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1 Flowering plant composition shapes pathogen infection intensity and reproduction in bumble bee
2 colonies

3

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25

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28 manuscript with feedback from all authors.

29

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31

32

33 **Abstract**

34 Pathogens pose significant threats to pollinator health and food security. Pollinators can transmit
35 diseases during foraging, but the consequences of plant species composition for infection is
36 unknown. In agroecosystems, flowering strips or hedgerows are often used to augment pollinator
37 habitat. We used canola as a focal crop in tents, and manipulated flowering strip composition
38 using plant species we had previously shown to result in higher or lower bee infection in short-
39 term trials. We also manipulated initial colony infection to assess impacts on foraging behavior.
40 Flowering strips using high-infection plant species nearly doubled bumble bee colony infection
41 intensity compared to low-infection plant species, with intermediate infection in canola-only
42 tents. Both infection treatment and flowering strips reduced visits to canola, but we saw no
43 evidence that infection treatment shifted foraging preferences. Although high-infection flowering
44 strips increased colony infection intensity, colony reproduction was improved with any flowering
45 strips compared to canola alone. Effects of flowering strips on colony reproduction were
46 explained by nectar availability, but effects of flowering strips on infection intensity were not.
47 Thus, flowering strips benefited colony reproduction by adding floral resources, but certain plant
48 species also come with a risk of increased pathogen infection intensity.

49

50 **Significance Statement**

51 Pollinator decline affects food security, and pollinators are threatened by stressors including
52 pathogens and insufficient food resources. Flowering strips are increasingly planted to increase
53 pollinator abundance and diversity in agricultural settings, but flowers can also be disease
54 transmission sites between pollinators. However, the effect of plant species composition on bee
55 disease is unknown. We compared the effect of flowering strips with high- or low-infection plant

56 species, or no flowering strips, on bee infection and reproduction in tents. Using high-infection
57 flowering strips doubled bee infection intensity compared to low-infection flowering strips.
58 However, bee reproduction was higher with any flowering strips. Thus, floral resources in
59 flowering strips benefited bees, but certain plants also come with a risk of increased pathogen
60 infection intensity.

61

62 **Introduction**

63 Community composition changes species dynamics, including the probability of disease
64 transmission between hosts. For pollinators, understanding how plants shape disease
65 transmission is important because pollination services contribute an estimated US\$235–577
66 billion to our global economy (1) and pathogens have been implicated as one of the factors
67 underlying pollinator declines (2). Governments, industry and private individuals worldwide are
68 investing in planting pollinator-friendly habitat (called ‘flowering strips’ hereafter to encompass
69 any supplemental floral resources) to mitigate pollinator declines. These habitats can increase
70 pollinator species richness and abundance (3, 4, e. g., 5, 6) and sometimes pollination and crop
71 yield (7-9). However, floral cover by particular plant groups, such as high-quality forage, can be
72 more important than overall floral cover for bumble bee family lineage survival across years
73 (10). Plant species vary in the amount and quality of resources for pollinators (e. g., 11, 12), and
74 particular plant groups can play larger roles than overall plant diversity for bee colony growth
75 (13). Thus, some plant species or groups may be more effective than others for managing
76 pollinator health.

77 In addition to providing resources, plants can be sites of disease transmission between
78 pollinators (e. g., 14, 15, 16). However, the role of plant species composition in shaping parasite

79 or pathogen infection in pollinators is largely unknown. Sown wildflower fields increased
80 prevalence of several bee pathogens as well as bee abundance in landscapes with few semi-
81 natural elements (17). In an observational study across 19 urban gardens, the number of trees and
82 shrubs was positively correlated with phorid fly parasitism in both honey and bumble bees (18).
83 In another study, the prevalence of deformed wing virus and black queen cell virus was higher in
84 bumble bees and on flowers near honey bee apiaries, suggesting that flowers are the site of virus
85 transmission from commercial honey bees to wild bumble bees (19). All of these studies suggest
86 that floral resources can increase both bee abundance and risks of pathogen or parasite infection,
87 but we do not yet know whether plant species composition plays significant roles in shaping bee
88 pathogen infection.

