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RESEARCH ARTICLE

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# Flowers as dirty doorknobs: Deformed wing virus transmitted between *Apis mellifera* and *Bombus impatiens* through shared flowers

**Phillip Alexander Burnham**[1,2] | **Samantha A. Alger**[3,4] | **Brendan Case**[2,5] | **Humberto Boncristiani**[6] | **Laurent Hébert-Dufresne**[2,5] | **Alison K. Brody**[1]

[1]Department of Biology, University of Vermont, Burlington, VT, USA

[2]Vermont Complex Systems Center, University of Vermont, Burlington, VT, USA

[3]Plant and Soil Science, University of Vermont, Burlington, VT, USA

[4]Vanasse Hangen Brustlin, Inc, South Burlington, VT, USA

[5]Computer Science Department, University of Vermont, Burlington, VT, USA

[6]Honeybee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, FL, USA

**Correspondence**
Phillip Alexander Burnham
Email: pburnham@uvm.edu

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## Abstract

1. In light of bee declines, the importance of pollination services from managed and native bees to our agriculture and economy is of great political, scientific and public interest. Viruses, first observed in honeybees, have been documented in bumblebees and the prevalence and load of some RNA viruses have been associated with managed honeybees. Shared flowers may be the bridge across which viruses pass between bees but no study has yet demonstrated that bumblebees can pick up viruses while foraging on contaminated flowers.
2. Here, through a series of mechanistic laboratory experiments and mathematical modelling, we ask whether viruses can be transmitted between bee genera on shared flowers and how transmission can be effectively mitigated.
3. We demonstrated that deformed wing virus (DWV) can be transmitted from infected honeybees to bumblebees through the use of shared red clover. We were also able to show that the route may work in reverse and bumblebees could contribute to the spread as well.
4. Our model showed that reducing vector-mediated transmission in honeybee colonies could potentially lead to a far greater reduction in bumblebee infection than simply reducing the number of honeybees. Additionally, we identified a dilution effect, whereby increasing floral abundance reduced transmission.
5. *Synthesis and applications*. In this study, we showed that DWV may be spread between bee genera through the shared use of flowers. Through mathematical simulation, we identified two practical management options for reducing spread. The combination of treating honeybees effectively for the *Varroa* mite, a known vector of DWV, and increasing floral abundance where honeybees and native pollinators share the landscape were shown to reduce the spread of DWV within bee communities in simulations.

KEYWORDS

bumblebees, deformed wing virus, disease ecology, disease transmission, honeybees health, mathematical disease model, RNA virus, virus spillover

  

## 1 | INTRODUCTION

The decline of important pollinators has garnered much attention (Goulson et al., 2015). However, much of the focus has been on the health of managed honeybees *Apis mellifera* (van Engelsdorp et al., 2008). Although native bumblebees are often better pollinators of wild plants and food crops, they are understudied compared to honeybees. Additionally, bumblebees face many of the same threats facing honeybees. Many previously common species are currently in decline (Cameron et al., 2011; Colla et al., 2012; Colla & Packer, 2008) and several are state or federally listed. The decline of these species may drastically disrupt pollination services, natural communities and human industries that rely upon them (Aizen et al., 2009; Koh et al., 2016).

Although many factors including habitat loss, pesticides and global change contribute to bumblebee losses (Cameron & Sadd, 2020; Crone & Williams, 2016; Ogilvie et al., 2017; Soroye et al., 2020; Whitehorn et al., 2012), pathogens and parasites are among the top threats (Goulson et al., 2008; Imhoof & Schmid-Hempel, 1999; Kissinger et al., 2011; Otterstatter et al., 2005). For example, the RNA virus, deformed wing virus (DWV), has been associated with increased mortality in bumblebees (Fürst et al., 2014; Graystock et al., 2016) as well as the presence of deformed wings as seen previously in honeybees (Genersch et al., 2006). These understudied RNA viruses may spillover from honeybees, increasing the risk to managed and wild bumblebees (Alger, Burnham, Boncristiani, et al., 2019; Alger, Burnham & Brody, 2019; Fürst et al., 2014; Manley et al., 2019; McMahon et al., 2015). When honeybees are present, bumblebees are infected with deformed wing virus (DWV) more often (Pritchard et al., 2021) and in higher loads than when honeybees are not present (Alger, Burnham, Boncristiani, et al., 2019). Although DWV is vectored by *Varroa destructor* within and among honeybee colonies, bumblebees are not known hosts of this parasite. Thus, it was hypothesized by us and others that these viruses spread between species on shared flowers (Dalmon et al., 2021; Fürst et al., 2014; Grozinger & Flenniken, 2019).

