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

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Abstract

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

Significance Statement

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

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

Introduction

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

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

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

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

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

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

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

Results

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

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

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

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

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

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

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

Discussion

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

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

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

Although flowering strips affected both pathogen infection intensity and bee reproduction, these effects were mediated by different mechanisms. The effect of flowering strips on bee reproduction was explained by estimated nectar availability in each tent (Fig. 2B, 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

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

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

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

Materials and Methods

Study system

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

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

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

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

Experimental Design Summary

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

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

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

Flowering strip treatments

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

Assessing infection and microcolony performance

Microcolonies were created from commercial colonies of *B. impatiens* (Biobest, Leamington, Ontario, Canada) approximately one week before deploying in field tents. All colonies were confirmed to be free of *C. bombi* before use by dissecting a subsample of workers. We used three colonies of origin per round. Each block in the field site contained microcolonies from the same colony of origin, so that the block factor includes genetic as well as spatial variation. Each microcolony was initiated with 11-17 workers, plus 2-9 pupal cells in the first 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 µl 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 µl 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^{o}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 µL 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^{o}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

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

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

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

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

Data Availability

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

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References

1. S. G. Potts *et al.*, Safeguarding pollinators and their values to human well-being. *Nature* **540**, 220-229 (2016).
2. D. Goulson, E. Nicholls, C. Botías, E. L. Rotheray, Bee declines driven by combined stress from parasites , pesticides , and lack of flowers. *Science* **2010**, 1-16 (2015).
3. D. Kleijn *et al.*, Scaling up effects of measures mitigating pollinator loss from local- to landscape-level population responses. *Methods in Ecology and Evolution* **9**, 1727-1738 (2018).
4. N. M. Williams *et al.*, Native wildflower plantings support wild bee abundance and diversity in agricultural landscapes across the United States. *Ecol. Appl.* **25**, 2119-2131 (2015).
5. R. Winfree, "The conservation and restoration of wild bees" in Year in Ecology and Conservation Biology 2010, R. S. Ostfeld, W. H. Schlesinger, Eds. (Wiley-Blackwell, 2010), vol. 1195, pp. 169-197.
6. L. A. Morandin, C. Kremen, Hedgerow restoration promotes pollinator populations and exports native bees to adjacent fields. *Ecol. Appl.* **23**, 829-839 (2013).

7. B. R. Blaauw, R. Isaacs, Flower plantings increase wild bee abundance and the pollination services provided to a pollination-dependent crop. *J. Appl. Ecol.* **51**, 890-898 (2014).
8. L. G. Carvalheiro, C. L. Seymour, S. W. Nicolson, R. Veldtman, Creating patches of native flowers facilitates crop pollination in large agricultural fields: mango as a case study. *J. Appl. Ecol.* **49**, 1373-1383 (2012).
9. L. A. Morandin, R. F. Long, C. Kremen, Pest control and pollination cost-benefit analysis of hedgerow restoration in a simplified agricultural landscape. *J. Econ. Entomol.* **109**, 1020-1027 (2016).
10. C. Carvell *et al.*, Bumblebee family lineage survival is enhanced in high-quality landscapes. *Nature* **543**, 547-549 (2017).
11. T. H. Roulston, J. H. Cane, Pollen nutritional content and digestibility for animals. *Plant Syst. Evol.* **222**, 187-209 (2000).
12. S. W. Nicolson, M. Nepi, E. Pacini, Eds., *Nectaries and nectar* (Springer, Dordrecht, The Netherlands, 2007), p 395.
13. W. Kämper *et al.*, How landscape, pollen intake and pollen quality affect colony growth in *Bombus terrestris*. *Landscape Ecol.* **31**, 2245-2258 (2016).
14. S. Durrer, P. Schmid-Hempel, Shared use of flowers leads to horizontal pathogen transmission. *Proceedings of the Royal Society of London Series B* **258**, 299-302 (1994).
15. P. Graystock, D. Goulson, W. O. H. Hughes, Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. *Proceedings of the Royal Society B-Biological Sciences* **282**, 20151371 (2015).