89 Variation in floral traits within and among plant species can change the likelihood of
90 vectoring or transmitting pathogens or parasitic mites (14, 15, 20, 21), and such variation can
91 have consequences for disease transmission dynamics (22). In particular, a recent study found
92 four-fold variation across 14 plant species in transmission of the gut pathogen *Crithidia bombi* to
93 foraging bumble bees (*Bombus impatiens*) (20), and defecation on flowers by infected bees
94 varied with plant species (23). However, we do not know whether these individual dynamics
95 scale up to plant community consequences for bumble bee colony-level pathogen infection and
96 reproduction.

97 The role of plant species in shaping infection intensity could be influenced by bee
98 behavior. If infected bees increase visitation to antimicrobial plant species as a form of self-
99 medication (24), such plant species could play a larger role than predicted in disease dynamics.
100 Alternatively, antimicrobial plant species may be less effective than expected if pathogens
101 manipulate host behavior to avoid such plants (25). Sunflower (*Helianthus annuus*) has pollen

102 that dramatically reduces *C. bombi* in *B. impatiens* (26, 27) and several plant species produce
103 nectar with secondary compounds that can reduce pathogens (28-30), although such effects are
104 not always consistent (31, 32). Only a few studies have assessed whether infection alters bee
105 preference. In the field, infected *B. impatiens* and *B. vagans* had greater preference than
106 uninfected bees for inflorescences with high nectar iridoid glycosides that can reduce pathogen
107 infection (33). However, a laboratory study with *B. terrestris* found only weak evidence that
108 infected bees had increased preference for nectar nicotine compared to uninfected bees (28).
109 Thus, there are conflicting results across species and compounds, and very few data overall to
110 assess whether infection changes foraging preferences.

111 We assessed whether flowering strip species composition would affect pathogen infection
112 intensity and bumble bee colony reproduction, and whether flowering strips and infection
113 affected foraging behavior and pollination services. We designed our experiment in an
114 agricultural context because flowering strips are increasingly used to promote pollinator
115 abundance and diversity and enhance pollination services in agriculture, although results are also
116 relevant for natural ecosystems. We used tent mesocosms with canola as a focal crop and
117 included three flowering strip treatments: canola only, high-infection strip, and low-infection
118 strip, crossed with presence/absence of infection. This experiment is the first to assess the
119 consequences of specific plant communities for bee colony infection and reproduction, which is
120 critical for making informed decisions about pollinator habitat management as well as
121 understanding how plant community composition structures species interactions.

122

123 **Results**

124 Pathogen infection. *C. bombi* cells replicated in hosts; in the infected treatment final raw counts
125 averaged 39.7 ± 30.4 cells per 0.02 ul (mean + s.d.; range 0-130), nearly 100 times more *C.*
126 *bombi* cells than the initial inoculation.

127 We asked how flowering strips affected infection, including only bees in the infected
128 treatment. There was no effect of flowering strips on the proportion of infected bees ($\chi^2 = 2.68$, P
129 $= 0.262$), but flowering strips did affect mean infection intensity ($\chi^2 = 7.99$, $P = 0.018$), with
130 higher mean infection in high-infection than low-infection tents. Mean infection intensity in
131 canola tents was intermediate and not significantly different from either flowering strip treatment
132 (Fig. 1A). When nectar availability per tent was included as a covariate, the effect of flowering
133 strip was still significant ($\chi^2 = 8.97$, $P = 0.011$) and the effect of nectar was not ($\chi^2 = 2.32$, $P =$
134 0.128 ; Fig. 1B), indicating that the effect of flowering strips on infection intensity was not
135 mediated by nectar availability.

136

137 Microcolony performance. Surviving adult workers, number of larvae produced and mean egg
138 weight were significantly affected by flowering strips (Table 1), with more adults and larvae and
139 heavier eggs with high- and low-infection strips than canola alone (Fig. 2A, C). Flowering strips
140 did not significantly affect egg number or larval weight (Table 1). Infection had a nonsignificant
141 tendency to reduce egg number; infection and the infection by flowering strip interaction did not
142 affect any other performance measures (Table 1).

143 When nectar resources were included as a covariate (Table 1), they were positively
144 related to number of surviving workers (Fig 2B), larvae produced (Fig. 2D), number of eggs and
145 egg weight. The effect of flowering strips was still borderline significant for number of larvae

146 but not other responses, and there was a borderline significant interaction between infection and
147 flowering strips for larval weight (Table 1). No other effects were significant (Table 1).

