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Sunflower plantings reduce a common gut pathogen and increase queen production in common eastern bumblebee colonies

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Community diversity can reduce the prevalence and spread of disease, but certain species may play a disproportionate role in diluting or amplifying pathogens. Flowers act as both sources of nutrition and sites of pathogen transmission, but the effects of specific plant species in shaping bee disease dynamics are not well understood. We evaluated whether plantings of sunflower (*Helianthus annuus*), whose pollen reduces infection by some pathogens when fed to bees in captivity, lowered pathogen levels and increased reproduction in free-foraging bumblebee colonies (*Bombus impatiens*). Sunflower abundance reduced the prevalence of a common gut pathogen, *Crithidia bombi*, and reduced infection intensity, with an order of magnitude lower infection intensity at high sunflower sites compared with sites with little to no sunflower. Sunflower abundance was also positively associated with greater queen production in colonies. Sunflower did not affect prevalence of other detected pathogens. This work demonstrates that a single plant species can drive disease dynamics in foraging *B. impatiens*, and that sunflower plantings can be used as a tool for mitigating a prevalent pathogen while also increasing reproduction of an agriculturally important bee species.

1. Introduction

The composition of a community can influence the prevalence and spread of disease among its members. Greater biodiversity generally reduces disease risk within communities [1–3], and specific taxa can play a disproportionate role in diluting the prevalence of disease in co-occurring species. In grassland communities, for example, the loss of disease-resistant grass species explained higher disease prevalence in remaining species [4,5], and the intensity of foliar fungal infections was greater in alpine meadow plants when low-competence hosts were lost [6]. Most documented dilution effects are the result of reduced encounter frequency or increased transmission interference, in which higher community diversity reduces opportunities for pathogens to interact with competent hosts (e.g. [7]). In some cases, organisms reduce risk of disease for community members more directly. For example, amphibians with greater filter-feeding ability can dilute chytrid fungus infections in other amphibians [8], and certain plant species reduce soil pathogens, benefiting neighbouring crop species in agroecosystems [9].

The role of pathogens in pollinator decline is an area of great interest and concern [10,11]. Reduced abundance and biodiversity of bees (Anthophila), in particular, pose a threat to our global food supply [12]. Flowers can be both critical sources of nutrition and sites of disease transmission [13–15]. Plant diversity is important for sustaining pollinator diversity broadly, as well as species with commercial and economic value, such as honeybees (*Apis mellifera*) and bumblebees

(*Bombus* sp.) [16,17]. However, we are only beginning to understand how the composition of flowering plant communities influences disease dynamics in pollinators.

Presently, only a few field studies relate the floral resource community to pathogen loads in bees. These efforts confirm that flowering plant taxa vary in both the type and amounts of bee pathogens they harbour in nature [18–20] and highlight characteristics of plant–pollinator networks that may contribute to transmission. Piot *et al*. [20] found that the centrality of plant taxa within plant–pollinator networks is associated with higher bee pathogen loads on flowers, while Figueroa *et al*. [18] found that more diverse network connections diluted pathogen prevalence in bumblebees. Similarly, Graystock *et al*. [19] reported lower pathogen prevalence in bees earlier in the season when the bee community was more diverse and less dominated by social taxa (i.e. bumblebees and honeybees). Experimental work also demonstrates that flowering plant species differentially harbour [21] and transmit certain pathogens to bee hosts [15,22].

Just as some flowering plants may serve as 'hotspots' for bee disease transmission because of increased bee visitation, pathogen deposition and/or transmission capacity [14,15,23], others might slow disease spread through dietary effects on pathogen infection in visiting pollinators. Flowering plants vary in the nutritional content of their pollen and nectar [24,25], which can influence immune function in bees [26,27], and some plants produce antimicrobial phytochemicals in floral rewards. For example, secondary metabolites in nectar, when fed to bees in captivity, can reduce pathogen infections [28–30]. Thus, specific plants might disproportionately contribute to pollinator disease via floral resources or traits that shape infection; however, the influence of specific plants on infection dynamics in wild bee populations remains largely unexplored (but see [31]).

One plant species with the potential to drive disease dynamics in bee populations is sunflower (*Helianthus annuus*), which was recently discovered to reduce pathogen infection in *Bombus impatiens*, the common eastern bumblebee. A diet of sunflower pollen, when fed to workers in captivity, consistently and dramatically reduced infection by *Crithidia bombi* (Trypanosomatidae), a common intestinal parasite of bumblebees, compared with wildflower or other monofloral pollen diets [32]. This effect was consistent across multiple *H. annuus* cultivars and wild accessions, and some sunflower relatives [33]. Furthermore, 50% *H. annuus* pollen mixed with wildflower pollen was still effective at reducing infection [34], suggesting that free-flying *B. impatiens* foraging on multiple plant species may experience medicinal benefits. The effects of sunflower pollen may also extend beyond *Crithidia*; sunflower pollen and honey reduced infection in honeybees by *Nosema ceranae* [32,35]. In separate work, sunflower pollen provisions were associated with reduced *Varroa* mites in honeybee colonies, but did not affect *Nosema* or virus intensities [36]. However, pollen with spiny exines, such as sunflower pollen, was also more likely to harbour pollen-associated viruses than smoother pollen in a large metagenomic survey [37]. Thus, while our work focuses on sunflower pollen and *Crithidia* infection owing to prior dramatic and consistent effects, sunflower pollen may influence infection by diverse pollinator pathogens in different ways.