16. S. H. McArt, H. Koch, R. E. Irwin, L. S. Adler, Arranging the bouquet of disease: floral traits and the transmission of plant and animal pathogens. *Ecol. Lett.* **17**, 624-636 (2014).

17. N. Piot *et al.*, Establishment of wildflower fields in poor quality landscapes enhances micro-parasite prevalence in wild bumble bees. *Oecologia* **189**, 149-158 (2019).

18. H. Cohen, R. D. Quistberg, S. M. Philpott, Vegetation management and host density influence bee-parasite interactions in urban gardens. *Environ. Entomol.* **46**, 1313-1321 (2017).

19. S. A. Alger, P. A. Burnham, H. F. Boncristiani, A. K. Brody, RNA virus spillover from managed honeybees (*Apis mellifera*) to wild bumblebees (*Bombus* spp.). *PLoS One* **14**, e0217822 (2019).

20. L. S. Adler *et al.*, Disease where you dine: plant species and floral traits associated with pathogen transmission in bumble bees. *Ecology* **99**, 2535-2545 (2018).

21. D. T. Peck, M. L. Smith, T. D. Seeley, *Varroa destructor* mites can nimbly climb from flowers onto foraging honey bees. *PLoS One* **11**, e0167798 (2016).

22. L. L. Truitt, S. H. McArt, A. H. Vaughn, S. P. Ellner, Trait-based modeling of multihost pathogen transmission: Plant-pollinator networks. *Am. Nat.* **193**, E149-E167 (2019).

23. L. L. Figueroa *et al.*, Bee pathogen transmission dynamics: deposition, persistence and acquisition on flowers. *Proceedings of the Royal Society B-Biological Sciences* **286**, 20190603 (2019).

24. J. Abbott, Self-medication in insects: current evidence and future perspectives. *Ecol. Entomol.* **39**, 273-280 (2014).

25. J. Moore, An overview of parasite-induced behavioral alterations - and some lessons from bats. *J. Exp. Biol.* **216**, 11-17 (2013).

26. J. J. Giacomini *et al.*, Medicinal value of sunflower pollen against bee pathogens. *Scientific Reports* **8**, 14394 (2018).
27. G. M. LoCascio, L. Aguirre, R. E. Irwin, L. S. Adler, Pollen from multiple sunflower cultivars and species reduces a common bumblebee gut pathogen. *Royal Society Open Science* **6**, 190279 (2019).
28. D. Baracchi, M. J. F. Brown, L. Chittka, Behavioural evidence for self-medication in bumblebees? [version 3; peer review: 3 approved]. *F1000Research* **4**, 1-15 (2015).
29. J. S. Manson, M. C. Otterstatter, J. D. Thomson, Consumption of a nectar alkaloid reduces pathogen load in bumble bees. *Oecologia* **162**, 81-89 (2010).
30. L. L. Richardson *et al.*, Secondary metabolites in floral nectar reduce parasite infections in bumblebees. *Proceedings of the Royal Society B-Biological Sciences* **282**, 20142471 (2015).
31. O. M. Biller, L. S. Adler, R. E. Irwin, C. McAllister, E. C. Palmer-Young, Possible synergistic effects of thymol and nicotine against *Crithidia bombi* parasitism in bumble bees. *PloS One* **10**, e0144668 (2015).
32. L. P. Thorburn, L. S. Adler, R. E. Irwin, E. C. Palmer-Young, Variable effects of nicotine and anabasine on parasitized bumble bees. *F1000Research* **4**, 880 (2015).
33. L. L. Richardson, M. D. Bowers, R. E. Irwin, Nectar chemistry mediates the behavior of parasitized bees: consequences for plant fitness. *Ecology* **97**, 325-337 (2016).
34. A. G. Dolezal, A. L. St. Clair, G. Zhang, A. L. Toth, M. E. O'Neal, Native habitat mitigates feast–famine conditions faced by honey bees in an agricultural landscape. *Proceedings of the National Academy of Sciences* **116**, 25147-25155 (2019).