148

149 Pollinator foraging behavior. Based on the ‘quick observations,’ both infection ($\chi^2 = 13.95$, $P <$
150 0.001) and flowering strips ($\chi^2 = 7.81$, $P = 0.02$) reduced the number of foragers on canola, with
151 38% fewer foragers in tents with infected bees and 30% fewer foragers in tents with flowering
152 strips (Fig. 3A). There was no significant interaction between infection and flowering strips ($\chi^2 =$
153 2.42 , $P = 0.30$). However, when considering foragers to all plants, the infection x flowering strip
154 interaction was significant ($\chi^2 = 6.16$, $P = 0.046$); infection reduced foraging on all plants in the
155 canola and low-infection strips, but not high-infection strips (Fig. 3B).

156 When considering bee behavior and movement between plants, we found that infection,
157 flowering strips and their interaction did not affect the number of plant switches per minute, the
158 proportion of switches to a new plant species, the number or proportion of visits to sunflower or
159 thyme plants. Treatments also did not affect the proportion of low- vs. high-infection plants or
160 flowers visited ($\chi^2 < 2.05$, $P > 0.15$ for all), except that bees visited a greater proportion of low-
161 infection plants and flowers in low-infection than high-infection flowering strips (plants, $\chi^2 =$
162 37.96 , $P < 0.0001$; flowers, $\chi^2 = 29.23$, $P < 0.0001$). Similarly, total visits and proportion of
163 visits to sunflowers was greater in low-infection flowering strips where sunflowers were more
164 numerous (total visits, $\chi^2 = 6.53$, $P = 0.011$; proportion of visits, $\chi^2 = 5.29$, $P = 0.022$).

165

166 **Discussion**

167 Although previous studies have shown that floral resources can increase bumble bee
168 parasites in some contexts (17, 18), to our knowledge our study is the first to demonstrate that
169 plant species composition shapes colony-level infection intensity in free-foraging bees. All
170 infected bees began trials with equal pathogen exposure, but after two weeks, bees in tents with
171 high-infection plant species had nearly twice the infection intensity as bees in tents with low-
172 infection species, with intermediate infection in canola-only tents (Fig. 1A). These results are
173 particularly important since flowering strips can provide important season-long nutrients in the
174 boom-bust resource context of agricultural fields (34). Effects of floral composition on pathogen
175 intensity could be due to changes in pathogen transmission at flowers (20) or impacts of floral
176 resources on bee-pathogen dynamics in the microcolonies. For example, lack of food resources
177 can suppress immune function (35) but also decrease pathogen counts (36-38). Secondary
178 compounds in nectar can reduce bee pathogens, as can certain types of pollen (e. g., 26, 30).
179 Importantly, although pollen diversity can improve honey bee health and pathogen tolerance
180 (39), the effect of floral composition on infection intensity in our study was not due to species
181 diversity since high- and low-infection flowering strips included the same plant species, just in
182 reversed ratios. If plant diversity drove bee-pathogen interactions, we would expect the canola-
183 only tents to have qualitatively different results than tents with flowering strips, but this was not
184 the case (Fig. 1A). These results are important because they suggest that choice of plant species
185 in flowering strips can influence bee disease dynamics, potentially increasing or decreasing
186 pathogen infection intensity depending on the plant species chosen.

187 Even though the highest pathogen intensity occurred in high-infection tents, having
188 enhanced floral resources from either flowering strip treatment improved bee performance
189 compared to canola-only tents (Fig. 2). Because experimental bees were confined to tents, they

190 were likely more food-limited than wild bees and so these results should be interpreted
191 cautiously. Nonetheless, it's interesting that bees in high-infection and low-infection tents had
192 similar survival and reproduction in spite of differences in infection intensity, suggesting that
193 food resources play a more critical role than *C. bombi* infection for reproduction in this bee
194 species under these experimental conditions. In support of this, we found little effect of infection
195 treatment on most reproductive measures, with a marginal tendency for infection to reduce egg
196 production. We note that other studies often find little impact of *C. bombi* infection on individual
197 or colony performance in the lab when bees have abundant food resources, but more negative
198 effects when bees are food-stressed (40, 41). Furthermore, wild colonies of *B. terrestris* with
199 higher *C. bombi* infection intensities were less likely to produce daughter queens (42), indicating
200 reproductive consequences of infection under natural conditions. Experimental infection did
201 reduce foraging in our study, consistent with a survey of wild foraging *Bombus* (43). Since bees
202 were resource-limited in our tents (demonstrated by greater microcolony reproduction with more
203 nectar availability), it is surprising that reduced foraging due to infection treatment did not affect
204 reproduction more strongly. Perhaps we would have seen more negative reproductive
205 consequences if we had conducted longer trials. Alternatively, high pathogen intensity could
206 signal bees to invest in reproduction prior to their demise. Longer-term studies are needed to
207 determine impacts of plant species on bee reproductive fitness via changes in resources *versus*
208 pathogen infection intensity.