In 2016, we demonstrated that honeybees are able to deposit RNA viruses, including DWV, on the flowers that they visit (Alger, Burnham, & Brody, 2019). Flowers facilitating the spread of pathogens between and within bee species have been observed in the literature (Adler et al., 2018; Truitt et al., 2019); however, no study has yet determined how RNA viruses are spread to bumblebee species or demonstrated that bumblebees can pick up viruses from flowers that have been visited by infected honeybees. We are only now scratching the surface of how the floral environment contributes to disease dynamics in this system. Although honeybees are known to be infected with RNA viruses from hosting *Varroa* mite vectors (Posada-Florez et al., 2019), and can deposit viruses on flowers while foraging (Alger, Burnham, & Brody, 2019), a detailed understanding of how these viruses move through bee communities requires a joint experimental and computational approach. Demonstrating a hypothesized transmission route in any disease system allows us to confirm how a pathogen moves within and between species. This, in turn, allows stakeholders and scientists to join forces to examine methods for mitigating transmission and spread.

In addition to their agricultural value, bees provide a valuable opportunity to study broad epidemiological concepts, making them an ideal candidate organism for developing more broadly applicable epidemiological models that examine transmission dynamics. More specifically, the honeybee–bumblebee virus transmission system is readily manipulated and measured, has analogues for vector and fomite-mediated transmission, and provides us a unique opportunity to examine how spillover events between species can occur in a real-world setting. Capitalizing on previous field work, we were able to design several mechanistic experiments to answer questions about flower-mediated transmission. Results from our surveys and experiments informed our modelling effort and gave us more insight into the potential dynamics and consequences thereof in this system. We therefore argue that leveraging the complementary benefits of field biology, laboratory experimentation and mathematical modelling is of the upmost importance in studying emerging infectious disease and promotes interdisciplinary collaboration.

In this study, we use this combined approach to examine the role of shared flowers on spillover of DWV from honeybees to bumblebees. We ask whether infected honeybees can deposit DWV on flowers and if so, can uninfected bumblebees pick up the virus from the flower. Additionally, we computationally examine the relative roles of flowers, honeybees and bumblebees in this system to look for potential ways to mitigate disease spread.

## 2 | MATERIALS AND METHODS

To examine whether DWV could be picked up by bumblebees on contaminated flowers and explore the mechanisms behind transmission, we designed three sets of experiments. In the first set, we examine transmission through flowers directly via honeybee and hand inoculation of flowers. In the second set, we examined virus pick up as a function of foraging time and dosage acquired. In the third experiment, we examined whether bumblebees could deposit viruses on flowers as well. Parameters from these studies and others were used to inform a mathematical model of the system to make recommendations for mitigation strategies.

### 2.1 | Experimental design

To ensure colonies started clean of viruses, 15 individuals from each of four commercial bumblebee colonies (*B. impatiens*) were tested for DWV using RT-qPCR and were found to be negative. Colonies were fed 30% sucrose solution and gamma-irradiated pollen to ensure no active DWV particles were introduced during the course of the study. All experimental and source colonies in this study were maintained in a controlled growth chamber at a constant temperature (26°C) and relative humidity (52%–55%). Infected honeybee

colonies were identified using RT-qPCR and microcolonies were created from these host colonies and kept active in a separate growth chamber using the same temperature and relative humidity. The virus inoculate used in this experiment was purified and the concentration of the stock solution and subsequent dilutions was confirmed through RT-qPCR.

To examine the full transmission route mechanistically, 12 microcolonies containing 15 workers were made from the four commercial bumblebee colonies and assigned randomly to four groups (3 colonies/group). Micro-colonies were pollen starved for 3 days and were transferred to $8'' \times 5'' \times 4''$ boxes and exposed to contaminated red clover *Trifolium pretense*. In the random flowers control group (RF), three colonies were exposed to a new set of three haphazardly selected red clover inflorescences from the field with no honeybee or hand inoculation for each of 3 days. In the hand-inoculated experiment (HI), three colonies were exposed to three sets of red clover inflorescences each inoculated with a field-realistic dose (Alger, Burnham, Boncristiani, et al., 2019) of 1 million genome copies spread in 100,000 increments between 10 haphazardly selected florets. A new set of red clover was presented on each of 3 days. In the honeybee inoculation experiment (HBI), flowers were inoculated by being placed in the infected honeybee micro-colonies for 3 days before being presented to bumblebee colonies. To control for potential viral contamination, handling control groups were presented with sets of three artificial flowers with a sham inoculation of pure 30% sucrose throughout the course of the experiment and assayed for DWV using RT-qPCR along with the other treatment groups. At the end of the experiment, virus loads on bees and flowers were determined using RT-qPCR with absolute quantification.