35. N. Roger, D. Michez, R. Wattiez, C. Sheridan, M. Vanderplanck, Diet effects on bumblebee health. *J. Insect Physiol.* **96**, 128-133 (2017).

36. T. J. Conroy, E. C. Palmer-Young, R. E. Irwin, L. S. Adler, Food limitation affects parasite load and survival of *Bombus impatiens* (Hymenoptera: Apidae) infected with *Crithidia* (Trypanosomatida: Trypanosomatidae). *Environ. Entomol.* **45**, 1212-1219 (2016).

37. C. J. Jack, S. S. Uppala, H. M. Lucas, R. R. Sagili, Effects of pollen dilution on infection of *Nosema ceranae* in honey bees. *J. Insect Physiol.* **87**, 12-19 (2016).

38. A. Logan, M. Rioz-Gonzales, M. Brown, The impact of host starvation on parasite development and population dynamics in an intestinal trypanosome parasite of bumble bees. *Parasitology* **130**, 637-642 (2005).

39. G. Di Pasquale *et al.*, Influence of pollen nutrition on honey bee health: Do pollen quality and diversity matter? *PloS One* **8**, e72016 (2013).

40. M. J. F. Brown, R. Loosli, P. Schmid-Hempel, Condition-dependent expression of virulence in a trypanosome infecting bumblebees. *Oikos* **91**, 421-427 (2000).

41. M. J. F. Brown, R. Schmid-Hempel, P. Schmid-Hempel, Strong context-dependent virulence in a host-parasite system: reconciling genetic evidence with theory. *J. Anim. Ecol.* **72**, 994-1002 (2003).

42. D. Goulson, S. O'Connor, K. J. Park, The impacts of predators and parasites on wild bumblebee colonies. *Ecol. Entomol.* **43**, 168-181 (2018).

43. J. A. Shykoff, P. Schmid-Hempel, Incidence and effects of four parasites in natural populations of bumble bees in Switzerland *Apidologie* **22**, 117-125 (1991).

44. E. C. Palmer-Young, B. M. Sadd, P. C. Stevenson, R. E. Irwin, L. S. Adler, Bumble bee parasite strains vary in resistance to phytochemicals. *Scientific Reports* **6**, 37087 (2016).

45. L. R. Shapiro, I. Seidl-Adams, C. M. De Moraes, A. G. Stephenson, M. C. Mescher, Dynamics of short- and long-term association between a bacterial plant pathogen and its arthropod vector. *Scientific Reports* **4**, 4155 (2014).

46. S. Gillespie, Factors affecting parasite prevalence among wild bumblebees. *Ecol. Entomol.* **35**, 737-747 (2010).

47. N. Cordes *et al.*, Interspecific geographic distribution and variation of the pathogens *Nosema bombi* and *Crithidia* species in United States bumble bee populations. *J. Invertebr. Pathol.* **109**, 209-216 (2012).

48. J. N. Tasei, O. Aupinel, Validation of a method using queenless *Bombus terrestris* micro-colonies for testing the nutritive value of commercial pollen mixes by comparison with queenright colonies. *J. Econ. Entomol.* **101**, 1737-1742 (2008).

49. P. Schmid-Hempel, S. Durrer, Parasites, floral resources and reproduction in natural populations of bumblebees. *Oikos* **62**, 342-350 (1991).

50. R. J. Gegear, M. C. Otterstatter, J. D. Thomson, Does parasitic infection impair the ability of bumblebees to learn flower-handling techniques? *Anim. Behav.* **70**, 209-215 (2005).