209 Although flowering strips affected both pathogen infection intensity and bee
210 reproduction, these effects were mediated by different mechanisms. The effect of flowering
211 strips on bee reproduction was explained by estimated nectar availability in each tent (Fig. 2B,
212 D), while the effect of flowering strips on pathogen infection intensity remained after accounting

213 for nectar availability (Fig. 1B). It is not surprising that reproduction correlated strongly with
214 floral resources in bees confined to tents, although this may still reflect larger-scale patterns
215 since resources increase bee abundance and diversity in widespread agricultural settings (4).
216 However, this contrasts with pathogen infection intensity, which was not related to nectar
217 resources. The original trials that designated plant species as ‘high-’ or ‘low-’ infection allowed
218 single uninfected workers to forage on an experimentally inoculated inflorescence for less than
219 20 minutes, and then assessed infection one week later (20). It is remarkable that these
220 categorizations of plant species based on brief foraging bouts still predicted infection intensities
221 for microcolonies foraging in tents over a two-week period, when bees could forage for pollen,
222 revisit flowers over several days, and interact with each other inside the colony.

223 Our previous trials identified surprisingly few floral traits associated with likelihood of
224 acquiring disease other than number of reproductive structures (20). However, subsequent work
225 suggests that floral architecture or surface compounds/trichomes, traits we did not measure in our
226 original study, may play a role in disease dynamics, since the location of inoculum placement
227 (inside flowers, outside flowers, on bracts) has variable effects on acquisition across plant
228 species (23). We also did not measure floral volatiles, some of which reduce *C. bombi* viability
229 (44). Nectar or pollen chemistry could also influence *C. bombi* infection intensity (26, 28, 29, 33,
230 44). Since the designation of ‘high-’ and ‘low-’ infection species accurately predicted colony
231 infection intensity over two weeks, this suggests that whatever traits influence short-term
232 transmission dynamics also play important roles over longer time periods in determining
233 infection intensities. Short-term dynamics can predict longer-term patterns in other systems; for
234 example, greater exposure to bacterial wilt (3 hr vs. 24 hr) increased vector beetle infection

235 likelihood after 5 days and persisted at 28 days, suggesting that interaction intensity affected
236 both short- and long-term ability to acquire this pathogen (45).

237 We saw little effect of infection treatment or flowering strips on pollination service to
238 canola, most likely because confining bees in tents resulted in excess pollen delivery to all
239 plants. However, infection reduced the number of workers foraging at any given time, which
240 could have more significant consequences for pollination on larger spatial scales given that
241 *Crithidia* can infect up to 80% of *Bombus* in western Massachusetts (46) and nearly 50% of *B.*
242 *terrestris* workers in Switzerland and the UK (42, 43). Flowering strips also reduced the number
243 of foragers on canola, but the high ratio of flowering strip to crop plants in our tents may
244 overestimate the magnitude of this effect in the field. Designs that incorporate flowering strips or
245 hedgerows on farms or in field trials would be more appropriate to assess effects on pollinator
246 service; such studies often find benefits of flowering strips for pollination services (7-9).

247 In conclusion, we found that bee colony-level infection intensity nearly doubled with the
248 addition of plant species known to increase pathogen acquisition, compared to low-infection
249 plant species. However, bee colony reproduction was substantially improved with any flowering
250 strips compared to canola alone. Variation in colony reproduction was explained by nectar
251 resources, but infection intensity was not. Thus, both flowering strip treatments benefited colony
252 reproduction by adding floral resources, but certain plant species may also increase pathogen
253 infection intensity. This should be a consideration when selecting plant species for augmenting
254 pollinator habitat.