Because *B. impatiens* is a social bee species, it is important to assess effects of sunflower pollen at the colony level, and to consider reproduction as well as pathogen infection. Sunflower pollen consistently and dramatically reduced *Crithidia* infection in individual bees in laboratory trials, and greater sunflower area on farms was associated with lower *Crithidia* infection in wild *B. impatiens* foragers [32]. However, the effects of sunflower abundance at the colony level are unknown. *Bombus impatiens* is the most abundant native bumblebee in eastern North America [38,39] and owing to its dominance may drive pathogen prevalence in other wild bee species [18,40]. It is also the most economically valuable wild pollinator worldwide, with benefits double that of any other species in a meta-analysis of 100 pollinator species [41]. Sunflower is both a North American native perennial [42] and a major oilseed crop grown worldwide (reviewed in [43]). Therefore, understanding the potential benefits of sunflower for *B. impatiens* health and reproduction is important for both natural and managed ecosystems.

The broader floral resource community may also shape pollinator health and performance, since floral abundance and diversity can influence disease dynamics [19]. Asteraceae are dominant species in most ecosystems, and Asteraceae pollen beyond sunflower could have antipathogenic effects. The mechanism underlying the effect of sunflower pollen on reduced *Crithidia* infection may be due to spiny exine rather than chemistry [44,45], and many Asteraceae species have spiny pollen [46,47], suggesting that species beyond sunflower could have similar effects. In support of this hypothesis, pollen from several other Asteraceae species reduced *Crithidia* infection in *B. impatiens* [33,45]. Asteraceae pollen provisions also reduced brood parasitism by wasps in *Osmia* bees (e.g. [48]). Thus, we hypothesized that Asteraceae abundance overall, rather than sunflower alone, could reduce infection.

We assessed whether sunflower plantings in agroecosystems can mitigate microparasite and viral infections in free-foraging colonies of the common eastern bumblebee (*Bombus impatiens*). Based on prior laboratory research, we hypothesized that sunflower abundance on farms would reduce infection prevalence and intensity of *Crithidia*, but there is less consistent information to drive hypotheses about other pathogens. Because other Asteraceae pollen reduces *Crithidia* and brood parasite infection, we hypothesized that Asteraceae floral resources more generally would be associated with reduced infection frequency and intensity. We also assessed how sunflower and the broader floral resource environment influenced colony performance (peak colony weight, queen production). Because sunflower pollen is considered a poor source of nutrition for bees [43,49], we hypothesized that higher sunflower abundance could come with a colony performance cost. Understanding whether sunflower can reduce infection in the field without negative reproductive consequences is important for conservation efforts, as sunflower plantings could be a simple tool that land managers could use to manage pollinator health.

2. Methods

(a) Overall approach

To test the hypotheses that sunflower pollen would reduce *Crithidia* infection and influence colony reproduction, we placed commercial colonies across 20 farms in western Massachusetts growing different amounts of sunflower, with the largest plantings representing the maximum amount (1–2 acres) typical in the region. Sampling pathogens in foragers from these colonies over several weeks allowed us to determine whether pathogen

infection decreased with increasing availability of sunflower or other floral resources. We evaluated colony performance by weighing colonies at each sampling visit and counting daughter queens produced.

(b) Study region and species

This study was conducted in western Massachusetts, which includes many small farms [50] that often grow diverse crops. Vegetable farms in the area often grow sunflower varieties as cut flowers; some plant sunflowers as cover crops. Sunflower is a late-blooming crop (mid-July until frost). In other parts of the USA where farms are larger in scale, sunflowers are grown for seed and oil production; they are also grown as a rotation crop to improve soil health and as a double crop to provide additional economic benefits in a season [51].

Bombus impatiens is native to the eastern USA and has a late-season phenology [52] with peak worker and queen production when blooms of sunflower and other Asteraceae are abundant. It is a dominant species with a wide diet breadth [52], making it likely to interact with flowers used by other pollinators. We chose this species because of its commercial availability, its phenological alignment with our study plant, its prominence in plant–pollinator networks [18] and its agricultural significance [41].