51. R. J. Gegear, M. C. Otterstatter, J. D. Thomson, Bumble-bee foragers infected by a gut parasite have an impaired ability to utilize floral information. *Proceedings of the Royal Society B-Biological Sciences* **273**, 1073-1078 (2006).

52. J. A. Shykoff, P. Schmid-Hempel, Parasites delay worker reproduction in bumblebees: Consequences for eusociality. *Behav. Ecol.* **2**, 242-248 (1991).

53. L. A. Morandin, M. L. Winston, Wild bee abundance and seed production in conventional, organic, and genetically modified canola. *Ecol. Appl.* **15**, 871-881 (2005).
54. L. A. Morandin, M. L. Winston, Pollinators provide economic incentive to preserve natural land in agroecosystems. *Agriculture Ecosystems & Environment* **116**, 289-292 (2006).
55. K. E. Hayter, J. E. Cresswell, The influence of pollinator abundance on the dynamics and efficiency of pollination in agricultural *Brassica napus*: implications for landscape-scale gene dispersal. *J. Appl. Ecol.* **43**, 1196-1202 (2006).
56. I. Ahman, A. Lehrman, B. Ekbom, Impact of herbivory and pollination on performance and competitive ability of oilseed rape transformed for pollen beetle resistance. *Arthropod-Plant Interactions* **3**, 105-113 (2009).
57. M. C. Otterstatter, J. D. Thomson, Within-host dynamics of an intestinal pathogen of bumble bees. *Parasitology* **133**, 749-761 (2006).
58. R Core Team (2018) R: A language and environment for statistical computing. (R Foundation for Statistical Computing, Vienna, Austria).
59. D. Bates, M. Machler, B. M. Bolker, S. C. Walker, Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**, 1-48 (2015).
60. B. M. Bolker *et al.*, Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* **24**, 127-135 (2009).
61. X. A. Harrison, Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* **2**, e616 (2014).