In addition to this examination of the route using real flowers, a series of experiments were conducted with artificial flowers to examine other aspects of virus pickup (Figure S2). To determine the number of viral particles that can be acquired as a function of foraging time, 31 bees were allowed to forage on artificial flowers that were inoculated on their cotton nectaries with $10^6$ genome copies of DWV. Bees were allowed to forage between 1 and 120 s on their inoculated flower. Foraging time was classified as the interval of time between when the bee began actively feeding from the artificial nectary and when the bee retracted its proboscis and stopped feeding. A handling control of 14 bees foraged on sterile sucrose-inoculated flowers. For all artificial flowers, cotton nectaries were extracted and DWV determined using RT-qPCR. Prevalence and load were analysed as a function of foraging time in a regression design. To create a dose curve and examine the potential for replication, the amount of virus required to retain high levels of virus after pickup was determined by inoculating 50 bees with 1, 3, 5 and 10 million genome copies of DWV. We pollen starved the bees and fed them only 30% sucrose. We tested the bees using RT-qPCR 72 hr later to ensure non-active virus particles in the pollen or sitting inactive in the gut were passed. To ensure no particles on the surface of the bee were detected, we conducted an exterior rinse (see Section 2.2). To determine whether the route might work in reverse (bumblebees to flowers), we allowed 12 orally inoculated bees (3 million genome copies) to forage on clean artificial flowers 72 hr after inoculation for 10 s each. Viral loads in the bees and on the floral nectaries were measured with RT-qPCR.

## 2.2 | Molecular viral assays

All samples were stored at −80°C and kept on liquid nitrogen in the laboratory. RNA extractions were conducted using Qiagen RNA mini kits. Prior to extraction, samples were rinsed with a sterile guanadine thiosyanate-based buffer for 30 s (buffer GITC) and then rinsed for 30 s with 70% ethanol to ensure no viral particles were on the exterior of the bee. The concentration of extracted RNA was quantified using photospectrometry and diluted to a constant of 20 ng/μl. All viral assays were conducted using RT-qPCR. Vetted primer sets for both DWV and the housekeeping gene, Actin, were used (Table S1). Standard curves were created for both targets (DWV and Actin) by creating tenfold serial dilutions using G-blocks (synthetic DNA) to quantify samples. Actin levels were used to normalize DWV loads across individuals and across qPCR runs. PCR product for several positive samples using these primers was purified and sequenced (Sanger sequencing) to ensure the amplicon was DWV. For all RT-qPCR protocols, the MIQE Guidelines were consulted and followed (Bustin et al., 2009).

## 2.3 | Data processing and analysis

Data were cleaned, processed and analysed using R statistical programming language (R Core Team, 2019). Viral load data for the DWV pickup (viral load by time) and reverse transmission (flower load by bumblebee load) experiments were analysed using linear models (LM). Estimates of virus acquisition were made by averaging successes and failures. In addition, 95% confidence intervals were calculated from the probability density function (PDF) of the appropriate $\beta$ distribution based on the number of successes and failures for each case. Models for dose curve data were built using an LM for virus load and a generalized linear model (GLM) fit to a binomial distribution with a link logit function for prevalence data. Significance for all models was determined by calculating the Type-II analysis of variance with the 'ANOVA' function in the 'car' package (Fox & Weisberg, 2019).

## 2.4 | Model details

To begin capturing the possible disease dynamics between bee populations through flowers, we designed a set of differential equations to study theoretical transmission dynamics within a honeybee population coupled with spillover of infection to a bumblebee population through shared foraging of flowers (Equations 1–3). Using a combination of our experimental results and existing literature (Table 1), we were able to fit our model to replicate the observed prevalence

| Symbol | Value | Units | Original source |
|---|---|---|---|
| $N_B$ | 145 | Num./100 m | Alger, Burnham, Boncristiani, et al. (2019), Alger, Burnham and Brody (2019) and Mandelik et al. (2012) |
| $N_H$ | 140 | Num./100 m | Alger, Burnham, Boncristiani, et al. (2019), Alger, Burnham and Brody (2019) and Mandelik et al. (2012) |
| $N_F$ | 2e6 | Num./100 m | Truitt et al. (2019) |
| $\alpha$ | 0.75 | Prob. | Transmission experiments |
| $\delta$ | 0.05 | Prop./day | Truitt et al. (2019) and references therein |
| $\xi$ | 0.1 | Prop./day | Primack (1985) |
| $\gamma$ | 4.0 | Num./day | Model fitting |
| $\beta$ | 0.8 | Prob. | Model fitting |

