The costs and benefits of sunflower pollen diet on bumble bee colony disease and health

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Abstract. Pathogen transmission between domesticated and wild host species has important implications for community ecology, agriculture, and wildlife conservation. Bumble bees provide valuable pollination services that are vital for both wildflowers and agricultural production. Intense concerns about pathogen spillover from commercial bumble bees to wild bee populations, and the potential harmful effects of pathogen spillback to commercial bees, has stimulated a need for practical strategies that effectively manage bumble bee infectious diseases. Here, we assessed the costs and benefits of a medicinal sunflower pollen diet (Helianthus annuus) on whole-colony bumble bee disease and performance using commercial colonies of the common eastern bumble bee, Bombus impatiens, and its protozoan pathogen, Crithidia bombi (Trypanosomatida). We first found that a 1:1 mixture of sunflower combined with wildflower pollen reduced C. bombi infection prevalence and intensity within individual B. impatiens workers by nearly 4-fold and 12-fold, respectively, relative to wildflower pollen. At the colony level, a 1:1 mixture of sunflower and wildflower pollen reduced C. bombi infection prevalence by 11% averaged over a 10-week period and infection intensity by 30% relative to wildflower pollen. Colony performance was similar between pollen diets and infection treatments, including the number of workers and immatures produced, and size and weight of workers, drones, and queens. Infection significantly reduced the probability of queen production in colonies fed a pure wildflower pollen diet, but not colonies fed a mixed sunflower pollen diet, suggesting that the medicinal benefits of a mixed sunflower pollen diet can reverse the negative effects of infection on reproductive success. This study provides evidence that sunflower pollen as part of a mixed pollen diet can reduce infection in individual bees and whole colonies with no significant nutritional trade-offs for colony worker production and most aspects of colony reproduction. A supplemental mixed sunflower pollen diet may provide a simple and effective solution to reduce disease and improve the health of economically and ecologically important pollinators.

Key words: bee health; conservation; disease ecology; parasitology; pollination biology; pollination services; tri-trophic interactions.

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INTRODUCTION

Many pathogens can infect both domesticated and wild host species, creating the potential for pathogen transmission (Daszak et al. 2000, Dobson and Foufopoulos 2001, Power and Mitchell 2004), which can have important implications for community ecology, agricultural production, and species conservation (Lafferty and Gerber 2002, McCarthy et al. 2007, Pedersen et al. 2007). Consequently, the control of pathogens in domesticated species is paramount. For example, bumble bees are critically important for the conservation of plant biodiversity and provide pollination services vital to agricultural production (Klein et al. 2007, Ollerton et al. 2011, Potts et al. 2016). Since the late 1980s, domesticated bumble bee colonies have been used extensively for crop pollination services (Shipp et al. 1994, Whittington and Winston 2004, Velthuis and Van Doorn 2006). The increasing use of domesticated bumble bee species both within and outside of their native ranges (Velthuis and Van Doorn 2006, Looney et al. 2019), along with high prevalence of infectious diseases (Arbetman et al. 2013, Graystock et al. 2013, Sachman-Ruiz et al. 2015), has generated concerns about pathogen spillover from domesticated to wild bee populations (Colla et al. 2006, Otterstatter and Thomson 2008). Furthermore, effective management of the pollination potential of commercial bumble bee colonies requires consideration of pathogen spillover. The transmission of pathogens from wild to domesticated bees could have deleterious effects on worker bee abundance, lifespan, and foraging behavior (Corman et al. 2012, Koch et al. 2017). Thus, strategies are needed that effectively manage bumble bee infectious diseases.

Diet may play a key role in mediating host-pathogen dynamics for bees. Pollen is a primary source of nutrition for bees and contains multiple nutritional components, including protein, lipids, amino acids, vitamins, and carbohydrates, each of which vary widely in composition and concentration among plant taxa (Roulston and Cane 2000, Yang et al. 2013). Various aspects of pollen quality and nutrition have been linked to bumble bee colony growth and reproduction (Schmid-Hempel and Schmid-Hempel 1998, Vaudo et al. 2018) and increasing evidence points to pollen’s role in bee-pathogen dynamics. For example, given that immune systems are energetically costly (Sheldon and Verhulst 1996), poor nutrition can weaken the bee immune system and increase symptoms of pathogen infection (Alaux et al. 2010, Di Pasquale et al. 2013, Roger et al. 2017). Conversely, poor host nutrition may affect the availability of resources for the pathogen, limiting pathogen growth and reproduction (reviewed in Pike et al. 2019). Pollen also contains a high diversity of plant secondary compounds, often orders of magnitude higher concentrations than found in nectar and vegetative tissues (Cook et al. 2013, Palmer-Young et al. 2018, 2019). Plant secondary compounds can mediate bee–pathogen interactions by reducing parasitism, although this effect has mostly been studied within the context of nectar (Manson et al. 2010, Richardson et al. 2015, Koch et al. 2019).

Optimizing bumble bee health management requires the careful consideration of key plant species that play disproportionate roles in protecting pollinators against pathogens, as well as nutritional needs associated with colony growth and reproduction. The consumption of sunflower pollen (Helianthus annuus) greatly reduces the intensity of infection by the trypanosome Crithidia bombi in bumble bee, Bombus impatiens, workers (Giacomini et al. 2018, LoCascio et al. 2019, Adler et al. 2020). Giacomini et al. (2018) found that more than two-thirds of bees that consumed sunflower pollen had no detectable infection after one week, and the intensity of infection was reduced 20- to 50-fold compared to other pollen diets. Sunflower pollen also resulted in greater bumble bee microcolony reproduction (i.e., production of male drones) compared to buckwheat (Fagopyrum cymosum) pollen, which matched sunflower pollen in crude protein content but did not reduce C. bombi infection (Giacomini et al. 2018). These results suggest that crude protein content is not the mechanism behind the medicinal effect of sunflower pollen in bumble bees. However, sunflower pollen is traditionally considered a poor-quality diet for bumble bees due multiple factors, including relatively low protein content (Yang et al. 2013), deficiency in
three essential amino acids for bee development (Nicolson and Human 2013), and conspicuous spines on the outer pollen wall (Blackmore et al. 2007), which may act as a digestive barrier that prevents nutrient assimilation (Vanderplanck et al. 2018). For sunflower pollen to be an effective treatment for reducing pathogens in domesticated bumble bee colonies, the benefits of pathogen reduction need to outweigh the nutritional costs of sunflower pollen consumption for colony growth and reproduction.

The goal of this study was to assess the costs and benefits of sunflower pollen on whole-colony bumble bee disease and performance. We used commercial colonies of the common eastern bumble bee B. impatiens and its protozoan pathogen C. bombi (Trypanosomatida) to ask the following questions: (1) Is the minimum dose of sunflower pollen mixed with wildflower pollen that provides medicinal benefits? Given that sunflower pollen is low in protein content and lacks some essential amino acids for bee development (Nicolson and Human 2013, Yang et al. 2013), it is not advisable to feed bumble bee colonies a diet exclusively of sunflower pollen. Instead, we needed to determine the minimum proportion of sunflower pollen mixed with wildflower pollen that would provide medicinal benefits to infected bees relative to a pure sunflower diet. We predicted that the medicinal effect of sunflower pollen would be significant at concentrations <100% sunflower pollen, but lacking at very low sunflower pollen concentrations. (2) We then used the minimum proportion of sunflower pollen mixed with wildflower pollen that provided a medicinal effect in a whole-colony experiment to ask: What are the costs and benefits of sunflower pollen on colony-level infection and performance? We predicted that a diet of sunflower mixed with wildflower pollen would minimize colony growth and reproductive costs that are associated with a poor nutritional diet, while providing medicinal benefits by reducing pathogen infection. (3) Finding that a diet with sunflower pollen could not completely clear infection in bumble bee colonies after 10 weeks of continuous pollen diet treatment, we asked: Can C. bombi develop resistance to the medicinal effects of sunflower pollen? Given that rapid evolution of resistance to medicines is documented in a wide variety of pathogens (Cohen 1992, D’Costa et al. 2011), we predicted that C. bombi would develop resistance to sunflower pollen.