Figure Legends

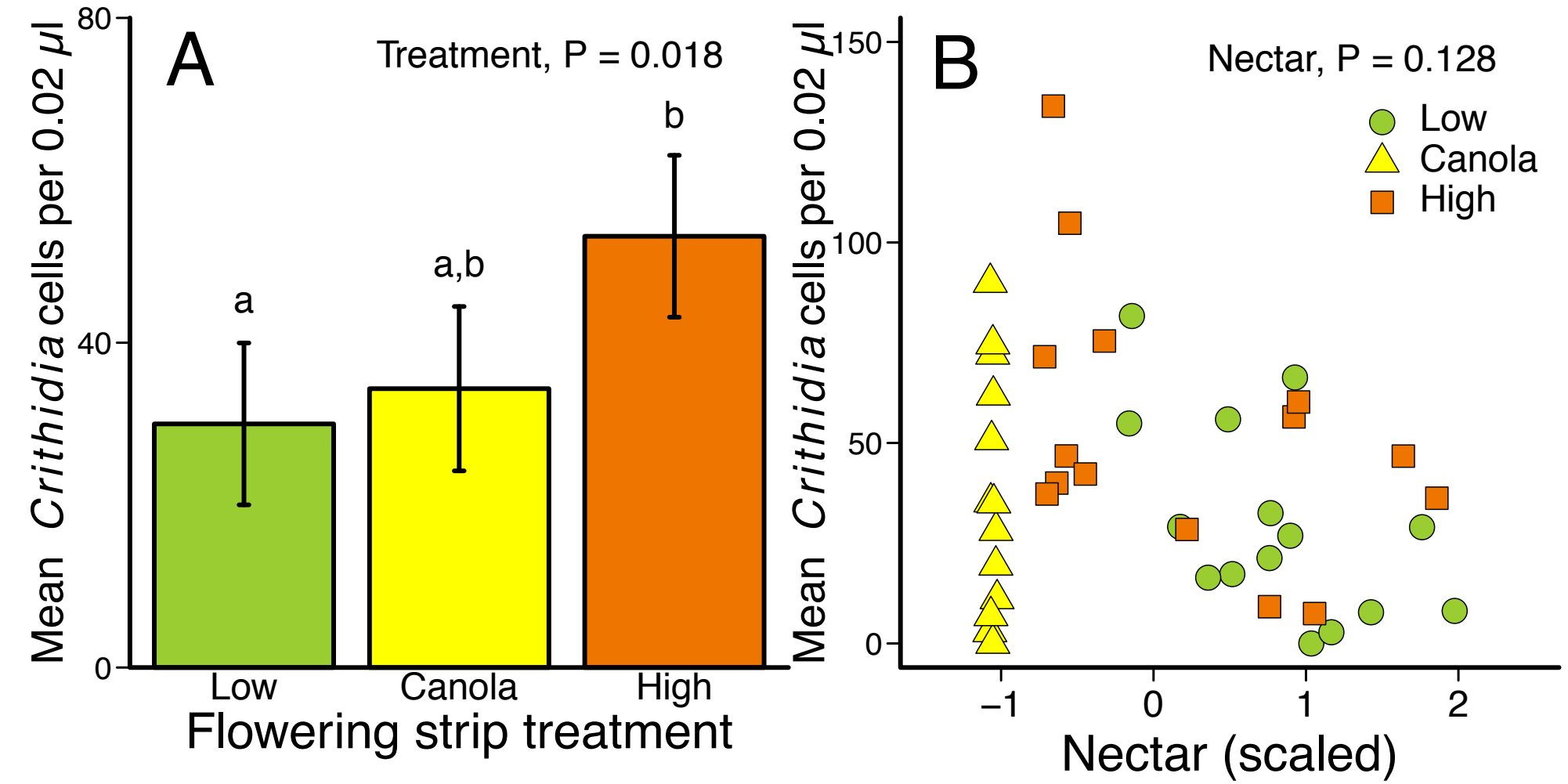
Figure 1. Effect of flowering strip treatment on pathogen infection in bumble bees in a field tent experiment. (A) Mean infection intensity (*Crithidia* cells per 0.02 µl) per tent in bumble bees (*B. impatiens*) that were originally infected and placed in microcolonies in tents with canola that either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