255

256 **Materials and Methods**

257

258 *Study system*

259 *Bombus impatiens* (Apidae, the common eastern bumble bee) is one of the most prevalent
260 bumble bee species in the eastern US (e. g., 46, 47), a generalist pollinator, and available
261 commercially. We used microcolonies of approximately 15 workers as our unit of replication;
262 microcolonies have been used successfully to estimate colony performance as a function of diet
263 (48).

264 *Crithidia bombi* (Zoomastigophora:Trypanosomatidae) is a protozoan gut parasite that
265 can be contracted at flowers via fecal-oral transmission (14, 49). *Crithidia bombi* reduces
266 learning and foraging efficiency in workers (50, 51), slows colony growth rates (52) and is
267 associated with reduced likelihood of reproduction in wild colonies (42). Stressful conditions
268 increase the mortality of infected workers (40) and reduce infected queen fitness (41). *C. bombi*
269 infection is locally common; for example, *C. bombi* infected over 60% of *B. impatiens* in western
270 Massachusetts (46).

271 Canola (*Brassica rapa* cultivar O. Eclipse; Spectrum Crop Development, Ritzville,
272 Washington; ‘canola’ hereafter) is obligately outcrossed and has improved yield from insect
273 pollinators (53, 54). Canola was grown on more than 1 million acres in the US with a production
274 value of \$357 billion in 2011 (National Agricultural Statistics Service, USDA), and so is a major
275 foraging source for bees as well as a valuable US crop. *Bombus* exhibit natural foraging
276 behaviors in tent enclosures, are common natural pollinators of canola (e. g., 55), and have been
277 successfully used to pollinate canola in greenhouse experiments previously (56). Thus, *Bombus*
278 are both relevant and tractable to examine the effects of pathogens and flowering strips on
279 pollination in canola.

280 We designated ‘low-’ and ‘high-infection’ plant species based on previous work in which
281 we inoculated flowers of each species with a known quantity of *C. bombi* inoculum, allowed an
282 uninfected bee to forage (typically less than 20 minutes), and then individually reared the bees
283 for one week and assessed pathogen cell counts (20). In most cases species were designated as
284 ‘low’ or ‘high’ infection based on both the probability of a bee becoming infected and the
285 severity of infection; mean *C. bombi* cells counts one week after foraging (including zeros) were
286 9.0-13.1 cells/0.02 μ L for ‘low’ and 18.9-36.2 cells/0.02 μ L for ‘high’ infection plant species
287 (20). High-infection species used in the current experiment were *Antirrhinum majus*
288 (Plantaginaceae), *Asclepias incarnata* (Asclepiadaceae), *Lobelia siphilitica* (Campanulaceae),
289 *Lythrum salicaria* (Lythraceae), *Penstemon digitalis* (Plantaginaceae), and *Solidago altissima*
290 ssp. *altissima* (Asteraceae). Low-infection species were *Digitalis purpurea* (Plantaginaceae),
291 *Helianthus annuus* (Asteraceae), *Linaria vulgaris* (Plantaginaceae), and multiple varieties of
292 *Thymus* (Lamiaceae). See SI Appendix for plant source and propagation details.

293

294 *Experimental Design Summary*

295 We manipulated *C. bombi* infection (yes/no) and flowering strips in a 2 x 3 factorial
296 design using tents in the field at the University of Massachusetts Center for Agriculture (South
297 Deerfield, MA, U.S.A., 42° 28.6’ N, 72° 34.8’ W). We used tents to constrain our experimental
298 bees to only forage on their provided treatment plants, although we acknowledge that this likely
299 resulted in more food limitation than free-foraging bees would experience. Our flowering strip
300 treatments were canola only, high-infection strip, and low-infection strip. These six treatment
301 combinations were replicated three times per round (18 mesh-sided tents; 2.44 m x 2.44 m; Delta
302 Canopy Inc., McKinney, Texas) and we conducted five, two-week rounds between 3 June and 27

303 Aug 2015. This provided a total of 88 replicate tents (two replicates of uninfected canola were
304 lost due to tent collapse). Tents were arranged in three blocks (rows) of six, with one replicate of
305 each treatment combination per block, and treatments were randomly assigned to tents within
306 blocks. The length of each round was approximately two weeks, long enough for colonies to
307 produce larvae but not newly emerged adults. Round length varied slightly across blocks, which
308 were always set up on the same day but taken down over 2-3 days. Tents each contained a single
309 microcolony of approximately 15 *B. impatiens* workers (see SI Appendix for microcolony
310 construction methods), bins of canola plants, and flowering strips when appropriate.
311 Microcolonies of bees were initially infected with *C. bombi* or received a sham infection. For
312 tents with infected microcolonies, our goal was to assess how flowering strips affected final
313 infection levels; our design did not allow us to distinguish between on-flower and within-colony
314 transmission pathways.