We screened bees for the following bumblebee pathogens: *Crithidia* sp., a common trypanosomatid gut pathogen that can impair learning in foragers [53] and reduce colony size and reproduction [54,55]; *Nosema bombi*, a potentially virulent intracellular fungus (Microsporidia) implicated in some bumblebee declines [38,56]; and *Apicystis bombi*, a protozoan (Neogregarinida) that infects and degrades the fat body of bumblebees with sublethal and lethal consequences [57]. We also screened for prevalent honeybee pathogens and viruses that may infect bumblebees [58–60] with some pathogenic consequences (e.g. [61,62]): *N. ceranae* and *Nosema apis* (Microsporidia), Black Queen cell virus (BQCV) and Deformed wing virus (DWV).

(c) Site selection, study design, and sampling scheme

Fieldwork took place between 6 July and 6 October 2019. We selected 20 mixed vegetable farms in western Massachusetts (Hampshire and Franklin counties) that ranged in the area of sunflower (*H. annuus*, in one case *Helianthus maximiliani*), from none or only a few rows to approximately 0.3–1.2 ha sunflower cover crops. Farms ranged in overall size, other crops and/or ornamentals cultivated, management practices (i.e. organic versus conventional), and sunflower planting establishment times (electronic supplementary material, tables S1 and S2). Small- to medium-sized plantings, grown as cut flowers, were sown and began blooming earlier in the season (mid-July) and typically bloomed for several weeks because of staggered planting and routine flower head removal resulting in new flower growth. Larger cover crop plantings were typically sown later in the season, with first blooms appearing in August and blooming for a shorter time, usually approximately three weeks.

We placed a pair of commercial common eastern bumblebee (*Bombus impatiens*) colonies (Koppert Biological Systems) at each farm (electronic supplementary material, figures S1 and S2), coinciding with the approximate start of sunflower bloom (electronic supplementary material, table S1). We used commercial colonies for logistical reasons and because they are commonly deployed in agricultural settings [63]; we also note that sunflower pollen had similar effects reducing *Crithidia* infection in wild and commercial *B. impatiens* [64]. We staggered colony deployment to sites without sunflower across the season. Prior to placement, we screened all colonies for *Nosema* sp., *Crithidia* sp. and *A. bombi* using visual detection methods (see below) and noted other signs of poor health (e.g. ejected larvae). Infected or otherwise poor-condition colonies were not used. We recorded initial colony weights on the day of deployment.

We returned to sites every 10–14 days until colonies entered either queen production or senescence. Most colonies were visited four times before being removed from the field. All sites had at least one surviving colony for the first three rounds. Across sites, peak sunflower bloom tended to occur between site visits 1 and 2 (electronic supplementary material, table S2). Thus, the period before site visit 1 can be considered 'pre-full bloom', between site visit 1 and 2 can be considered 'full bloom', and between site visit 2 and 3 can be considered 'post-full bloom'. Cover crop plantings were always out of flower by site visit 3, and so we did not analyse data from site visit 4.

At each visit, we (i) recorded colony weights to track growth, (ii) collected approximately 10 corbicular loads from returning foragers (per site) to assess usage of sunflower and other Asteraceae, and (iii) collected 10 returning foraging workers from each colony entrance for later pathogen analysis. Foragers were initially collected into vials and stored in a cooler with ice packs. We collected one corbicular load from every bee with pollen, and then placed all bees on dry ice. Bees were immediately transferred from dry ice to −80°C storage upon returning to the laboratory. We chose to collect foragers (versus randomly sample bees inside the colony box) because we expected these bees to have the greatest pathogen exposure.

Colonies were brought in from the field when weight dropped over two consecutive visits or when visual inspection indicated decline. Colonies were kept in cold storage and later dissected to count daughter queens produced, which included both eclosed and uneclosed cocoons. Queen cocoons can be distinguished from worker/male cocoons by their larger size [65].

(d) Pathogen assessments

Of the 10 bees collected from each colony at each sampling date, five were retained for visual detection and quantification of *Crithidia* sp. and *Nosema* sp. using phase-contrast microscopy. The other five were used for molecular detection of a wider suite of pathogens, including trypanosomatids, *Apicystis*, *N. ceranae*, *N. bombi*, BQCV and DWV. We note that this sampling level is similar to other studies; for example, Imhoof & Schmid-Hempel [66] sampled four workers per colony per sampling date to assess colony infection. Visual detection was used for samples collected during the first three visits. Based on initial findings, molecular screening was carried out for samples from the second and third visits only (detailed methods in electronic supplementary material). We note that molecular detection (qPCR) of *C. bombi* and other trypanosomatids was more sensitive than visual assessment, detecting this pathogen in a much higher fraction of bees (37 versus 13%). It is expected that lighter infections would evade detection using visual methods [67–69]. In addition, qPCR does not distinguish between dead and living pathogen cells, whereas only live *Crithidia* cells are counted using visual assessment. Furthermore, the qPCR primers we used in this study are not specific to *Crithidia*, as they can also amplify *Lotmaria* DNA [70]. We thus use '*Crithidia*' to refer to visual assessments, and 'trypanosomatid' to refer to molecular detection.

