*, 172(4), 774–783. <https://doi.org/10.1111/j.1469-8137.2006.01880.x>

473 Cunningham, S. A. (2000). Depressed pollination in habitat fragments causes low fruit set.
474 *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267(1448),
475 1149–1152. <https://doi.org/10.1098/rspb.2000.1121>

476 Dargas, J. H. F., Chaves, S. R., & Fischer, E. (2016). Pollination of lark daisy on roadsides
477 declines as traffic speed increases along an Amazonian highway. *Plant Biology*, 18(3),
478 542–544. <https://doi.org/10.1111/plb.12437>

479 Dornier, A., & Cheptou, P.-O. (2012). Determinants of extinction in fragmented plant
480 populations: *Crepis sancta* (asteraceae) in urban environments. *Oecologia*, 169(3), 703–
481 712. <https://doi.org/10.1007/s00442-011-2229-0>

482 Dulac, J. (2013). *Global Land Transport Infrastructure Requirements: Estimating Road and*
483 *Railway Infrastructure Capacity and Costs to 2050: Information Paper*. International
484 Energy Agency;

485 Ewing, R. (2008). Traffic calming in the United States: Are we following Europe’s lead?
486 *URBAN DESIGN International*, 13(2), 90–104. <https://doi.org/10.1057/udi.2008.20>

487 Fitch, G., & Vaidya, C. (2021). Data from: Impacts of roads on bee movement. Open Science
488 Framework. <https://osf.io/az6qv>.

489

- 490 Fitch, G., Glaum, P., Simao, M.-C., Vaidya, C., Matthijs, J., Iuliano, B., & Perfecto, I. (2019).
491 Changes in adult sex ratio in wild bee communities are linked to urbanization. *Scientific*
492 *Reports*, 9(1), 1–10. <https://doi.org/10.1038/s41598-019-39601-8>
- 493 Fitch, G., Wilson, C. J., Glaum, P., Vaidya, C., Simao, M.-C., & Jamieson, M. A. (2019). Does
494 urbanization favour exotic bee species? Implications for the conservation of native bees
495 in cities. *Biology Letters*, 15(12), 20190574. <https://doi.org/10.1098/rsbl.2019.0574>
- 496 Franzén, M., Larsson, M., & Nilsson, S. G. (2009). Small local population sizes and high habitat
497 patch fidelity in a specialised solitary bee. *Journal of Insect Conservation*, 13(1), 89–95.
498 <https://doi.org/10.1007/s10841-007-9123-4>
- 499 Gardiner, M. M., Riley, C. B., Bommarco, R., & Öckinger, E. (2018). Rights-of-way: A potential
500 conservation resource. *Frontiers in Ecology and the Environment*, 16(3), 149–158.
501 <https://doi.org/10.1002/fee.1778>
- 502 Gilbert-Norton, L., Wilson, R., Stevens, J. R., & Beard, K. H. (2010). A Meta-Analytic Review
503 of Corridor Effectiveness. *Conservation Biology*, 24(3), 660–668.
504 <https://doi.org/10.1111/j.1523-1739.2010.01450.x>
- 505 Greenleaf, S. S., Williams, N. M., Winfree, R., Kremen, C., Greenleaf, S. S., & Williams, N. M.
506 (2007). Bee foraging ranges and their relationship to body size. *Oecologia*, 153(3), 589–
507 596. <https://doi.org/10.1007/s00442-007>
- 508 Grobler, B. A., & Campbell, E. E. (2020). Pollinator Activity and the Fecundity of a Rare and
509 Highly Threatened Honeybush Species along a Highway in the Cape Floristic Region.
510 *International Journal of Plant Sciences*, 181(6), 581–593. <https://doi.org/10.1086/708385>
- 511 Hanley, M. E., & Wilkins, J. P. (2015). On the verge? Preferential use of road-facing hedgerow
512 margins by bumblebees in agro-ecosystems. *Journal of Insect Conservation*, 19(1), 67–
513 74. <https://doi.org/10.1007/s10841-014-9744-3>
- 514 Hopwood, J. L. (2008). The contribution of roadside grassland restorations to native bee
515 conservation. *Biological Conservation*, 141(10), 2632–2640.
516 <https://doi.org/10.1016/j.biocon.2008.07.026>
- 517 Huang, H. F., Stewart, J. R., & Zegeer, C. V. (2002). Evaluation of Lane Reduction “Road Diet”
518 Measures on Crashes and Injuries. *Transportation Research Record*, 1784(1), 80–90.
519 <https://doi.org/10.3141/1784-11>
- 520 IRF. (2017). *World Road Statistics 2016*. International Road Federation.