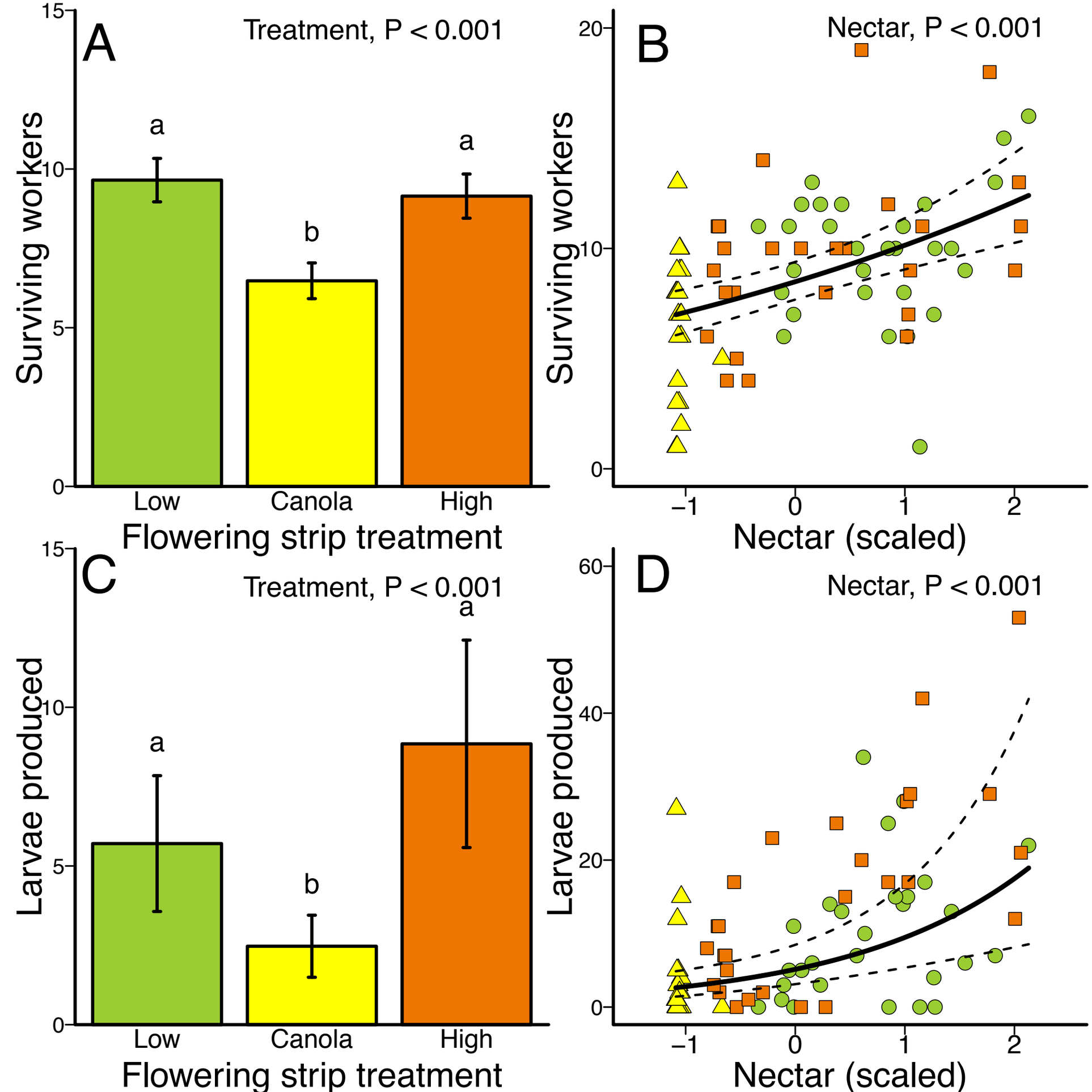
Figure 2. Effect of flowering strip treatment on bumble bee adult survival and colony reproduction in a field tent experiment. (A) Number of surviving adult workers per tent in each flowering strip treatment; samples sizes are 30, 26 and 30 for low, canola and high tents respectively. (B) Number of surviving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