315 At the end of each round we removed microcolonies, counted *C. bombi* cells of all
316 surviving workers, and recorded worker survival and microcolony reproduction. We then
317 randomly reassigned tents within blocks to new treatments, with the constraint that tents with
318 infected bees in the first round were maintained as ‘infected’ tents for the entire experiment to
319 avoid contamination, even though contamination is unlikely because *C. bombi* does not survive
320 long outside the host (23). Plants were rearranged or replaced as needed, again with the
321 constraint that plants from ‘infected’ tents were only used in other ‘infected’ tents. New
322 microcolonies were used in each round.

323

324 *Flowering strip treatments*

325 All tents contained bins of canola as the focal crop, with approximately 20 plants per bin.
326 We included 15 bins of canola per tent in the canola-only treatment, and 12 bins per tent in the
327 flowering strip treatments to reflect that flowering strips could result in less space for a crop (see
328 SI Appendix, Fig. S1 for physical layout). Flowering strips included 12 potted plants; there were
329 typically three low- and three high-infection species per round, but in some cases a single species
330 was used twice (i.e., at double concentration) per round due to flowering plant availability (SI
331 Appendix, Table S1). Tents with a ‘low-infection’ flowering strip contained three individuals of
332 each low-infection species and one of each high-infection species; tents with a ‘high-infection’
333 flowering strip used three of each high-infection species and one of each low-infection species.
334 Thus, high- and low-infection tents included the same plant species, just in reversed ratios.
335 Manipulating the ratio of high:low-infection plant species, rather than species composition,
336 allowed us to avoid confounding plant species composition and diversity with flowering strip
337 treatments. High- and low-infection species varied between rounds based on phenology (SI
338 Appendix, Table S1). See SI Appendix Table S2 for plant sources and propagation methods.

339

340 *Assessing infection and microcolony performance*

341 Microcolonies were created from commercial colonies of *B. impatiens* (Biobest,
342 Leamington, Ontario, Canada) approximately one week before deploying in field tents. All
343 colonies were confirmed to be free of *C. bombi* before use by dissecting a subsample of workers.
344 We used three colonies of origin per round. Each block in the field site contained microcolonies
345 from the same colony of origin, so that the block factor includes genetic as well as spatial
346 variation. Each microcolony was initiated with 11-17 workers, plus 2-9 pupal cells in the first
347 two rounds only. We chose this range of initial workers to be large enough to facilitate

348 microcolony survival, but small enough to have sufficient floral resources in the tents.
349 Microcolonies were randomly assigned to infected or uninfected treatments and inoculated with
350 *C. bombi* at microcolony initiation. In infected colonies, each bee was fed 10 μ L of inoculum with
351 6000 *C. bombi* cells in 25% sucrose solution. This concentration is well within the range of
352 natural variation (38, 57). We made inoculum fresh daily following Richardson *et al.* (30). Bees
353 in uninfected microcolonies were fed a 10 μ L droplet of 25% sucrose without *C. bombi* cells to
354 control for handling effects. Bees that did not consume their entire droplet in either treatment
355 were discarded. Microcolonies were maintained in a growth chamber in darkness at 27°C for
356 approximately one week before deployment at the field site. We only used microcolonies in the
357 field that had initiated egg laying; we recorded the number of workers in each microcolony when
358 they were moved to the field. See SI Appendix for microcolony inoculation, construction and
359 field deployment details.