521 Jha, S., & Kremen, C. (2013). Urban land use limits regional bumble bee gene flow. *Molecular*
522 *Ecology*, 22(9), 2483–2495. <https://doi.org/10.1111/mec.12275>

523 Keilsohn, W., Narango, D. L., & Tallamy, D. W. (2018). Roadside habitat impacts insect traffic
524 mortality. *Journal of Insect Conservation*, 22(2), 183–188.
525 <https://doi.org/10.1007/s10841-018-0051-2>

526 Menzel, R., Tison, L., Fischer-Nakai, J., Cheeseman, J., Balbuena, M. S., Chen, X., Landgraf, T.,
527 Petrasch, J., Polster, J., & Greggers, U. (2019). Guidance of Navigating Honeybees by
528 Learned Elongated Ground Structures. *Frontiers in Behavioral Neuroscience*, 12.
529 <https://doi.org/10.3389/fnbeh.2018.00322>

530 Munguira, M. L., & Thomas, J. A. (1992). Use of Road Verges by Butterfly and Burnet
531 Populations, and the Effect of Roads on Adult Dispersal and Mortality. *Journal of*
532 *Applied Ecology*, 29(2), 316–329. <https://doi.org/10.2307/2404501>

533 Nobarinezhad, M. H., Challagundla, L., & Wallace, L. E. (2019). Small-Scale Population
534 Connectivity and Genetic Structure in Canada Thistle (*Cirsium arvense*). *International*
535 *Journal of Plant Sciences*, 181(4), 473–484. <https://doi.org/10.1086/706882>

536 Osgathorpe, L. M., Park, K., & Goulson, D. (2012). The use of off-farm habitats by foraging
537 bumblebees in agricultural landscapes: Implications for conservation management.
538 *Apidologie*, 43(2), 113–127. <https://doi.org/10.1007/s13592-011-0083-z>

539 Phillips, B. B., Gaston, K. J., Bullock, J. M., & Osborne, J. L. (2019). Road verges support
540 pollinators in agricultural landscapes, but are diminished by heavy traffic and summer
541 cutting. *Journal of Applied Ecology*, 56(10), 2316–2327. <https://doi.org/10.1111/1365-2664.13470>

543 Phillips, B. B., Wallace, C., Roberts, B. R., Whitehouse, A. T., Gaston, K. J., Bullock, J. M.,
544 Dicks, L. V., & Osborne, J. L. (2020). Enhancing road verges to aid pollinator
545 conservation: A review. *Biological Conservation*, 108687.
546 <https://doi.org/10.1016/j.biocon.2020.108687>

547 R Core Team. (2020). *R: a language and environment for statistical computing* (4.0.2)
548 [Computer software].

549 Remon, J., Chevallier, E., Prunier, J. G., Baguette, M., & Moulherat, S. (2018). Estimating the
550 permeability of linear infrastructures using recapture data. *Landscape Ecology*, 33(10),
551 1697–1710. <https://doi.org/10.1007/s10980-018-0694-0>

552 Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A.,
553 Gemmill-Herren, B., Greenleaf, S. S., Klein, A. M., Mayfield, M. M., Morandin, L. A.,
554 Ochieng', A., & Viana, B. F. (2008). Landscape effects on crop pollination services: Are
555 there general patterns? *Ecology Letters*, *11*(5), 499–515. [https://doi.org/10.1111/j.1461-](https://doi.org/10.1111/j.1461-0248.2008.01157.x)
556 [0248.2008.01157.x](https://doi.org/10.1111/j.1461-0248.2008.01157.x)