Taken together, results from this study suggest that sunflower pollen supplements in a mixed pollen diet could reduce pathogens in domesticated colonies of bumble bees, reducing the potential for pathogen transmission between domestic and wild bees.

**Methods**

**Study system**

*Bombus impatiens* is a native eusocial bee species in North America and ranges from Maine to Ontario to the eastern Rocky Mountains and south through Florida (Kearns and Thomson 2001). Colonies are annual, founded by single inseminated queens. The main bumble bee species domesticated in North America is *B. impatiens*. Standard commercial *B. impatiens* colonies come with a queen and 50–100 workers and reach a peak of several hundred individuals typically within 12 weeks (J. J. Giacomini, personal observation). Later in the colony lifecycle, colonies switch from rearing workers to the production of males and daughter queens. In wild colonies, the males and new queens disperse and mate, and the inseminated new queens overwinter (Goulson 2003).

*C. bombi* (Zoomastigophora:Trypanosomatidae) is an infectious protozoan gut pathogen that can be contracted at flowers via fecal transmission and can also be horizontally transmitted within colonies (Schmid-Hempel and Durrer 1991, Durrer and Schmid-Hempel 1994). *C. bombi* infection reduces learning and foraging efficiency in worker bumble bees (Gegear et al. 2005, 2006), is correlated with slower colony growth rates, especially early in the colony life cycle (Shykoff and Schmid-Hempel 1991a), and is correlated with the reduced likelihood of successful reproduction in wild colonies (Goulson et al. 2018). *C. bombi* infection is common; for example, *C. bombi* infected over 60% of wild-caught *B. impatiens* in western Massachusetts (MA; Gillespie 2010), suggesting potential for pathogen transmission among wild and commercial bees.

Domesticated sunflower (*H. annuus*) is a major oilseed crop cultivated worldwide and a native U.S. wildflower (Reagon and Snow 2006). Nearly two million acres of sunflowers are planted in

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**DISEASE ECOLOGY**

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Domesticated sunflower (*H. annuus*) is a major oilseed crop cultivated worldwide and a native U.S. wildflower (Reagon and Snow 2006). Nearly two million acres of sunflowers are planted in
the USA (Holcomb 2015), and ten million acres are planted in Europe annually (Strange et al. 2016). The high abundance of cultivated sunflowers combined with large nectar and pollen yields make it an important resource for bees, despite being considered a poor-quality diet (Nicolson and Human 2013). Many bee species, including bumble bees, are known to visit sunflowers (Aslan and Yavuksuz 2010, Riedinger et al. 2014, but see Tepedino and Parker 1982, Fell 1986), and we have identified *H. annuus* pollen from the corbiculae of wild-caught *B. impatiens* workers foraging on sunflower (J. J. Giacomini et al., unpublished data).

**Experimental methods**

*Preparing C. bombi inoculum.*—Experiments used *C. bombi* originally harvested from three wild *B. impatiens* workers collected near Stone Soup Farm, Hadley, MA, USA (42.363911 N, −72.567747 W) and housed in commercial colonies of *B. impatiens*. The *Crithidia* species was identified in a previous study by our group (Figueroa et al. 2019). Briefly, the CB-SSU rRNA F2 and CB-SSU rRNA B4 primers and PCR conditions described in Schmid-Hempel and Tognazzo (2010) were used to Sanger sequence the 18S small subunit rRNA gene. BLAST searches were then conducted against the National Center for Biotechnology Information’s nr/nt database. The sequences matched *C. bombi* and had the same level of divergence to *C. expokei* as reported in Schmid-Hempel and Tognazzo (2010), confirming the species identity as *C. bombi*. The commercial colonies were free of the pathogen prior to infection with the field-collected *C. bombi*. Both the *C. bombi* source colonies and experimental colonies (described below) were from BioBest LTD (Leamington, Ontario, Canada). Colonies were fed with 30% sucrose solution and mixed wildflower pollen throughout their lifetimes and housed in a dark room at 21–24°C and ~50% rh. We made *C. bombi* inoculum for each experiment using an established protocol (Manson et al. 2010, Richardson et al. 2015, Giacomini et al. 2018). Briefly, bee digestive tracts, excluding the honey crop, were removed with forceps, placed into a 1.5-mL microcentrifuge tube with 300 μL of distilled water and ground with a pestle. We allowed each sample to rest at room temperature for 4–5 h so that gut material settled and the *C. bombi* cells could ascend into the supernatant. Flagellate *C. bombi* cells were counted from a 0.02-μL sample of supernatant per bee with a Neubauer hemocytometer under a compound light microscope at 400× magnification. We then mixed 150 μL of the supernatant with distilled water to achieve a concentration of 2400 cells/μL. The sample was then mixed with an equal volume of 50% sucrose solution to yield inoculum with 1200 cells/μL in 25% sucrose.

*Measuring C. bombi infection.*—Each experimental bee was dissected using similar techniques as *Preparing C. bombi inoculum* with the addition that all tools were washed with 70% ethanol and thoroughly dried between bees. We counted flagellate *C. bombi* cells from a 0.02-μL sample of supernatant per bee with a Neubauer hemocytometer (Manson et al. 2010, Richardson et al. 2015, Giacomini et al. 2018). The proportion of flagellate vs. aflagellate *C. bombi* cells changes as infections develop in pollen-fed bees (Logan et al. 2005). However, assessing infection levels based solely on flagellate *C. bombi* should not alter interpretation of the results since infection age was consistent across our different treatments. Prevalence was recorded as the presence (1 or more *C. bombi* cells) or the absence of *C. bombi* cells per 0.02 μL of each sample. *C. bombi* infection intensity was measured as the number of flagellate *C. bombi* cells per 0.02 μL, and only included bees with at least 1 or more *C. bombi* cells. We removed the right forewing of each bee and mounted them on glass slides to measure marginal cell length, a proxy for bee size (Nooten and Rehan 2020).

What is the minimum dose of sunflower pollen mixed with wildflower pollen that provides medicinal benefits?