(e) Quantifying sunflower and other floral resources

Flowering resources typically visited by bumblebees were quantified at each farm twice to characterize abundance and diversity. Surveys were usually spaced approximately three weeks apart, with the first survey coinciding with the first colony sampling event. At some sites, we conducted interim sunflower surveys to ensure we captured peak bloom. Prior to surveying, we created digital maps of each farm in Google Earth Pro (v. 7.3.3.7786), designating areas (hereafter 'polygons') that appeared to be actively cultivated, fallow, semi-natural (e.g. meadow) or mowed based

on satellite imagery and ground-truthing; we calculated the area (m^2) of all polygons. Flowering row crops were quantified by estimating the flowers or inflorescences per row metre and multiplying by the length and number of rows for that crop. Quadrat sampling was used to assess the density of inflorescences in cover crops, fallow fields and mowed areas. Sampling effort was proportional to total polygon area. Flowering forest edges, which tended to include wild floral resources often used by *B. impatiens* like goldenrod (*Solidago* spp.) [71], flower beds and community gardens were included in surveys; actively grazed areas were excluded, as they contained few inflorescences and were often inaccessible.

(f) Pollen analysis

We determined whether the frequency of sunflower and other non-*Helianthus* Asteraceae pollen in corbicular loads was related to the local abundance of these plants and/or to infection status of foraging bees. About 8.5% of bees were carrying sunflower pollen and 46% carried Asteraceae pollen. Although these rates of collection exceeded the proportional abundance of *Helianthus* and Asteraceae inflorescences across sites (electronic supplementary material, table S3), none of the relationships we tested between pollen composition, individual infection status and sunflower availability at farms was statistically significant (detailed methods and results in the electronic supplementary material).

(g) Statistical analysis

All analyses were carried out in R Core Program v. 4.0.2 [72].

(i) Pathogen prevalence and intensity in relation to sunflower and other floral metrics

We evaluated the influence of sunflower abundance and other floral resource metrics on *Nosema* (visual inspection) and *Crithidia*/trypanosomatid infections (visual inspection or qPCR, respectively) using a hurdle model in which we (i) first assessed whether predictors influenced disease status (presence/absence) of bees using generalized linear mixed models (*glmer*, lme4 package [73]) with binomial error distribution, and (ii) subsequently assessed how predictors influenced infection intensity (cells per 0.02 µl for visual inspection or copies of *C. bombi* DNA, normalized to total extracted DNA for qPCR) in infected bees only using linear mixed models (*lmer*, lme4 package [73]) with Gaussian error distributions. *Crithidia*/trypanosomatid intensity was $\log_{10}$-transformed to scale this variable and normalize model residuals.

We also assessed the influence of sunflower and other floral metrics on the prevalence (presence/absence) of *N. ceranae*, *Apicystis* and BQCV using generalized linear mixed models (*glmer*) with a binomial error distribution.

In all models, sunflower abundance was represented by the maximum number of sunflower heads recorded at a site. Other predictors included site visit, maximum abundance of non-*Helianthus* Asteraceae (excluding common taxa, e.g. *Erigeron* spp., not frequently used by bumblebees), overall floral resource density, floral resource diversity (Shannon index) and bee body size (length of marginal cell of right wing). In all models, sunflower abundance, non-*Helianthus* Asteraceae abundance and floral density were $\log_{10}$-transformed to linearize and scale these variables. Intercorrelation of main effects was assessed for each model; variance inflation factors (VIFs) were low (less than 5), so all terms were retained. For most pathogens, initial models included two-way interactions between site visit and abundance of sunflower, non-*Helianthus* Asteraceae, and floral density to account for changes over time; non-significant interactions were excluded from final models. Non-significant main effects were excluded from final models if their removal improved Akaike information criterion (AIC; $\Delta < -2$). All models initially included the random effect of colony identity nested within site. In some cases, the nested effect of colony identity or the entire random effect was removed when it explained zero variance in the data and caused singularity issues as a result.

We used *emtrends* (emmeans package [74]) in R to produce coefficients for all fixed effect interaction terms across models. We employed the effects [75] and ggplot2 [76] packages for visualizations. All parameter values can be found in electronic supplementary material, tables S5–S7.

(ii) Colony performance in relation to sunflower and other floral metrics

We evaluated whether sunflower abundance, non-*Helianthus* Asteraceae abundance, floral density and floral diversity influenced peak colony weights using a linear mixed model (*lmer*, lme4 package [73]) and daughter queen counts using a negative binomial generalized linear model (*glm.nb*, MASS package [77]) to account for overdispersion. All floral metrics were $\log_{10}$-transformed for scaling purposes. Site was initially included as a random effect in both models but was removed from the model for queen count to resolve singularity issues. For the analysis of peak weights, we included colony deployment date and initial weight as covariates. Because colony peak weight and deployment date were correlated (Spearman's $\rho = 0.485$, $p = 0.002$), we did not include both terms in the model for queen production. Peak weight resulted in a lower AIC than deployment date ($\Delta < -2$), so we used the former as a covariate.