557 Ries, L., & Debinski, D. M. (2001). Butterfly responses to habitat edges in the highly fragmented
558 prairies of Central Iowa. *Journal of Animal Ecology*, *70*(5), 840–852.
559 <https://doi.org/10.1046/j.0021-8790.2001.00546.x>

560 Ries, L., Debinski, D. M., & Wieland, M. L. (2001). Conservation Value of Roadside Prairie
561 Restoration to Butterfly Communities. *Conservation Biology*, *15*(2), 401–411.
562 <https://doi.org/10.1046/j.1523-1739.2001.015002401.x>

563 Riva, F., Acorn, J. H., & Nielsen, S. E. (2018). Localized disturbances from oil sands
564 developments increase butterfly diversity and abundance in Alberta's boreal forests.
565 *Biological Conservation*, *217*, 173–180. <https://doi.org/10.1016/j.biocon.2017.10.022>

566 Saarinen, K., Valtonen, A., Jantunen, J., & Saarnio, S. (2005). Butterflies and diurnal moths
567 along road verges: Does road type affect diversity and abundance? *Biological*
568 *Conservation*, *123*(3), 403–412. <https://doi.org/10.1016/j.biocon.2004.12.012>

569 Smith, S. M., & Deng, Z. (2012). Pollen-mediated Gene Flow from *Coreopsis tinctoria* to
570 *Coreopsis leavenworthii*: Inheritance of Morphological Markers and Determination of
571 Gene Flow Rates as Affected by Separation Distances. *Journal of the American Society*
572 *for Horticultural Science*, *137*(3), 173–179. <https://doi.org/10.21273/JASHS.137.3.173>

573 Strutz, T. (2016). *Data fitting and uncertainty: A practical introduction to weighted least squares*
574 *and beyond*. Springer Vieweg.

575 Van Der Ree, R., Smith, D. J., & Grilo, C. (2015). *Handbook of road ecology*. John Wiley &
576 Sons, Ltd.

577 Zurbuchen, A., Bachofen, C., Müller, A., Hein, S., & Dorn, S. (2010). Are landscape structures
578 insurmountable barriers for foraging bees? A mark-recapture study with two solitary
579 pollen specialist species. *Apidologie*, *41*(4), 497–508.
580 <https://doi.org/10.1051/apido/2009084>