Experimental adult worker bumble bees (*n* = 120 bees) were obtained from three commercial *B. impatiens* colonies (40 bees/colony) that were determined to be uninfected by screening five workers from each colony using the methods described in *Measuring C. bombi infection*. Workers were removed from their colonies of origin and placed into individual plastic containers (7.5 × 10 × 5 cm) with mesh screen flooring. We starved the bees for 4–6 h and then fed each a 10-μL drop of inoculum that contained 12,000 *C. bombi* cells, which is within the
concentration range bees are exposed to when foraging on flowers in the wild (Schmid-Hempel and Schmid-Hempel 1993). Only bees that consumed the entire droplet were used in the experiment. Bees were then randomly assigned, within colony of origin, to one of four experimental pollen diets: (1) pure sunflower pollen diet (100% Sun), (2) 1:1 mixture of sunflower pollen to wildflower pollen (50% Sun), (3) 1:3 mixture of sunflower to wildflower pollen (25% Sun), or (4) pure wildflower pollen (100% Wild). We based ratios on the weight of honey bee-collected pollen pellets. Sunflower pollen was obtained from Changge Huading Wax Industry (China). We sorted honey bee-collected sunflower pollen pellets by color to remove impurities and visually identifying the lack of non-sunflower pollen with a compound light microscope at 400× magnification. Honey bee-collected mixed wildflower pollen pellets were purchased from Koppert Biological Systems (Howell, Michigan, USA) and microscopically confirmed to contain <5% Asteraceae pollen, identified by having spines on the exine (Blackmore et al. 2007). The chemical and nutritional quality of wildflower pollen can vary based on plant species composition. While we did not identify the pollen species that made up the wildflower mixture used in this study, several studies have used wildflower pollen mixtures of unknown species composition as a control, showing sunflower pollen reduces C. bombi infection relative to wildflower pollen (Giacomini et al. 2018, LoCascio et al. 2019). Pesticide residues in pollen used in this study were not measured. However, the same sunflower and wildflower pollen lots were used for all experiments throughout this study and are from the same suppliers as in Giacomini et al. (2018), which did measure pollen pesticide levels. More pesticide residues were found in wildflower compared to sunflower pollen, all but two of which were at trace levels. The two that were above trace levels were both miticides used to treat varroa mites in honey bee colonies. Sunflower pollen also contained a different miticide used to treat varroa in honey bees. Given that pesticide levels were low overall and higher in wildflower than sunflower pollen, it seems unlikely that pesticides mediated the results.

Experimental pollen diets were provided to bees as a paste produced by mixing ground pollen pellets, weighed to the appropriate ratios of sunflower:wildflower pollen, and adding distilled water to achieve a uniform consistency. Each day for a week we fed inoculated bees fresh pollen paste packed into an inverted lid of a 1.5-mL microcentrifuge tube, and 1 mL of 30% sucrose via a filled and inverted plastic 1.5-mL microcentrifuge tube plugged with cotton (Richmond Dental and Medicine, Charlotte, North Carolina, USA). On day 7, we assessed C. bombi prevalence and infection intensity (see Measuring C. bombi Infection). The 7-d period allowed sufficient C. bombi growth to quantify infection intensity within host bumble bees (Schmid-Hempel and Schmid-Hempel 1993, Otterstatter and Thomson 2006).

Statistical analyses.—All statistical analyses here and below were conducted using R version 3.5.2 (R Core Team 2014). All figures were generated using the ggplot2 package (Wickham 2016). We used generalized linear mixed models (GLMMs) to analyze how pollen diets affected C. bombi infection prevalence (presence/absence) and intensity (cells per 0.02 µL in infected bees only) using the package glmmTMB (Brooks et al. 2017). C. bombi prevalence models were fit with a binomial distribution and infection intensity models were fit with a negative binomial distribution. Models included pollen diet (100% sunflower, 50% sunflower, 25% sunflower or wildflower mixture) and bee size estimated as marginal cell length (covariate) as fixed effects and experimental bee colony as a random effect. Significance of fixed effect terms was evaluated with a likelihood ratio chi-squared test, implemented via the drop1() function in R. Tukey’s Honest Significantly Differ- ence tests were used for post hoc pairwise comparisons between pollen diets using the emmeans package (Lenth 2020). All bees that died before their scheduled dissection date were excluded from the C. bombi infection analysis. A total of 90 bees survived until dissec- tion date ($N_{100\%} = 23$, $N_{50\%} = 21$, $N_{25\%} = 21$, $N_{0\%} = 25$). We tested how pollen diets affected bee survival using mixed-effects Cox proportional hazards models (Therneau 2015), with pollen diet and bee size as fixed effects, and experimental bee colony as a random effect. We used Analysis of
Deviance type III tests from the car package to test the significance of terms.

What are the costs and benefits of sunflower pollen on colony-level infection and colony performance?

We conducted a two-way factorial laboratory experiment crossing *C. bombi* infection (yes/no) with pollen diet treatment. *Bombus impatiens* colonies were randomly assigned to a *C. bombi* infection treatment (*C. bombi* inoculum [infected] or a sham inoculum [uninfected]) and pollen diet treatment (50% sunflower [1:1 sunflower:wildflower pollen] or wildflower pollen) for 10 weeks, which is within the range of a natural life cycle for *B. impatiens*. A 1:1 ratio of sunflower to wildflower pollen (50% sunflower pollen) provided similar medicinal benefits as 100% sunflower pollen for individual bees (see Results) and so was used as the sunflower pollen diet in this whole-colony experiment (hereafter referred to as sunflower pollen diet for simplicity within the context of this experiment). We used 9–11 replicate colonies per treatment (*N* = 45 total colonies), split into four blocks of 9–11 colonies. Samples sizes per treatment were not identical because a small number of colonies were damaged upon receipt from the commercial supplier. Blocks corresponded to day of the week that we performed initial colony inoculations and weekly parasite screenings. On any given day of the week, there was at least one replicate of each treatment combination represented so that treatment was not confounded with block. All colonies were confirmed to be *C. bombi*-free by screening ten workers from each colony upon receipt.

Infection treatment.—A fresh *C. bombi* inoculum was made for each block of colonies on their assigned inoculation day (as in Preparing *C. bombi* inoculum). We made the sham inoculum following the same procedure used to make the *C. bombi* inoculum, but using uninfected worker bumble bees from a different *B. impatiens* colony that was confirmed to be *C. bombi*-free. From each experimental colony, we removed 15 adult worker bees and placed each into seven-dram snap cap vials (Qorpak, Clinton, Pennsylvania, USA). We starved the bees on the laboratory bench for 3–5 h. We then hand-fed all bees 10 μL of *C. bombi* or sham inoculum as appropriate. After consuming the entire drop, all inoculated bees were returned to their colonies. To ensure infections established in the infected treatments, we gave each colony an additional 5 mL of either *C. bombi* or sham inoculum via an open-faced petri dish (35 mm) placed directly into the colony for 24 h.

Pollen treatment.—The sunflower pollen diet was made by mixing an equal portion (1:1 ratio by weight) of sunflower and wildflower pollen. The wildflower pollen diet solely contained honey bee-collected wildflower pollen. We gave all colonies their assigned pollen treatment (sunflower or wildflower) one week after the infection treatment started, thus allowing *C. bombi* infection to spread and become established within the colonies. Colonies received fresh pollen diet (~50 g balls of ground pellets mixed with distilled water to form a paste) and 30% sucrose solution (750 mL) weekly until the termination of the experiment at 10-week post-inoculation.