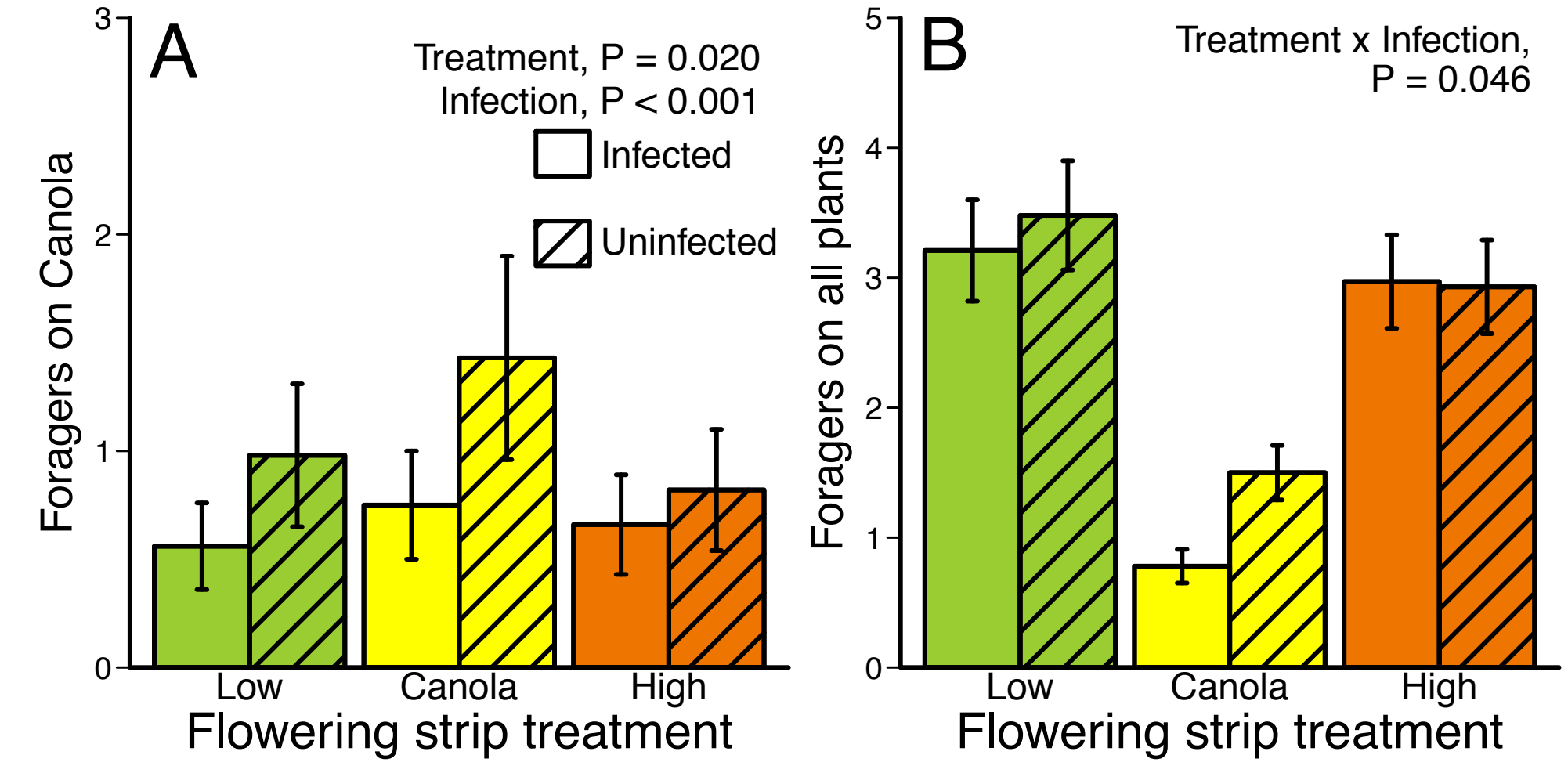
620 Yellow triangles represent tents with only canola, orange squares represent high infection, and
621 green circles represent low infection tents.
622
623 Figure 3. Effect of infection, flowering strip treatment (low infection plants, high infection
624 plants, or only canola) and their interaction 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	0.289
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


A
Treatment, P = 0.018
Mean Crithidia cells per 0.02 µl
80
40
0
a
a,b
b
Low
Canola
High
Flowering strip treatment
B
Nectar, P = 0.128
Low
Canola
High
Mean Crithidia cells per 0.02 µl
150
100
50
0
−1
0
1
2
Nectar (scaled)


A
Treatment, P < 0.001
Surviving workers
15
10
5
0
a
b
a
Low
Canola
High
Flowering strip treatment
B
Nectar, P < 0.001
Surviving workers
20
10
0
−1
0
1
2
Nectar (scaled)
C
Treatment, P < 0.001
Larvae produced
15
10
5
0
a
b
a
Low
Canola
High
Flowering strip treatment
D
Nectar, P < 0.001
Larvae produced
60
40
20
0
−1
0
1
2
Nectar (scaled)


A
Treatment, P = 0.020
Infection, P < 0.001
Infected
Uninfected
Foragers on Canola
3
2
1
0
Low
Canola
High
Flowering strip treatment
B
Treatment x Infection,
P = 0.046
Foragers on all plants
5
4
3
2
1
0
Low
Canola
High
Flowering strip treatment