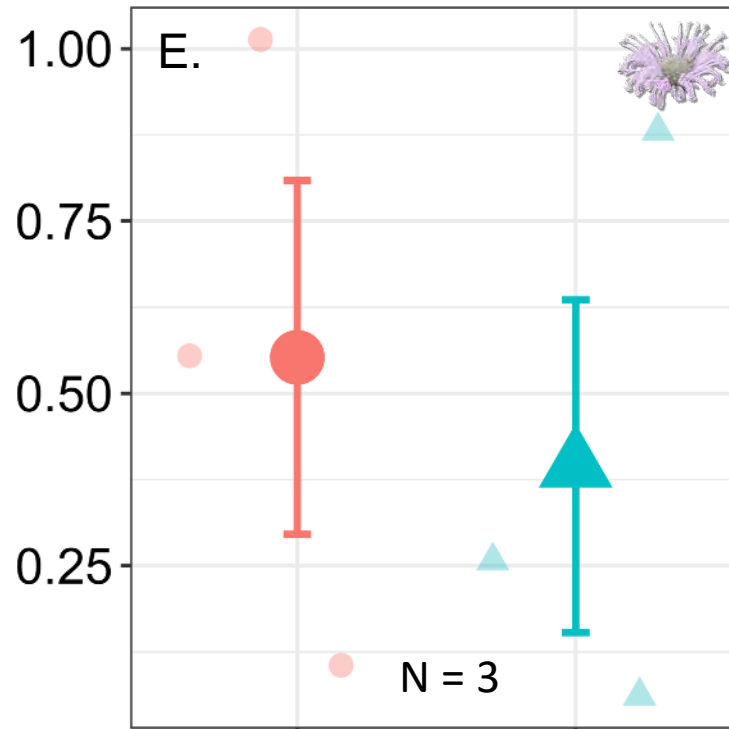
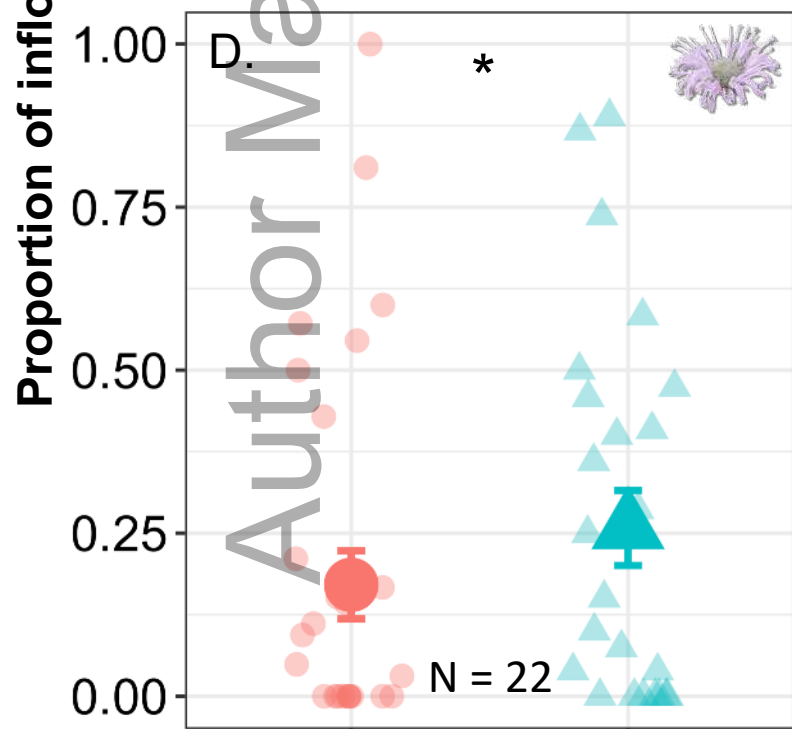
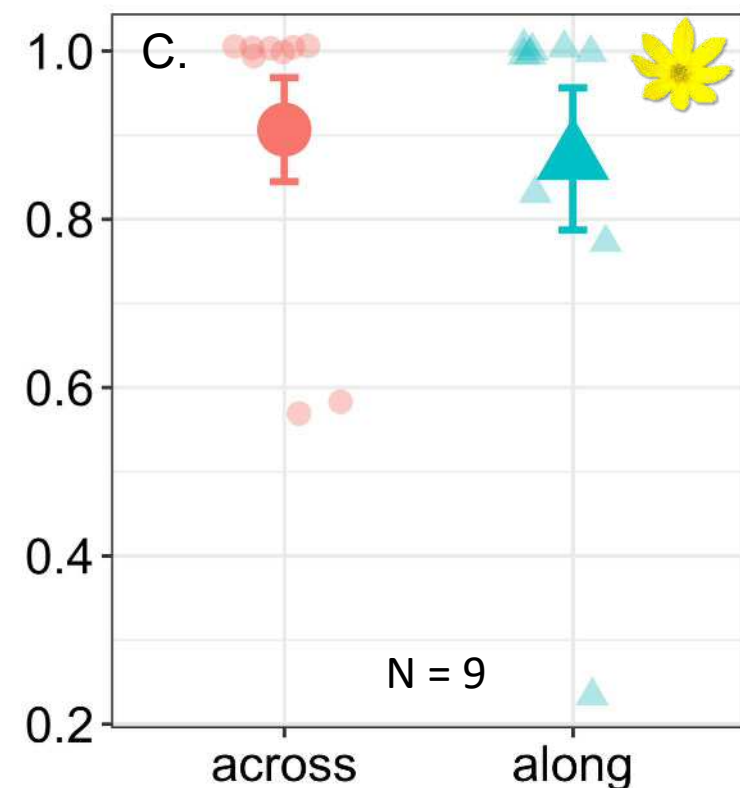