Measuring *C. bombi* infection and colony performance.—Starting one-week post-inoculation, each week we removed 10% of the workers (up to 10) from each colony to measure *C. bombi* prevalence and infection intensity (see Measuring *C. bombi* infection). Weekly, each colony box, excluding the sucrose reservoir, was weighed to the nearest 0.1 g, and drones were recorded and removed. Upon termination of the experiment, colonies were weighed (final weight) and then immediately frozen and stored at −18°C. We dissected frozen colonies and counted and weighed eggs, larvae, pupae, workers, drones, and new queens. We combined the number of eggs, larvae, and pupae into a single variable that represented the number of immature stages. The right forewings of all drones and queens (*n* = 0–93 per colony) from each colony were removed and mounted on glass slides to measure the length of the marginal cell to estimate bee size of each caste. The average weight of workers, drones, queens, and immatures was calculated by dividing the total weight of each by the number counted for each colony. Post-dissection, we pressure washed each plastic colony box to remove organic material and weighed each box to the nearest 0.1 g to subtract the box weights from the colony weights.

Statistical analyses.—Generalized linear mixed-effects models (GLMMs) using the package
glmmTMB (Brooks et al. 2017) were used to analyze how pollen diets affected C. bombi infection prevalence within colonies (fit with a binomial distribution) and infection intensity (fit with a negative binomial distribution), only for colonies experimentally inoculated with C. bombi \((n = 20)\). Throughout the experiment, we did not detect C. bombi within colonies given the sham inoculum. The models included pollen diet, week, and their interaction as fixed effects, bee size as a covariate and both block and experimental colony as random effects. AIC scores were used to evaluate model fits using the AICtab() function in the bbmle package (Bolker and R Development Core Team 2020) to select random effect terms that produced the best fit model (i.e., lowest AIC score by 2 units). We checked for autocorrelation between adjacent weekly intervals using the acf() function in the base R stats package (R Core Team 2014). Linear contrasts using Tukey’s method for \(P\)-value adjustment were used for post hoc pairwise comparisons between pollen diets at each weekly interval, or between weekly intervals, using the emmeans package (Lenth 2020).

Differences between pollen diets and infection treatments in the number, size, and weight of adult bees and immature stages were analyzed either with linear mixed-effects models using the lmerTest package (Kuznetsova et al. 2017) for response variables that met the assumptions of normality or GLMMs using the glmmTMB package for those that followed a negative binomial. The probability of producing new queens and the probability of producing any drones were modeled using GLMMs fit with a binomial distribution. The models included pollen diet, infection treatment and their interaction as fixed effects, and block as a random effect. Colony weight gain over time was analyzed with a GLMM, which included pollen diet, infection treatment and sample week (time), and two- and three-way interactions as fixed effects, and block and colony as random effects. Using the emmeans package, we computed estimated marginal means and pairwise comparisons using Tukey’s \(P\)-value adjustment for pollen diets, infection treatments and sample week, when applicable. We applied the Benjamini-Hochberg method to control false discovery rate and reduce the chance of type 1 errors from multiple testing of correlated dependent variables (Benjamini and Hochberg 1995).

**Can C. bombi develop resistance to the medicinal effects of sunflower pollen?**

We tested for the development of sunflower pollen-resistant C. bombi at the end of the 10-week whole-colony experiment. We first created a potentially resistant (PR) lineage of C. bombi using bees that had been exposed to sunflower pollen for 10 weeks from the whole-colony experiment, and a non-resistant (NR) lineage sourced from the original C. bombi used to create the infection treatment for the whole-colony experiment. We then conducted a \(2 \times 2\) factorial experiment crossing inoculum type (PR or NR) with pollen diet (100% sunflower vs. wildflower) and measured subsequent C. bombi infection. We hypothesized that C. bombi previously exposed to sunflower pollen (PR) would have greater ability to infect and grow in new sunflower-fed hosts, compared to C. bombi with no prior exposure to sunflower pollen (NR). If evolved resistance to sunflower comes with a tradeoff and reduces the ability to infect hosts consuming other diets, then the PR lineage may have reduced infection compared to NR in hosts fed wildflower pollen.

To create the PR inoculum, we randomly chose three colonies from the whole-colony experiment from the C. bombi infection/sunflower pollen diet treatment combination. We removed 15 workers from each colony and placed them as groups into plastic containers with screen floors to establish microcolonies (i.e., one 15-worker microcolony established from each parent colony). The microcolonies were established to house the PR infection until we were ready to run the experiment. Each microcolony was fed 30% sucrose solution and sunflower pollen daily for two weeks. We dissected seven bees from each microcolony (see Preparing C. bombi inoculum) and counted C. bombi cells (see Measuring C. bombi infection). Mean C. bombi counts were similar across microcolonies \((F_{2,18} = 0.319, P = 0.731)\), suggesting an even representation of C. bombi cells from each of the three colonies. Therefore, we combined the samples to produce the PR C. bombi inoculum that was continuously exposed to sunflower pollen. A non-resistant (NR) inoculum was created using infected bees sourced from the same...
ancestral colony that the PR lineage started from, but were never exposed to sunflower pollen. An alternative approach would have been to create NR inoculum from infected wildflower colonies from the whole-colony experiment; we did not do so because we kept the ancestral source colony (which was also fed wildflower pollen) and creating the NR inoculum from the ancestral colony allowed us to start the experiment more quickly. For the NR inoculum, the supernatant of 21 bees (same number used to make the PR inoculum) was mixed together using the same procedure as for the PR inoculum. The mean C. bombi cell counts of the combined supernatant prior to diluting with distilled water and sucrose solution for the two inocula (PR and NR) were similar (9950 and 10,550 cells/μL, respectively).

To conduct the factorial experiment, we removed 40 B. impatiens workers each from three new uninfected parent colonies, placed them into seven dram snap-cap vials, and starved them for 4–6 h. Each bee (N = 120 total) was then handed 10 μL of either PR or NR inoculum. Incubated bees were transferred to individual plastic containers as in question 1 and then randomly assigned to a pollen diet treatment (100% sunflower vs. wildflower) to yield 10 bees per colony-treatment combination. Fresh pollen and sucrose were provided for 7 d. All bees were sacrificed on day 7, at which point we measured infection prevalence, intensity, and bee size (see Measuring C. bombi infection). A nearly even number of bees died per treatment before the scheduled dissection date (8–11 bees per treatment). A total of 19 bees from the NR-Sunflower treatment, 22 PR-Sunflower, 19 NR-Wildflower, and 22 PR-Wildflower were in the final analysis.

Statistical analysis.—We used GLMMs to analyze how treatments affected C. bombi infection prevalence and intensity, as in question 1. Pollen diet (sunflower or wildflower), inoculum type (PR or NR), and their interaction were included as fixed effects and bee size as a covariate. Experimental bee colony was included as a random effect. Significance of fixed effect terms and post hoc pairwise comparisons were conducted as in question 1. All bees that died before their scheduled dissection date were excluded from the C. bombi infection analysis. We tested how pollen diets affected bee survival using mixed-effects Cox proportional hazards models (Therneau 2015), with pollen diet, inoculum type, their interaction and bee size as fixed effects, and experimental bee colony as a random effect. We used analysis of deviance type III tests from the car package to test the significance of terms.

Results

What is the minimum dose of sunflower pollen mixed with wildflower pollen that provides medicinal benefits?