3. Results

(a) Pathogen summary

Using visual assessment, we found 13% of bees were infected with *Crithidia* across the season. *Crithidia* infections were most prevalent during the second site visit (17% infected in site visit 2 versus 10.2 and 14.5% during site visits 1 and 3). Using molecular detection in site visits 2 and 3 only, we found trypanosomatids in 37% of bees ($n = 152$ of 410 bees), *A. bombi* in 11% ($n = 47$) and BQCV in 73% of bees ($n = 306$). *Nosema bombi* and DWV did not appear in any of the screened samples using molecular detection. Because recent work suggests that *N. ceranae* does not replicate in *B. impatiens* or other *Bombus* ([70,78,79]; but see [80]), we report *N. ceranae* statistical analyses and results in the electronic supplementary material.

(b) Pathogen prevalence and intensity in relation to sunflower and other floral metrics

Statistical results for full and reduced models are reported in electronic supplementary material, table S4, as are parameter values for all effects in reduced models (electronic supplementary material, tables S5–S7).

(i) *Crithidia*/trypanosomatids

Higher sunflower abundance was associated with reduced likelihood of infection during the second visit, when *Crithidia* was most prevalent and sunflowers were closest to peak bloom (electronic supplementary material, table S1; see electronic supplementary material, figure S3 for first and third visit). This was the case using both visual and molecular assessments (table 1a, figure 1*a*,*c*), though the effect using the latter method was marginal (electronic supplementary material, table S5c). During the second site visit, visually assessed