360 At the end of each round, we assessed microcolony infection and performance. We
361 quantified *C. bombi* infection by macerating bee guts and counting cells in a 0.02 μ L sample in a
362 hemocytometer (see SI Appendix). Overall, in the uninfected treatment only 2.3% of bees had
363 detectable *C. bombi* at the end of trials compared to 66% of bees in the infected treatment,
364 indicating that the infection treatment was effective. Uninfected bees in the ‘infected’ treatment
365 may be because our inoculation did not successfully infect, or because bees recovered from their
366 infection. The small number of infected bees in the ‘uninfected’ treatment may be due to a few
367 bees we observed escaping and returning to their tents. The number of living workers per
368 microcolony was used as a measure of survival, scaled by the number at the start of the round. In
369 addition, we froze each microcolony at -20°C and then counted eggs, and counted and weighed
370 all larvae and pupae. Since each round only ran for 14 days, no adults emerged.

371

372 *Measuring foraging behavior and pollination service*

373 We conducted two types of pollinator observations to ask how infection and flowering
374 strips affected visits to plants, and whether infection shifted foraging preferences. Detailed
375 observations were conducted 2-3 times per round for 30-min periods per tent by observing
376 individual bees and recording the plant species (high- and low-infection treatments only),
377 flowers probed per plant visit, and time per plant visit in seconds. Time per flower was
378 calculated by dividing the flowers probed by the time per plant visit. For canola, it was not
379 possible to distinguish individual plants and so inflorescences were the unit of observation.
380 Whenever a tent was observed, we also recorded floral resource availability by counting or
381 estimating the number of open flowers of each species. Beginning in round 3, we also conducted
382 ‘quick observations’ 2-3 times per observation day, in which a single observer would visit each
383 tent and record the number of bees foraging on each plant species at that moment. This provided
384 a larger dataset to ask whether infection and flowering strips affected the total number of
385 foragers and their foraging preferences at a given time point.

386 We found no evidence that treatments affected pollination service, measured as pollen
387 deposition and pollen limitation for fruit and seed set, and therefore report methods and results in
388 the SI Appendix.

389

390 *Statistical analysis*

391 Overview. We used R version 3.5.2 for all analyses (58). Most models were generalized linear
392 mixed models (GLMMs) using the glmer function in lme4 (59), including the fixed effects of
393 flowering strips (canola, low- or high-infection), infection (yes/no), and their interaction as fixed

394 effects. Count data (e.g., number of eggs) were analyzed with Poisson distributions, proportional
395 data (e.g., proportion of bees infected per microcolony) with binomial distributions, and
396 continuous data (e.g., larval weight) with Gaussian distributions. For responses with a single
397 value per tent (e.g., larvae per microcolony), we used block nested within round as a random
398 effect. For responses with multiple values per tent (e.g., *C. bombi* cells per bee per microcolony),
399 we included tent nested within block within round as a random effect. When Poisson or binomial
400 data were overdispersed, we included an observation-level random effect (60, 61). We used
401 likelihood ratio tests to evaluate fixed factors (the interaction term, then flowering strip, then
402 infection treatment).

403

404 Pathogen infection. Effects of flowering strips on the proportion of bees infected and mean
405 infection level (cells per 0.02 μ L) were analyzed in the infected treatment only. To determine
406 whether flowering strip effects on infection were due to resource availability, we reanalyzed
407 responses including estimated nectar availability in each tent as a covariate. Nectar availability
408 was estimated as the average number of open flowers per species in each tent (recorded during
409 pollinator observations) multiplied by the previously determined mean nectar volume per flower
410 for each species (20). We did not consider variation in sugar concentration since these values
411 were unavailable for most species.

412

413 Microcolony performance. Microcolony performance was measured as number of eggs, larvae
414 and surviving workers (including number of workers at the start of the round as a covariate), and
415 mean egg and larval weight. As with pathogen infection, we reanalyzed responses with estimated
416 nectar availability in each tent as a covariate.

417
418 Pollinator foraging behavior. We analyzed ‘quick observations’ of total bees on each plant
419 species per tent and observations of individual bee behavior. We used the ‘quick observations’ to
420 ask whether infection and flowering strips affected mean foragers on canola and on all plants.
421 We used our observations of individual foraging bouts to ask whether infection and flowering
422 strips changed foraging behavior, including only tents with flowering strips. Responses included
423 the total switches between plants (any species) per minute, the proportion of switches to a new
424 plant species, the proportion of flowering strip species visited or flowers probed that were low-
425 vs. high-infection, and the number and proportion of visits to sunflower and thyme plants (using
426 the number of open sunflower or thyme flowers as covariates). We singled out sunflower since
427 its pollen dramatically reduced *C. bombi* (26), and thyme since thymol reduced *C. bombi* at
428 natural nectar concentrations *in vivo* (30) and *in vitro* (44).