Pollen diet had a significant effect on C. bombi prevalence ($\chi^2_{(3)} = 38.426, P < 0.0001$) and infection intensity ($\chi^2_{(3)} = 18.675, P = 0.0003$). Prevalence of infection ranged from 13% to 92% of bees with a detectable infection, with the highest prevalence in a 100% wildflower pollen diet and the lowest in a 100% sunflower pollen diet (Fig. 1a). Post hoc pairwise comparisons revealed significant differences in prevalence of infection between 100% sunflower and both the 100% wildflower (0% sunflower) and 25% sunflower treatments ($Z = -4.51, P < 0.0001; Z = -2.93, P = 0.018$; respectively), but not between 100% sunflower and 50% sunflower treatments ($Z = -1.63, P = 0.36$). The 50% sunflower treatment reduced C. bombi prevalence by nearly 4-fold compared to 100% wildflower pollen ($Z = -3.61, P = 0.0018$). For C. bombi infection intensity, pairwise comparisons revealed a similar pattern (Fig. 1b); 100% sunflower significantly reduced infection intensity compared to 100% wildflower and 25% sunflower treatments ($Z = -4.48, P < 0.0001; Z = -2.82, P < 0.025$; respectively), but not 50% sunflower ($Z = -0.83, P = 0.84$). The 50% sunflower treatment reduced C. bombi infection intensity by nearly 12-fold compared to 100% wildflower pollen ($Z = -3.28, P = 0.0057$). We found no effect of pollen diet on bee survival ($\chi^2_{(3)} = 4.889, P = 0.180$).

What are the costs and benefits of sunflower pollen on colony-level infection and colony performance?

C. bombi infection.—We analyzed the effect of pollen diet and time on infection dynamics in infected colonies only. Pollen diet significantly affected colony-level C. bombi infection (Fig. 2). Models indicated a significant interaction between pollen diet and time on prevalence ($\chi^2_{(8)} = 19.049, P = 0.015$; Fig. 2a). C. bombi infection prevalence
Fig. 1. *Crithidia bombi* infection prevalence and intensity for *Bombus impatiens* workers fed varying ratios of sunflower to wildflower pollen mix. (a) Average infection prevalence (the proportion of parasitized bees) and (b) average infection intensity of infected bees only (cells per 0.02 μL) were significantly lower in bees fed 100% sunflower (Sun) and a 50% sunflower:wildflower pollen mixture compared to wildflower pollen. Different letters above bars indicate statistically significant differences based on pairwise comparisons using Tukey’s HSD tests. Bars and error bars indicate binomial (a) and negative binomial (b) model means and standard errors, back-transformed from the scale of the linear predictor.

was similar for sunflower and wildflower pollen diets at the first sampling period ($t_{(1393)} = 0.641$, $P = 0.522$), suggesting that inoculated colonies started with comparable infection levels prior to receiving pollen treatments. Infection prevalence within wildflower colonies then increased over the 10 weeks of sampling, culminating with 90–100% of bees sampled from inoculated wildflower colonies testing positive for infection. In comparison, prevalence ranged from 33% to 100% within sunflower colonies by week 10. Post hoc linear contrasts revealed significant differences between pollen diets on *C. bombi* infection prevalence for weeks 5, 6, 7, 9, and 10 ($t_{(1393)} > 1.9$, $P < 0.049$ in all cases), ending with the largest difference between treatments at week 10. There was a spike in *C. bombi* prevalence in sunflower colonies at week 8, which muted the statistical difference between pollen diets in this week ($t_{(1393)} = -0.169$, $P = 0.866$). Moreover, variation in the prevalence of infection within colonies was much lower for wildflower colonies compared to sunflower colonies, revealed by a significant Fligner-Killeen test of homogeneity of variances ($\chi^2_{(1)} = 33.568$, $P = 0.0059$), and the variance decreased significantly throughout the 10-week experiment in wildflower ($\chi^2_{(8)} = 34.769$, $P < 0.0001$) but not sunflower ($\chi^2_{(8)} = 6.521$, $P = 0.589$) colonies.

Pollens diet and time also affected *C. bombi* infection intensity ($\chi^2_{(1)} = 5.665$, $P = 0.017$; $\chi^2_{(8)} = 36.044$, $P < 0.0001$, respectively; Fig. 2b), but the interaction was not significant ($\chi^2_{(8)} = 11.134$, $P = 0.194$). Averaged across sampling weeks, *C. bombi* infection intensity for sunflower colonies was 30.3% lower than wildflower colonies (sunflower = 29.9 ± 3.75 cells per 0.02 μL; wildflower = 42.9 ± 4.80 cells per 0.02 μL, mean ± SE). Variation in *C. bombi* infection intensity was also lower for sunflower compared to wildflower colonies, revealed by a Fligner-Killeen test of homogeneity of variances ($\chi^2_{(1)} = 37.387$, $P < 0.0001$). For the significant effect of time, post hoc pairwise tests revealed that week 4 was significantly different from all other weeks ($P < 0.0215$ for all comparisons), with lower infection levels by at least 40%. The covariate bee size had a significant effect on both infection prevalence ($\chi^2_{(1)} = 3.969$, $P = 0.046$) and intensity ($\chi^2_{(1)} = 4.022$, $P = 0.045$), although in opposite directions. Larger bees were less likely to be infected ($\beta = -0.7659$), but had greater infection intensities if they were infected ($\beta = 0.2852$).

Colony performance.—There was a significant interaction between pollen diet and time on colony weight gain ($\chi^2_{(7)} = 14.098$, $P = 0.0495$; Fig. 3), but not between infection treatment and time ($\chi^2_{(1)} = 7.580$, $P = 0.3711$) or between pollen diet and infection treatment ($\chi^2_{(1)} < 0.001$, $P = 0.983$). At the start of the experiment, the average colony weight was 629.2 ± 1.5 g (mean ± SE) and was not significantly different between pollen diets ($t_{(285)} = -0.073$, $P = 0.942$) or infection treatment ($t_{(285)} = -0.242$, $P = 0.809$). After 10 weeks, colonies gained an average of
Post hoc pairwise comparisons revealed that wildflower colonies surpassed sunflower colonies in terms of weight gain starting at week 7 and continuing through the end of the experiment ($t_{285} = -2.459, P < 0.015$ for weeks 7, 9, and 10), with the exception of week 8 ($t_{285} = -1.627, P = 0.105$). By the end of the experiment (week 10), wildflower colonies gained approximately 4% more weight than sunflower colonies. Infection treatment did

**Fig. 2.** *Crithidia bombi* infection prevalence for *Bombus impatiens* (a), measured as the proportion of bees sampled from an infected colony, and average infection intensity of infected bees only (cells per 0.02 μL) (b) over 10 weeks for experimentally infected bumble bee colonies fed either a sunflower pollen diet (1:1 ratio of sunflower to wildflower pollen; yellow squares) or wildflower pollen diet (black triangles). Points and error bars indicate binomial (a) and negative binomial (b) model means and standard errors, back-transformed from the scale of the linear predictor.
not significantly affect colony weight gain ($\chi^2_{1} = 0.361, P = 0.548$).