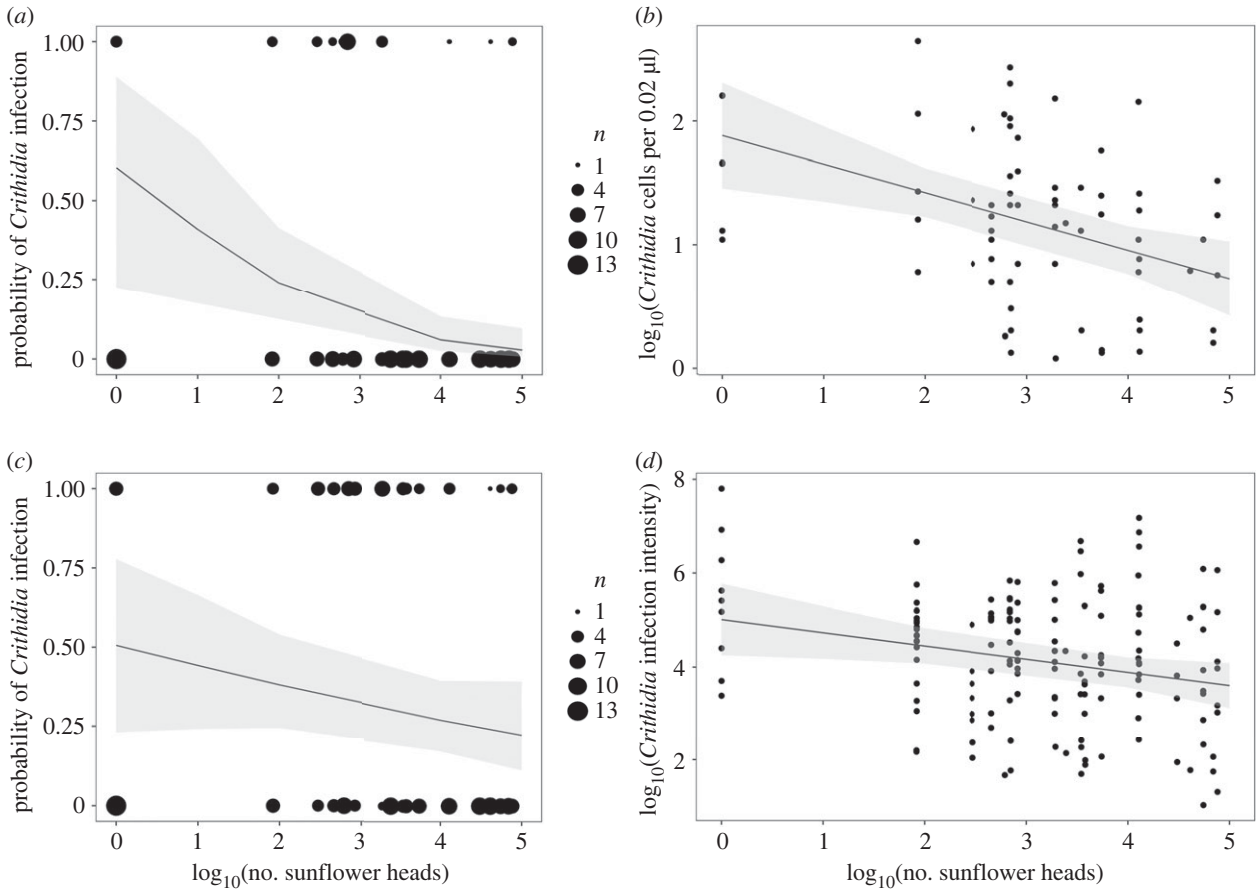

Figure 1. Probability of infection by *Crithidia bombi*, whether assessed visually (*a*) or using qPCR (*c*), decreased with sunflower abundance during the second site visit, which generally occurred just after peak bloom. Circles represent the number of samples infected (1) or not (0). There was no relationship between sunflower abundance and *Crithidia* prevalence (assessed visually or with qPCR) in site visits 1 and 3 (electronic supplementary material, figure S3). The intensity of *Crithidia* infections declined with sunflower abundance, irrespective of site visit, when assessed visually (*b*) or using qPCR (*d*). The shaded grey area around each trendline represents the 95% confidence interval.

Table 1. Wald χ^2 and *p*-values from final models predicting (*a*) *Crithidia*/trypanosomatid prevalence and infection intensity using data from visual assessments and (*b*) qPCR, and (*c*) the prevalence of BQCV. 'Non-sf Asteraceae' refers to abundance of non-sunflower Asteraceae. 'Floral density' refers to overall floral density. Dashes represent model terms that were excluded from the final model. Italics indicate $p < 0.05$, but include one case where $p = 0.052$. n.a., not applicable. Additional model results, including test statistics for all full models, are presented in electronic supplementary material, tables S4–S7.

	a. *Crithidia* (visual)				b. Trypanosomatids (qPCR)				c. BQCV	
	prevalence		intensity		prevalence		intensity		prevalence	
terms	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
site visit	4.034	0.133	1.092	0.579	1.874	0.171	3.783	*0.052*	3.928	0.066
$\log_{10}$(sunflower)	3.275	0.070	11.886	*<0.001*	0.305	0.581	6.255	*0.012*	0.516	0.472
$\log_{10}$(non-sf Asteraceae)	2.425	0.119	—	—	—	—	—	—	4.752	*0.010*
$\log_{10}$(floral density)	0.195	0.659	3.92	*0.047*	0.136	0.713	2.219	0.136	3.127	0.067
floral diversity, *H*	—	—	—	—	—		—	—	0.322	0.57
site visit ∗ $\log_{10}$(sunflower)	8.578	*0.014*	—	—	4.907	*0.026*	—	—	—	—
site.visit ∗ $\log_{10}$(Asteraceae)	—	—	—	—	—	—	—	—	—	—
site visit ∗ floral density	12.994	*0.002*	—	—	—	—	—	—	—	—
marginal cell length	—	—	—	—	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Crithida prevalence averaged 0.603 ($CI_{95\%}$: 0.225, 0.889) at sites with no sunflower, compared with 0.028 ($CI_{95\%}$: 0.008, 0.0971) at sites with the largest plantings (10 000–100 000 heads; figure 1*a*). Similarly, trypanosomatid prevalence assessed using qPCR ranged from 0.537 ($CI_{95\%}$: 0.256, 0.797) at sites with no sunflower to 0.216 ($CI_{95\%}$: 0.107, 0.388) at sites with

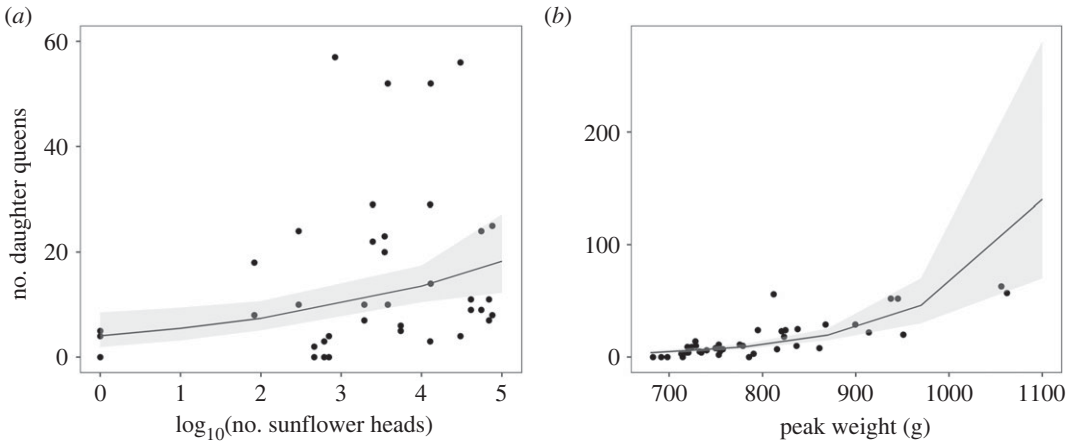

Figure 2. (*a*) The relationship between sunflower abundance and the number of daughter queens produced by colonies. Queen production increased by approximately 30% with every unit increase in sunflower on a $\log_{10}$ scale (electronic supplementary material, table S5f). (*b*) The relationship between colony peak weight and colony queen production. Shaded areas indicate 95% confidence intervals.

the largest plantings (figure 1*c*). Prevalence declined with overall floral density, but only during the third site visit using visually assessed *Crithidia* counts (table 1a; electronic supplementary material, figure S4).