429

430 **Data Availability**

431 All data and R scripts will be deposited in Dryad upon acceptance.

432

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434

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445

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596

597 **Figure Legends**

598 Figure 1. Effect of flowering strip treatment on pathogen infection in bumble bees in a field tent
599 experiment. (A) Mean infection intensity (*Crithidia* cells per 0.02 μ l) per tent in bumble bees (*B.*
600 *impatiens*) that were originally infected and placed in microcolonies in tents with canola that
601 either had only canola, or included wildflower strips of predominantly high-infection or low-
602 infection plant species. Values are least-square means, error bars indicate standard error, and
603 different letters above bars indicate significantly different means in post-hoc tests at $P < 0.05$.
604 Sample sizes are 15, 14, and 15 for low, canola and high tents respectively. (B) Mean infection
605 intensity when available nectar resources were included as a covariate; the main effect of
606 flowering strip treatments is still significant. Each point represents the mean value for one tent.
607 Sample sizes are 15, 13, and 15 for low, canola and high tents respectively.

608
609 Figure 2. Effect of flowering strip treatment on bumble bee adult survival and colony
610 reproduction in a field tent experiment. (A) Number of surviving adult workers per tent in each
611 flowering strip treatment; samples sizes are 30, 26 and 30 for low, canola and high tents
612 respectively. (B) Number of surviving adult workers when nectar resources were included as a
613 covariate; samples sizes are 29, 22 and 27 for low, canola and high tents respectively. (C)
614 Number of larvae produced per tent in each flowering strip treatment; samples sizes are 30, 28
615 and 30 for low, canola and high tents respectively. (D) Number of larvae produced when nectar
616 resources were included as a covariate; samples sizes are 28, 22 and 27 for low, canola and high
617 tents respectively. For (A) and (C), values are back-transformed least-square means, error bars
618 indicate standard error, and different letters above bars indicate significantly different means in
619 post-hoc tests at $P < 0.05$. For (B) and (D), each point represents the mean value for one tent.

620 Yellow triangles represent tents with only canola, orange squares represent high infection, and
621 green circles represent low infection tents.

622

623 Figure 3. Effect of infection, flowering strip treatment (low infection plants, high infection
624 plants, or only canola) and their interaction on bumble bee foraging behavior. (A) Number of
625 foragers observed on canola inflorescences during frequent ‘quick observations’ of each tent;
626 there are 73-77 data points per infection/flowering strip combination. (B) Number of foragers
627 observed on all plants during ‘quick observations’; there are 76-79 data points per
628 infection/flowering strip combination. Values are least-square means and error bars indicate
629 standard error.

Table 1. Effect of *C. bombi* infection (yes/no), flowering strip treatment (high-infection wildflower, low-infection wildflower, or canola only) and their interaction (all fixed effects) on multiple measures of *B. impatiens* microcolony performance using generalized linear mixed models. Surviving workers, number of larvae, and number of eggs were analyzed with Poisson distributions, and larval and egg weight with Gaussian distributions. The lower values show the same analysis with available nectar resources per tent included as a covariate. Bold indicates $P < 0.05$; italics indicates $P < 0.055$.

	Surviving workers		Number of larvae		Larval weight		Number of eggs		Egg weight	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
	infection	0.04	0.845	0.97	0.326	0.01	0.930	3.01	0.083	1.12
treatment	15.77	<0.001	13.70	0.001	0.84	0.657	2.79	0.248	10.72	0.005
inf x treat	3.32	0.190	2.22	0.33	5.03	0.081	0.37	0.833	0.65	0.722
with nectar as a covariate:										
nectar	15.39	<0.001	5.84	0.016	0.95	0.330	7.68	0.021	7.23	0.007
infection	0.28	0.600	1.81	0.178	0.01	0.940	3.26	0.071	1.47	0.225
treatment	3.34	0.188	5.92	0.052	4.97	0.083	0.06	0.97	3.49	0.175
inf x treat	3.84	0.146	2.42	0.298	5.95	0.051	0.41	0.816	0.66	0.719