By the end of the experiment, colonies contained on average 189.05 ± 17.68 workers (mean ± SE), but queen and drone production were highly variable (see Table 1 for means and statistical tests of colony performance metrics). Pollen diet and infection treatments did not have significant main effects on worker production or the average weight or size of workers, nor on the

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**Table 1.** Mean numbers, sizes, and weights of adult bees and immature stages in *Bombus impatiens* colonies either inoculated (Inf) with the protozoan gut parasite *Crithidia bombi* (Inf) or a sham control (Uninf) and fed either sunflower pollen (1:1 ratio of sunflower to wildflower pollen) or wildflower pollen mix, along with statistical test results.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Inf/Sun</th>
<th>Inf/Wild</th>
<th>Uninf/Sun</th>
<th>Uninf/Wild</th>
<th>Pollen</th>
<th>Inoc</th>
<th>Pollen x Inoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. workers</td>
<td>126 (39.51)</td>
<td>227 (36.34)</td>
<td>202 (37.46)</td>
<td>190 (37.46)</td>
<td>1.72 (35)</td>
<td>0.20</td>
<td>0.34 (33)</td>
</tr>
<tr>
<td>Worker wt. (mg)</td>
<td>138 (12.88)</td>
<td>145 (11.61)</td>
<td>130 (12.05)</td>
<td>152 (12.05)</td>
<td>1.33 (36)</td>
<td>0.26</td>
<td>0.00 (36)</td>
</tr>
<tr>
<td>No. drones</td>
<td>35 (22.07)</td>
<td>8.09 (4.68)</td>
<td>15.2 (9.14)</td>
<td>47.7 (28.5)</td>
<td>0.06 (1)</td>
<td>0.80</td>
<td>0.78 (1)</td>
</tr>
<tr>
<td>Prob. of drones</td>
<td>0.56 (0.17)</td>
<td>0.45 (0.15)</td>
<td>0.8 (0.13)</td>
<td>0.9 (0.09)</td>
<td>0.00 (1)</td>
<td>0.98</td>
<td>4.51 (1)</td>
</tr>
<tr>
<td>Drone wt. (mg)</td>
<td>66.4 (27)</td>
<td>107 (28.64)</td>
<td>108 (24.9)</td>
<td>94.7 (20.04)</td>
<td>0.33 (15)</td>
<td>0.57</td>
<td>0.40 (1)</td>
</tr>
<tr>
<td>No. queens</td>
<td>26.1 (13.17)</td>
<td>18.3 (8.92)</td>
<td>7.23 (3.69)</td>
<td>25.1 (12.34)</td>
<td>1.13 (1)</td>
<td>0.29</td>
<td>1.29 (1)</td>
</tr>
<tr>
<td>Prob. of queens</td>
<td>0.8 (0.15)</td>
<td>0.46 (0.19)</td>
<td>0.38 (0.19)</td>
<td>0.92 (0.09)</td>
<td>0.10 (1)</td>
<td>0.76</td>
<td>0.01 (1)</td>
</tr>
<tr>
<td>Queen wt. (mg)</td>
<td>366 (71.07)</td>
<td>513 (67.44)</td>
<td>600 (79.29)</td>
<td>496 (66.42)</td>
<td>0.09 (32)</td>
<td>0.76</td>
<td>2.44 (30)</td>
</tr>
<tr>
<td>No. immatures</td>
<td>152 (53.65)</td>
<td>217 (50.79)</td>
<td>197 (51.81)</td>
<td>153 (51.81)</td>
<td>0.08 (34)</td>
<td>0.78</td>
<td>0.07 (33)</td>
</tr>
<tr>
<td>Immature wt. (mg)</td>
<td>189 (47.91)</td>
<td>164 (38.82)</td>
<td>136 (30.86)</td>
<td>308 (69.8)</td>
<td>2.40 (28)</td>
<td>0.13</td>
<td>0.51 (27)</td>
</tr>
<tr>
<td>Worker size (mm)</td>
<td>2.53 (0.02)</td>
<td>2.54 (0.02)</td>
<td>2.5 (0.02)</td>
<td>2.53 (0.02)</td>
<td>1.39 (1)</td>
<td>0.24</td>
<td>0.72 (1)</td>
</tr>
<tr>
<td>Queen size (mm)</td>
<td>3.7 (0.11)</td>
<td>3.63 (0.13)</td>
<td>3.66 (0.14)</td>
<td>3.82 (0.12)</td>
<td>0.11 (14)</td>
<td>0.74</td>
<td>0.33 (14)</td>
</tr>
<tr>
<td>Drone size (mm)</td>
<td>2.79 (0.08)</td>
<td>2.81 (0.09)</td>
<td>2.76 (0.1)</td>
<td>2.71 (0.06)</td>
<td>0.02 (18)</td>
<td>0.89</td>
<td>0.62 (18)</td>
</tr>
<tr>
<td>Colony wt. (g)</td>
<td>718 (5.72)</td>
<td>733 (5.72)</td>
<td>714 (5.72)</td>
<td>729 (5.72)</td>
<td>5.22 (1)</td>
<td>0.02*</td>
<td>0.36 (1)</td>
</tr>
<tr>
<td>No. colonies (N)</td>
<td>9</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: Differences between treatments were analyzed using linear mixed-effects models, with the exceptions of worker size, colony weight, and drone and queen production, which were analyzed using generalized linear models. For linear mixed-effects models, F-scores (St) and denominator degrees of freedom are listed in parentheses under St; the numerator degrees of freedom were always one. For generalized linear models, Type II Wald chi-square values are listed (St). Statistically significant factors ($P < 0.05$) marked with an asterisk. Values under treatments are mean with SE in parentheses.
number of immature stages counted at the end of the experiment (Table 1). However, there was a significant interaction between pollen diet and infection treatment on the average weight of combined immature stages, with uninfected wildflower colonies having nearly double the weight of all other treatments. There was also a significant interaction between pollen diet and infection treatment on the number of drones produced, with sunflower pollen rescuing the negative effects of *C. bombi* infection on drone production. None of the treatments had a significant effect on average drone weight or size. There was a significant interaction between pollen diet and infection treatment on the probability of producing new queens, with uninfected sunflower and infected wildflower colonies nearly half as likely to produce new queens compared to infected sunflower and uninfected wildflower colonies. There was no effect of pollen diet, infection treatment, or their interaction on queen size or weight. Overall, these analyses show similar colony performance between pollen diets and infection treatments, with the addition that a mixed sunflower pollen diet rescued the negative effect of infection on queen and drone production.

*Can C. bombi develop resistance to the medicinal effects of sunflower pollen?*

Consistent with our first experiment, there was a significant effect of pollen diet on *C. bombi* prevalence ($\chi^2_{1} = 25.066, P < 0.0001$) and infection intensity ($\chi^2_{1} = 4.695, P = 0.0303$), with sunflower-fed bees having 69% lower *C. bombi* prevalence and nearly threefold lower infection intensity than wildflower-fed bees (Fig. 4). However, there was no effect of inoculum type on prevalence ($\chi^2_{1} = 1.538, P = 0.2149$) or infection intensity ($\chi^2_{1} = 0.013, P = 0.9110$), nor were there significant interactions between pollen diet and inoculum type for prevalence ($\chi^2_{1} = 0.305, P = 0.5809$) or intensity of infection ($\chi^2_{1} = 0.0003, P = 0.9860$). We also found no effect of pollen diet, inoculum type, or their interaction on bee survival ($\chi^2_{1} < 1.9, P < 0.2$ for all).