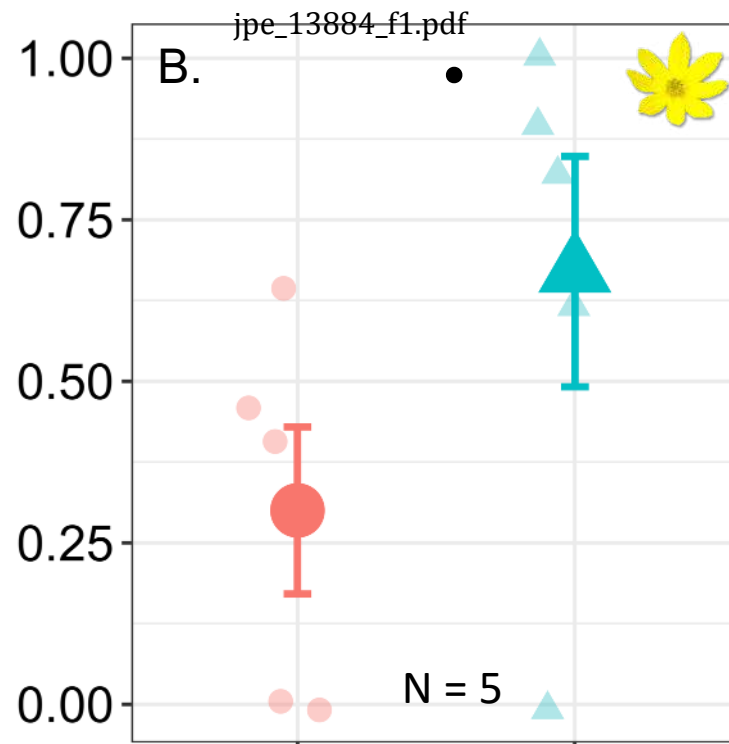
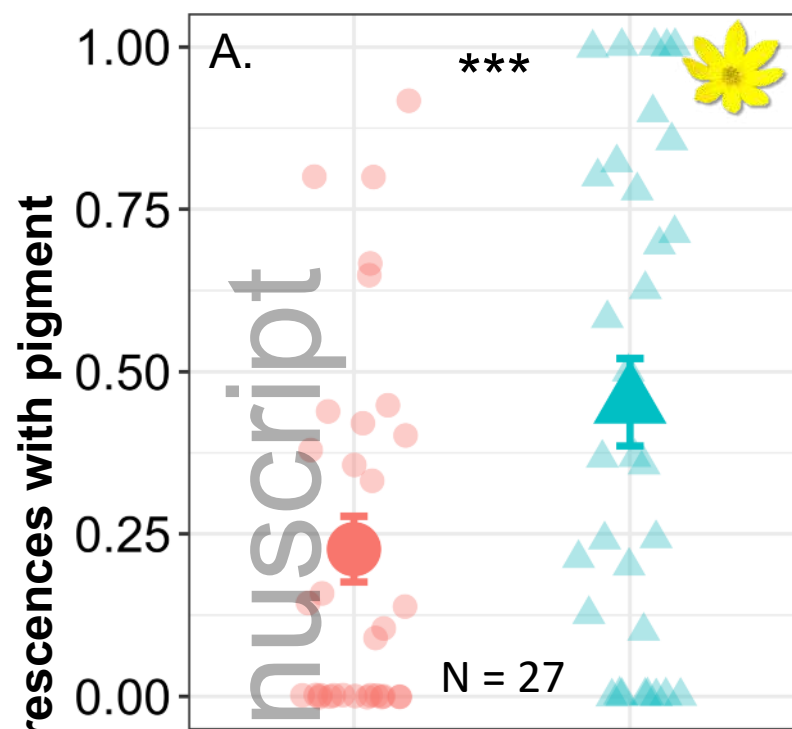
581
582