**TABLE 1** Parameter settings used to fit our model to observations from Alger, Burnham, Boncristiani, et al. (2019). 'Num./100 m' refers to the number of individuals in a circle of radius 100 m, the typical foraging radius of many wild bees. Fixed parameters were based on previous literature, relative to our chosen system size and time. $N_B$, $N_H$ and $N_F$ refer to the numbers of bumblebees, honeybees and flowers in the system, respectively. $\gamma$ is the probability of viral acquisition or deposition between bees and flowers. $\alpha$ and $\beta$ are the probabilities of viral replication and within-apiary transmission, respectively. $\delta$ and $\xi$ represent the bee death rates and the floral senescence rate, respectively

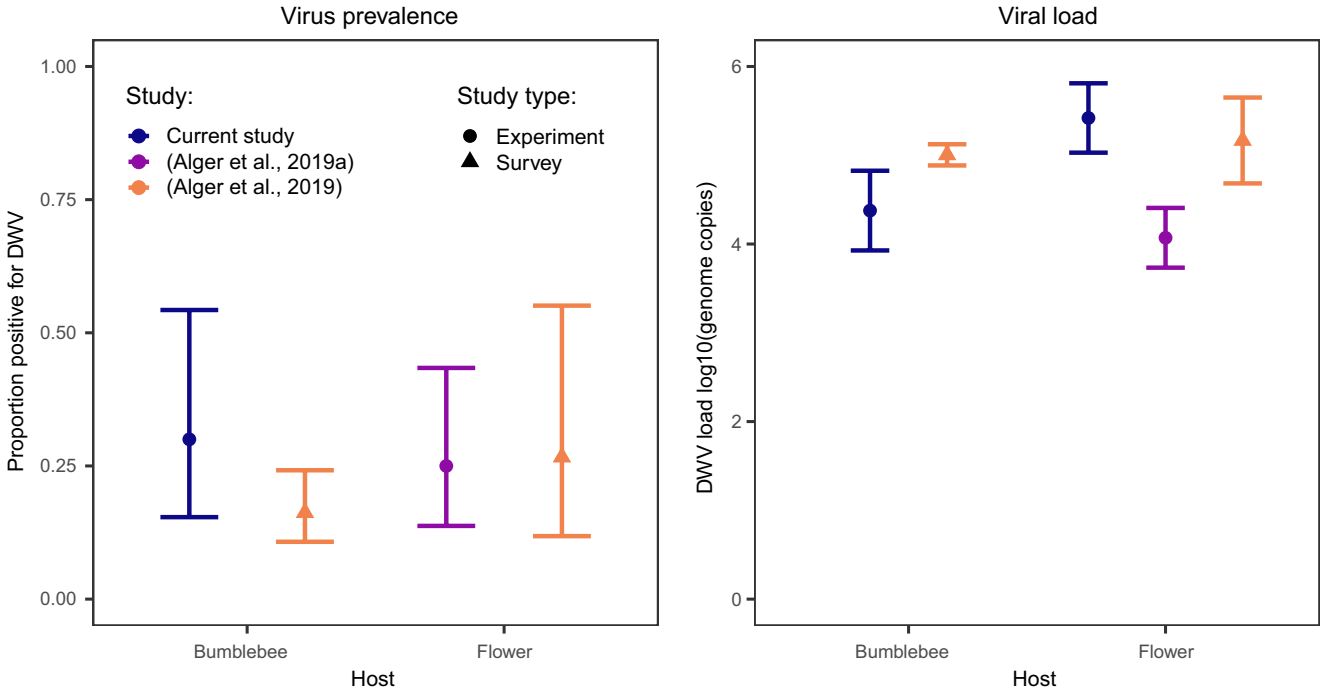


**FIGURE 1** The proportion of flowers and bumblebees positive for DWV as well as the respective viral loads from our previous field studies and experiments and values from this study. Other studies have measured viral load in bumblebees and found similar values as well (Fürst et al., 2014; Manley et al., 2019). The points denote the prevalence for the left panel and the mean viral load for the right panel. Triangles and circles indicate survey and experimental results, respectively. Error bars are derived from the associated beta distribution based on the number of successes and failures for prevalence data and standard error is shown for viral load

of infection in honeybees and wild bumblebees in the area surrounding Vermont apiaries (Alger, Burnham, Boncristiani, et al., 2019; Figure 1).