**DISCUSSION**

The goal of this study was to assess the costs and benefits of sunflower pollen on bumble bee colony disease and performance. Given concerns of pathogen transmission between domesticated and wild bee populations (Power and Mitchell 2004, Colla et al. 2006, Sachman-Ruiz et al. 2015).
and that *C. bombi* infection levels in *B. impatiens* can drive pathogen dynamics in other wild bee species (Figueroa et al. 2020), understanding how to control pathogens in managed populations of bees is critically important. This study provides evidence that a 1:1 mixture of sunflower combined with wildflower pollen reduces *C. bombi* infection prevalence and intensity in both individual *B. impatiens* workers and at the colony level, supporting our prediction that a mixed sunflower pollen diet could provide similar medicinal effects compared to a pure sunflower pollen diet. In a follow-up laboratory experiment, *C. bombi* was unable to rapidly evolve resistance to sunflower pollen over the course of the whole-colony experiment. A supplemental mixed sunflower pollen diet may provide a simple and effective solution to reduce disease and improve the health of economically and ecologically important pollinators.

Mixing sunflower with wildflower pollen can be an effective treatment for reducing *C. bombi* infection without sacrificing colony performance, which may reduce the risk of pathogen spillover from commercial colonies to wild bees, or spillover from wild bees to commercial colonies. Top-down negative effects of pathogens on pollination services can result from either reduced bee population sizes or deleterious effects on bee foraging behavior. Numerous studies have found that *C. bombi*-infected bumble bees are less efficient foragers, with reduced pollen collection rates and weaker ability to learn floral reward associations and flower handling techniques (Shykoff and Schmid-Hempel 1991b, Gegear et al. 2005, 2006). While the influence of bee pathogens and diseases on pollination services is poorly understood, infection in bees can be negatively correlated with pollen movement between flowers, reducing plant reproduction (Gillespie and Adler 2013, Lach et al. 2015). In contrast, Theodorue et al. (2016) did not find an indirect effect of *C. bombi* infection on plant reproduction in urban ecosystems, which may have been the result of greater bee abundance in urban vs. rural areas. In our whole-colony experiment, a mixed sunflower pollen diet reduced pathogen levels without the risk of a negative tradeoff in worker production, which is the major pollination workforce within a bumble bee colony. Whether sunflower pollen benefits pollination services by mediating host–pathogen interactions in bumble bees remains an open question.

By consuming pollen and nectar, pollinators can be considered herbivores that specialize on certain plant tissues. Generalist herbivores may benefit from a mixed diet when some components of the diet compensate for nutrient deficiencies or dilute toxic plant secondary compounds (Bernays et al. 1994). Similarly, generalist pollinator species may benefit from consuming mixed diets comprised of complementary pollen species that balance nutrient demands and dilute negative effects of nutritionally poor pollen, such as sunflower pollen (Eckhardt et al. 2013, Nicolson and Human 2013). For example, in a recent study using bumble bee microcolonies, bees that consumed a pure sunflower pollen diet had a significantly shorter lifespan than bees that consumed broad bean (*Vicia faba*, Fabaceae), rapeseed (*Brassica napus*, Brassicaceae) or Cucurbitaceae pollen, but the negative effects were eliminated when bees consumed a mixed pollen diet with 50% sunflower pollen (McAulay and Forrest 2019). Some pollen diets are inadequate for generalist bumble bee development (Génissel et al. 2002, Tasei and Aupinel 2008), and many studies demonstrate that worker foraging preferences are largely based on nutritional quality of pollen (Ruedenauger et al. 2016, Vaudo et al. 2016, Kriesell et al. 2017). Pollen diets vary considerably in nutrient content and concentrations (Roulston and Cane 2000), as well as secondary metabolites (Palmer-Young et al. 2018) and digestibility (Vanderplanck et al. 2018), each of which can have profound impacts on colony growth. Our study provides further support that a 1:1 mixture of sunflower and wildflower pollen can compensate for nutrient deficiencies of monofloral sunflower pollen at the bumble bee colony level, without significantly sacrificing the medicinal benefits of sunflower pollen. Our experiment was not designed to test whether the medicinal benefits of sunflower pollen in mixed pollen diets show a linear or step-function relationship; visual inspection of the relationships suggests a linear relationship for prevalence but a step-function relationship for intensity (compare Fig. 1a, b). An experiment with finer gradations in sunflower pollen proportions would be needed to test these patterns. Nonetheless, our
results demonstrate that sunflower pollen as part of a mixed wildflower pollen diet can reduce disease and maintain production of colonies, and by extension, sunflowers planted as part of a diverse wildflower mixture may allow bees to naturally resist pathogen infection.

Compared to a wildflower diet, we found that the sunflower diet significantly reduced negative effects of infection on queen and drone production. Infection significantly reduced the probability of queen production in wildflower but not sunflower colonies, while infection significantly reduced the probability of drone production independent of pollen diet. However, infected sunflower colonies that did produce drones yielded on average four times as many as infected wildflower colonies. Similarly, in a previous study using B. impatiens microcolonies, sunflower pollen consumption alleviated the negative effects of C. bombi infection on egg production compared to buckwheat pollen (Giacomini et al. 2018). Taken together, these results strongly suggest that the medicinal benefits of a mixed sunflower pollen diet can reverse the negative effects of C. bombi infection on reproductive success. While queen and drone production play an important role in bumble bee population dynamics and provide a practical estimate of reproductive success (Muller and Schmid-Hempel 1992, Pelletier and McNeil 2003, Crone and Williams 2016), further investigation of the effects of a mixed sunflower pollen diet and C. bombi infection on mating success, queen overwintering, and colony founding success is needed to better understand how a sunflower pollen diet ultimately affects bumble bee reproductive success.

In the whole-colony experiment, body size and mass of all adult castes (i.e., workers, drones, queens) were similar for pollen diets with and without sunflower, suggesting that a mixed sunflower pollen diet can minimize adverse effects on larval development associated with a pure Asteraceae diet (Tasei and Aupinel 2008, Vanderplanck et al. 2018). Maintenance of bee body size has important consequences for pollination services, as evidence suggests that intra-specific variation in body size can drive patterns in pollination efficacy, such that larger-bodied bees are more effective pollinators (Jauker et al. 2016). Body size and mass of workers are also important factors for determining bumble bee colony reproduction since larger workers are able to forage more effectively than smaller workers and can carry larger nectar and pollen loads per foraging trip (Goulson et al. 2002), although smaller workers are less vulnerable to starvation (Couvillon and Dornhaus 2010). Size and mass of queens also are important. Larger queens are more successful in nest usurpation contests (Richards 1978), better able to thermoregulate (Heinrich 1979), and have higher overwintering survival (Holm 1972).

Body size and mass in the whole-colony experiment were not affected by C. bombi infection. Energetic costs associated with the host’s ability to defend against parasites can affect host body size and mass under stressful conditions (Van Heugten et al. 1996, Moret and Schmid-Hempel 2000, Bonneaud et al. 2003), such as a nutritionally deficient diet. Under favorable conditions, bumble bees can tolerate C. bombi infection without adverse effects (Brown et al. 2000, 2003). However, when infection is combined with nutritional stress, the risk of mortality increases and resource allocation patterns within the colony change, resulting in bees that dedicate more energy into their fat body and less into their reproductive system (Brown et al. 2000, 2003). The nutritional profile of the sunflower pollen treatment in the whole-colony experiment was likely adequate since it was a mixture of sunflower and wildflower pollen. In addition, all colonies in this study received consistent access to abundant nectar and pollen throughout the experiment, making it less likely that we would observe negative effects of infection on bee size or mass. Nonetheless, habitat loss and lack of floral resources play major roles in bee declines globally (Goulson et al. 2015), warranting further investigation of the interaction between floral resources, sunflower pollen, and C. bombi infection to shed light on colony growth consequences under field conditions.