583 **Figure legends**

584 **Figure 1.** Effect of position relative to road or path on pigment deposition in coreopsis (A,B,C)
585 and monarda (D,E). Large points represent means and error bars ± 1 SE; small points represent
586 observations from individual sites, jittered to improve legibility. Significance codes, according to
587 paired t-tests: *** $p < 0.001$; * $p < 0.05$; • $p = 0.05$.

588
589 **Figure 2.** Relationship between road characteristics and pigment transfer in coreopsis (A-D) and
590 monarda (E-H). Red circles represent data from plants located across the road/path from the
591 pigment-laden plant, while cyan triangles represent plants alongside the road/path. Lines show
592 best-fit regression according to binomial GLMs; shading indicates 95% confidence intervals.

593
594 **Figure 3.** Effect of road characteristics on pigment transfer in coreopsis and monarda. Points
595 represent β estimates and bars represent \pm standard error for each predictor, derived from
596 binomial GLMs. Numbers to the right are R^2 values for that model. In the top panel, β and R^2
597 values are for model including plant position; all other panels show values for model using
598 residuals from models in top panel as the response variable. Significance codes: * $p < 0.05$,
599 ** $p < 0.005$, *** $p < 0.001$.

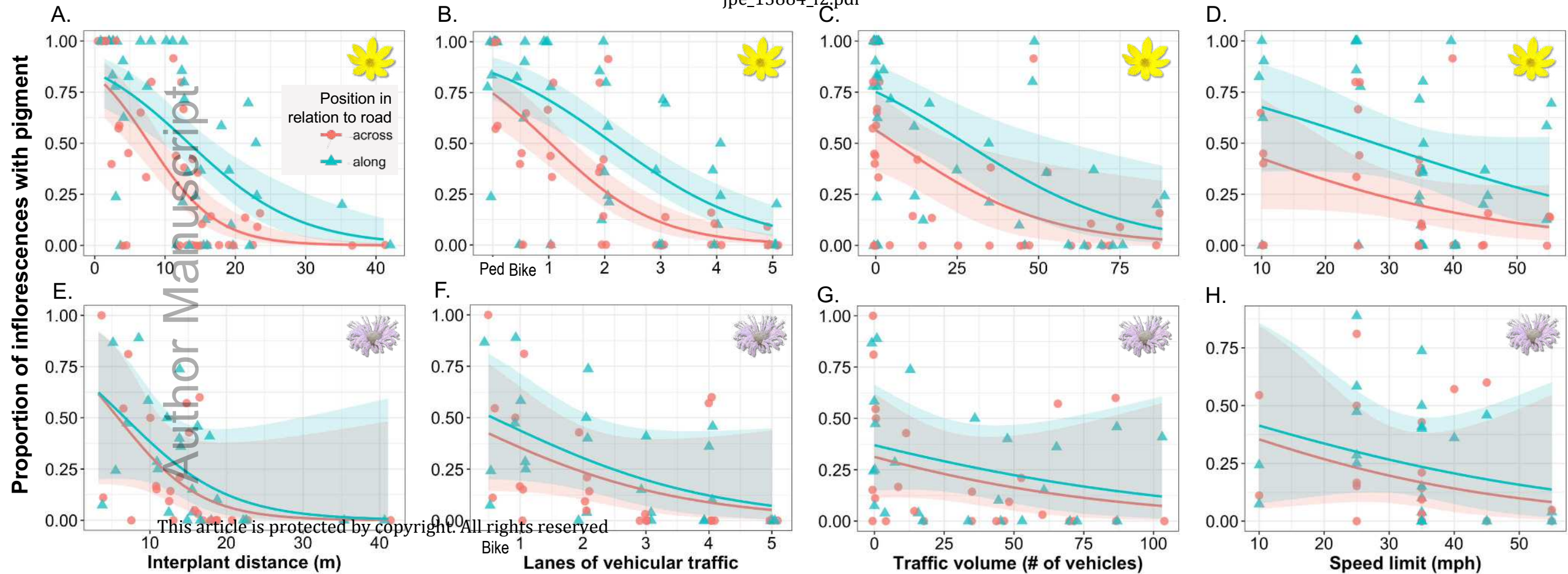


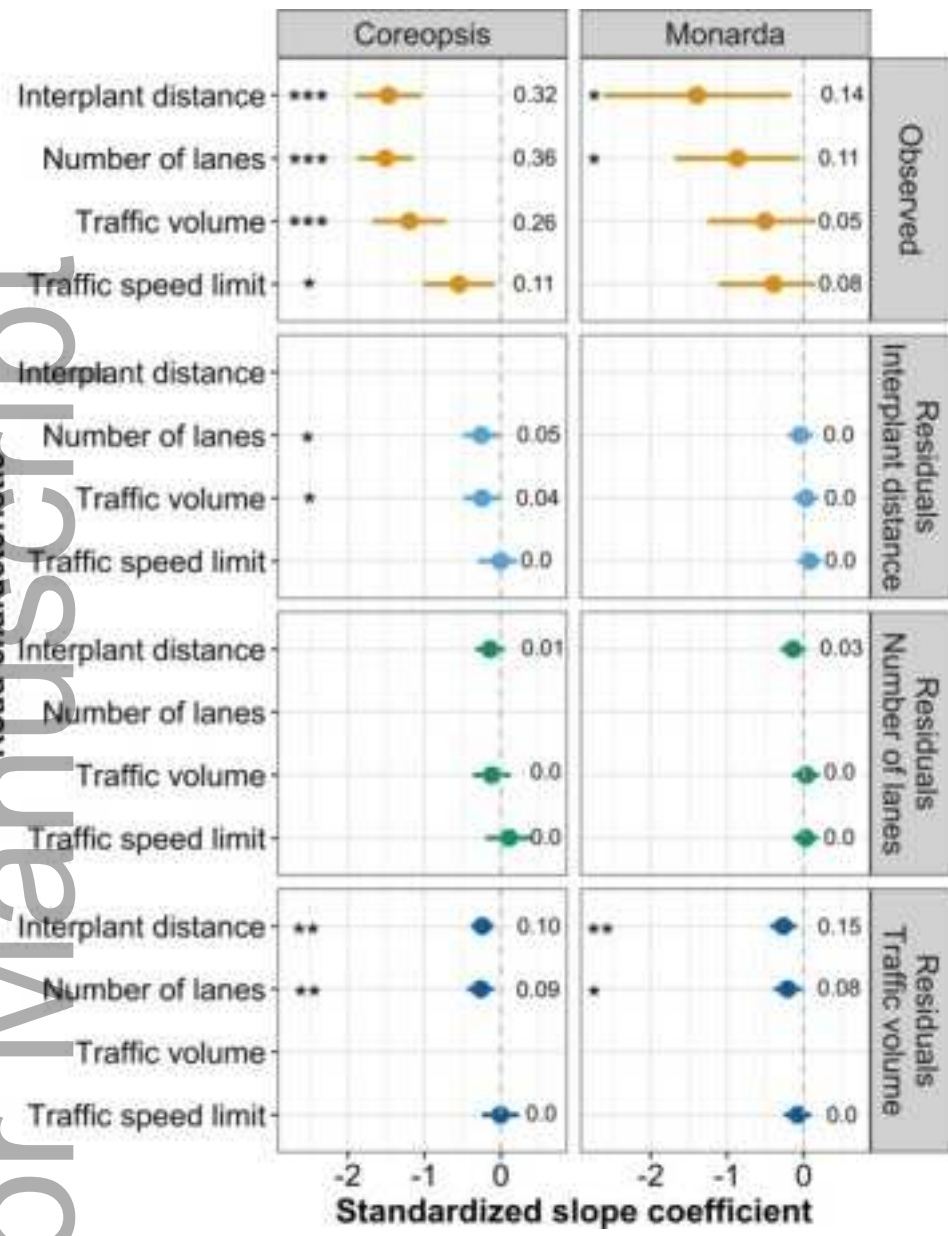
Position relative to pedestrian path

This article is protected by copyright. All rights reserved

Position relative to road

Position relative to bike path





jpe_13884_f3.png