Infection intensity also declined with increasing sunflower at a site, regardless of site visit. This result was consistent for both visual ($\beta = -0.232$, $CI_{95\%:}$ −0.365, −0.101, table 1a, figure 1*b*) and molecular methods ($\beta = -0.264$, $CI_{95\%:}$ −0.445, −0.082, table 1b, figure 1*d*), with an order-of-magnitude difference in predicted infection intensity between sites with no sunflower and sites with the greatest abundances (electronic supplementary material, figure S5). In addition, visually assessed infection intensity was positively associated with overall floral density (table 1a; electronic supplementary material, figure S6).

(ii) *Apicystis* and Black Queen cell virus

These pathogens were assessed with molecular methods only. Sunflower abundance and other floral resource metrics were not significantly related to *Apicystis* prevalence (electronic supplementary material, table S4c). Sunflower abundance was not related to the prevalence of BQCV (electronic supplementary material, table S4c). Increasing non-*Helianthus* Asteraceae abundance was associated with lower BQCV prevalence (table 1c; electronic supplementary material, figure S7). Two of the most abundant non-*Helianthus* Asteraceae genera were goldenrod (*Solidago* spp.) and joe-pye weeds (*Eutrochium* sp.), which occurred in fallow fields and field margins. In separate models, we found no relationship between BQCV prevalence and the abundances of these genera tested separately or together ($\chi^2 \leq 0.512$, $p \geq 0.090$ for all).

We note that overall floral diversity was not a significant predictor of prevalence or intensity of infections for any of the pathogens evaluated.

(c) Colony performance in relation to sunflower and other floral metrics

Queen production increased approximately 30% with every order-of-magnitude increase in sunflower abundance ($\beta = 0.301$, $CI_{95\%}$: 0.118, 0.486, $\chi^2 = 10.067$, d.f. = 1, $p = 0.002$; figure 2*a*) and was strongly associated with colony peak weight ($\chi^2 = 64.916$, d.f. = 1, $p < 0.0001$; figure 2*b*). Queen production was not influenced by the abundance of non-*Helianthus* Asteraceae or overall floral density and diversity ($\chi^2 < 0.611$, d.f. = 1, $p > 0.434$ for all). Excluding colonies from sites with no sunflower, which generated very few queens, did not affect results. Queen production was negatively correlated with average *Crithidia* colony infection intensity (assessed visually) over the season ($r = -0.39$, $p = 0.012$). Colony peak weight was not associated with any floral resource predictor ($\chi^2 < 0.31$, d.f. = 1, $p > 0.5$ for all). Peak weight tended to increase with initial weight, though this was not a significant effect ($\chi^2 = 3.097$, d.f. = 1, $p = 0.078$), and increased with deployment date ($\chi^2 = 7.732$, d.f. = 1, $p = 0.005$).

4. Discussion

Plant traits and community composition likely regulate disease dynamics of bees and other pollinators [30,81], but the role of particular host plants influencing the health and success of bee populations is largely unknown. Our study demonstrates that a single plant species can mitigate field-level pathogen loads in *B. impatiens*, the most abundant bumblebee species in eastern North America. Consistent with our original hypothesis, *C. bombi* infections tended to be less frequent and were less intense when sunflower was abundant, dropping an order of magnitude when plantings had at least approximately 10 000 heads (= approximately 0.5–0.75 acres). To the best of our knowledge, only one other study has linked the natural occurrence of a particular plant species to reduced pathogen loads; the prevalence of *A. bombi* was reduced in wild-caught bumblebee workers (*Bombus pascuorum*) at sites with the invasive plant *Impatiens glandulifera* [31]. That the medicinal effect of sunflower observed in a laboratory setting [32–34,82] is realized in the field demonstrates the potential for this native and widespread crop to mitigate a common pathogen in a dominant pollinator.

Contrary to our initial hypothesis, sunflower abundance was positively associated with queen production. Sunflower pollen is low in protein and therefore considered a poor source of bee nutrition [43,49], and we hypothesized that consuming it could come with a performance cost. Daughter queens were initially noticed in colonies about 40 days after

field placement; the larval feeding stage accounts for the first 20 days of development [65]. Thus, on farms with abundant sunflower, colonies experienced peak sunflower bloom and reduced pathogen loads during a critical and energy-intensive point in colony development. Previous work indicates that *Crithidia* infection reduces queen production in bumblebees [54,55] and can impair learning in workers [53], reduce pollen collection [83] and decrease foraging on some host plants [15]. Peak colony weight was not affected by sunflower abundance or other metrics of floral resource availability. Colonies were mature when placed in the field (as evidenced by the rapid switch to reproduction), and their earlier resource 'environment' in the laboratory likely determined ultimate growth [84]. Overall, it appears that sunflowers as a medicinal resource outweighed costs of potentially low-quality pollen.