At the end of the whole-colony experiment, we discovered that colonies inoculated with C. bombi and fed mixed sunflower pollen had lower mean parasite loads than colonies fed wildflower pollen, but infection was not reduced as dramatically as in previous individual bee experiments (see Giacomini et al. 2018). Several non-mutually exclusive hypotheses may explain the inability of
sunflower mixed with wildflower pollen to eliminate infection at the whole-colony level. One explanation could be rapid development of *C. bombi* resistance to the medicinal effects of sunflower pollen within a colony. Numerous studies have demonstrated the rapid evolution of infectious microbes to repeated antibiotic treatments (Van den Bergh et al. 2016, Capela et al. 2019, Liu et al. 2020). In bumble bees, *C. bombi* lineage (defined loosely as a unique genotype) plays a major role in the ability to establish an infection within a colony. In one study, bumble bee colonies that were given a mixture of *C. bombi* lineages filtered out less infective lineages within just five serial passages between workers (i.e., transmission between hosts), or just 35 d (Youth and Schmid-Hempel 2006). *C. bombi* also evolved resistance to inhibitory phytochemicals after only 6 weeks of exposure (Palmer-Young et al. 2017). Thus, it is plausible that a sunflower pollen diet could rapidly select for a novel *C. bombi* lineage resistant to the medicinal effects of sunflower pollen. However, we did not detect any differences in infection prevalence or intensity between *C. bombi* lineages (e.g., inoculum types) that were exposed to sunflower pollen or not, suggesting that *C. bombi* was unable to rapidly evolve resistance to sunflower pollen during the 10-week experiment.

Our finding that sunflower pollen did not completely clear infection within a colony could be because bees were confined to a box with increasing density of individuals over time. For fecal-orally transmitted parasites, such as *C. bombi*, the probability of susceptible hosts becoming infected should increase with increasing host density (Anderson and May 1979), due to increased density of social interactions between infected and susceptible hosts (Otterstatter and Thomson 2007). Domesticated bumble bees are typically confined to a single container for a significant period of their life cycle prior to being placed in greenhouses or outdoors for crop pollination. Confinement and greater density of individuals likely increases the deposition of contaminated bee feces and the likelihood of repeated exposure to susceptible individuals. Hygienic behaviors, such as localized deposition of feces or deposition away from the nest, are well known in eusocial insects and are thought to convey anti-parasite benefits (Michener and Michener 1974, Weiss 2006). It is thus plausible that a multi-box system for commercially reared bumble bees, in combination with a mixed sunflower pollen diet, may separate contaminated feces from the brood and reduce *C. bombi* transmission between individuals and colonies. Such a hypothesis warrants further investigation, although the space requirements needed for a multi-box system in a commercial rearing facility may be cost prohibitive.

Recent work suggests that the effect of sunflower pollen on *C. bombi* may be mediated in part by the bee host rather than via a direct effect of pollen on the pathogen. For example, sunflower pollen extract increased rather than suppressed *C. bombi* growth in vitro (Palmer-Young 2017). While the mechanism underlying the medicinal effect of sunflower pollen is unknown (Adler et al. 2020) and outside the scope of this study, it is plausible that sunflower pollen reduces *C. bombi* infection via changes in host physiological functions, such as an immune response, or via direct interactions with the *C. bombi* cells within the host, such as nutrient limitation. Brunner et al. (2014) found that pollen-starved bumble bees showed reduced immune responses to infection, including the upregulation of energetically costly antimicrobial peptides and putative immune signaling molecules. A sunflower pollen diet with low protein content and missing essential amino acids would be expected to negatively affect host immunocompetence and thus increase parasite growth. However, changes in host nutrition may affect the availability of resources for the pathogen and subsequently limit pathogen growth and reproduction. Logan et al. (2005) reported higher *C. bombi* infection levels in bumble bees fed pollen compared to pollen-starved bees. The bumble bee colonies in our study had access to a sufficient quantity of pollen, evident by each colony’s inability to completely finish the weekly pollen balls that were supplied to them. Moreover, the sunflower pollen treatment consisted of a 1:1 mixture of sunflower:wildflower pollen to increase the nutritional profile of the sunflower pollen treatment. Thus, it seems unlikely that poor nutrition was the sole cause of reduced *C. bombi* growth within sunflower pollen-fed bees. Sunflower pollen could also mediate infection through physiological changes in the host

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induced by the spiny morphology of the pollen grains, or, since *C. bombi* is a gut parasite that attaches to the hindgut wall (Gorbunov 1996), sunflower pollen could reduce infection by scouring the hindgut of parasite cells (Huffman and Caton 2001). Further work aimed at establishing how a sunflower pollen diet reduces *C. bombi* infection may open up new areas of inquiry into mechanisms mediating bee health, as well as identifying floral traits that could be incorporated into pollinator landscapes.

Despite the evidence that sunflower pollen reduces *C. bombi* infection in commercial *B. impatiens* colonies, several obstacles should be addressed prior to applying a medicinal sunflower pollen diet. First, since a mixed sunflower pollen diet reduces but does not eliminate infection, it is important to identify how such a reduction relates to the rate of pathogen spillover from commercially managed colonies to wild bees. Contact rate, rather than the duration of contact, may drive risk of *C. bombi* infection for bumble bees (Otterstatter and Thomson 2007, Sah et al. 2018), suggesting that reduced *C. bombi* prevalence and intensity within commercially managed bumble bee colonies will concordantly reduce pathogen spillover rates. Similarly, sunflower pollen supplements could increase resistance to pathogen spillback from wild bees to commercial colonies. Second, it is important to identify cost-effective sources of environmentally friendly sunflower and wildflower pollen that are not contaminated with pesticides or pathogens. Pesticides are commonly used on sunflower crops to suppress weeds, herbivorous insects, and plant pathogens (Elbert et al. 2008) and can pose a substantial risk for bees (Whitehorn et al. 2012). Moreover, honey bee-collected pollen used for feeding commercially managed bumble bees comes with the risk of pathogen contamination (Gravstock et al. 2016, de Sousa Pereira et al. 2019). Sterilization of non-local pollen used to feed bumble bees should be encouraged to reduce the transmission of infectious bee diseases among managed and wild bees.

**CONCLUSION**

Managing pollinator populations requires the careful consideration of key plant species that play disproportionate roles in protecting against pathogens, as well as nutritional needs associated with growth and reproduction. This study provides evidence that sunflower pollen as part of a mixed pollen diet can reduce infection in individual bees and at the whole-colony level and recover negative effects of infection on colony reproduction, with no significant nutritional trade-offs for colony worker production. The reduction of pathogens within bumble bee colonies is a significant concern for commercial producers of domesticated bumble bees, growers that use bumble bee colonies for pollination, and conservation biologists worldwide. We conclude that a mixed sunflower pollen diet could be an effective strategy for reducing bumble bee disease.

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**LITERATURE CITED**


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bumble bee colony growth and queen production.

**DATA AVAILABILITY**

Data and R scripts for the statistical analyses are available from Zenodo: https://doi.org/10.5281/zenodo.4770863.