It is important to emphasize that our findings support inclusion of sunflower as one component of a diverse flowering landscape. Even our largest cover crop planting (1.5–2 acres) was only a small fraction of the area typically planted by larger agricultural operations in other parts of the USA, and nearly all plantings were situated within diversified vegetable farms with flowering tree lines and/or fallow habitat. Because sunflower is lacking in essential nutrients for bee development [43], benefits from this crop may diminish in a low diversity or monoculture setting. Additional study is required to identify the conditions that constrain sunflower's advantages for bumblebee health.

It is also important to acknowledge that we demonstrate the influence of sunflower on disease in a single bumblebee species, *B. impatiens*, which has a stable and expanding population [38]. We need to understand whether sunflower may have similar effects on pathogen loads in other bee species, particularly those experiencing population declines [85]. We recently found that consuming sunflower pollen had weaker effects on *C. bombi* infection in *Bombus vagans* and *Bombus bimaculatus* and no detectable effect in *Bombus griseocollis* [64], suggesting that benefits vary between bee species. That said, decreasing pathogen loads in *B. impatiens* might positively influence disease dynamics in congeners. *Bombus impatiens* workers have a wide diet breadth and may therefore play a strong role in spreading disease to other bee species through resource sharing [18]. *Crithidia* survives between years in infected queens that hibernate with the pathogen and spread it in the spring. Reducing *Crithidia* prevalence in *B. impatiens* colonies and new queens is thus potentially important for mitigating transmission between years. Although we did not measure *Crithidia* levels in daughter queens produced by our colonies, previous laboratory work indicates that sunflower pollen reduced *Crithidia* infection in *B. impatiens* queens [82].

Other plants in the Asteraceae family may have medicinal effects, including antiparasitic properties [33,48,86]. We hypothesized that total Asteraceae abundance might drive disease levels but found no evidence that non-sunflower Asteraceae reduced *Crithidia* loads. While other Asteraceae may produce rewards with medicinal properties, it is possible that their effect is countered by their influence on disease transmission. For example, the pollen of goldenrod (*Solidago* sp.), which was common across our sites, reduced *Crithidia* in laboratory trials [33], but goldenrod was also identified as a 'high transmission' plant that more easily passes *Crithidia* to foraging bumblebees [22], perhaps outweighing its medicinal effects. Exploration of these interactive dynamics in individual plant species and in relation to plant functional traits is critical to understand mechanisms of disease transmission in plant–pollinator networks.

Surprisingly, non-sunflower Asteraceae abundance was associated with reduced prevalence of BQCV, a virulent honeybee pathogen that kills developing honeybee queens and can be found in bumblebees, sometimes at high rates (e.g. [60]). Owing to little previous work and inconsistent results regarding the relationship between Asteraceae pollen and viruses [36,37], we had no *a priori* hypothesis about the direction of this relationship. Although more work is required to verify and identify the possible mechanism(s) involved in this effect, our finding indicates the potential for other Asteraceae species to mitigate a harmful and widespread bee pathogen. Direct fitness effects of the virus on bumblebees and other wild species are not well understood [87]; at a minimum, bumblebees may serve as reservoirs for the virus and transmit it to susceptible hosts. Managing landscapes to reduce its transmission is an important consideration for future research.

Understanding how the presence of particular plant species, and host plant composition generally, influences disease and other aspects of bee population health is important for habitat management and restoration efforts. Installation of wildflower strips on farmland is a common conservation practice; even though wildflower plantings promote bee diversity and pollination services to adjacent crops (e.g. [88,89]), they may also serve as hubs for disease transmission. Bumblebees had higher pathogen prevalence in low-resource landscapes [90] and wildflower strips increased disease prevalence when placed within low-quality landscapes [91]. Incorporation of medicinal and low-transmission plants into seed mixes may be a way to combat this issue. Our work indicates that sunflower, a widely distributed species native to North and Central America, is a strong candidate for incorporation into pollinator habitat for this purpose. We encourage further exploration of plant species that may have a similar impact on pollinator diseases.

Data accessibility. All data and R scripts used to generate analyses for this manuscript, along with ReadMe files, are publicly available at Scholarworks @UMass Amherst, a digital repository: https://doi.org/10.7275/x9pj-g047 [92].

Data are also provided in the electronic supplementary material [93].

Authors' contributions. R.L.M.: data curation, formal analysis, investigation, methodology, project administration, supervision, writing—original draft, writing—review and editing; Q.S.M.: data curation, investigation, methodology, supervision, writing—review and editing; G.L.: investigation, writing—review and editing; R.E.I.: conceptualization, funding acquisition, writing—review and editing; L.S.A.: conceptualization, data curation, funding acquisition, project administration, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed herein.

Conflict of interest declaration. We declare we have no competing interests.

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