This deterministic compartmental model was constructed by assigning organisms to groups, or compartments, with the assumption that all organisms in a compartment are homogeneous (Keeling et al., 2008). We divided organisms based on their infection status (either *susceptible* or *infected*), and whether they were a bumblebee, honeybee or flower. This allowed us to easily model the number of infected bumblebees, infected honeybees and flowers that carry the virus, which we, respectively, denote as *B*, *H* and *F*. Since the model is intended to predict dynamics over a short time period (i.e. no more than a single reproductive season), we further assume the total number of bumblebees, honeybees and flowers are given by constants $N_B$, $N_H$ and $N_F$. Therefore, the number of susceptible bumblebees, for example, can be expressed as $N_B - B$ so that changes in susceptible compartments are implicitly defined by their corresponding change in infected compartments (e.g. $d[N_B - B]/dt = -dB/dT$). This allows

us to sufficiently describe a full epidemiological model tracking only infected compartments. Let

$$\frac{dB}{dt} = \alpha\gamma\frac{F}{N_F}\left(N_B - B\right) - \delta B, \quad (1)$$

$$\frac{dH}{dt} = \alpha\gamma\frac{F}{N_F}\left(N_H - H\right) + \beta H\left(N_H - H\right) - \delta H, \quad (2)$$

$$\frac{dF}{dt} = \gamma\left(\frac{N_F - F}{N_F}\right)(B + H) - \xi F, \quad (3)$$

where $\gamma$ is the rate of viral acquisition/deposition between bees and flowers, $\alpha$ is the probability that the virus replicates in an exposed bee, $\delta$ is the death rate for bees, $\beta$ is the *Varroa*-mediated between honeybee transmission rate (hereafter referred to as 'within-apiary transmission') and $\xi$ is the rate at which a virus is cleared from a flower based upon the average floral longevity of flowers in temperate climates (1–14 days; Primack, 1985). Our model has a single possible steady state, which we denote ($B^*$, $H^*$, $F^*$). We obtained approximations of these values for a particular combination of parameters using numerical integration with the deSolve package (Soetaert et al., 2010) in R (R Core Team, 2019).

For parameter selection for our model, we used the parameters calculated in Truitt et al. (2019) where applicable. Floral and bee abundance are determined relative to a system area of a circle with radius 100 m. While bumblebees can forage up to 2 km from their colony (Osborne et al., 2008) and honeybees even farther at 15 km (Beekman & Ratnieks, 2000), studies have shown that DWV-infected bumblebees are higher in prevalence when caught near honeybee colonies (Alger, Burnham, Boncristiani, et al., 2019; Fürst et al., 2014; McMahon et al., 2015) and contaminated flowers have only been documented at apiary sites (Alger, Burnham, Boncristiani, et al., 2019). As such, we elected to model this system within the area contained in a 100 m radius, which is consistent with the area that flower samples were collected in near apiaries in Alger, Burnham, Boncristiani, et al. (2019).

In Mandelik et al. (2012), it was found 60–120 foraging bees per hectare is typical during foraging seasons. Scaling by the average proportion of honey and bumblebees captured in (Alger, Burnham, Boncristiani, et al., 2019) and to our system radius of 100 m, we determined $N_B = 145$ and $N_H = 140$. For $\alpha$, the probability an exposed bee becomes infected, we took the viral prevalence in exposed bumblebees from our direct inoculation experiment (pooled across all initial dose levels). Finally, since we did not have sources to motivate a choice of parameters $\gamma$ and $\beta$, we compared the model's steady state under possible parameter settings to pooled field data from Alger (2017) and Alger, Burnham, Boncristiani, et al. (2019). Namely, we created a grid of pairs $\gamma \in [0, 100]$ and $\beta \in [0, 1]$, and calculated the mean squared error between $B^*/N_B$ and $H^*/N_H$ and the average infection prevalence in bumblebees in the presence of apiaries (16.4%), average prevalence in honeybees (100%) in areas near apiaries and average prevalence in bumblebees when no honeybees were present (0%). The model fit of these three situations was best within the region $3.5 \leq \gamma \leq 4.5$ and $\beta \geq 0.8$; therefore, we chose $\gamma = 4$ and $\beta = 0.8$ for all further simulations.

## 3 | RESULTS

Through laboratory experiments, we were able to demonstrate, for the first time, that bumblebees can pick up DWV from flowers visited by infected honeybees. We then built a mathematical model of this transmission route which suggests that the route is sufficient to describe DWV levels we see in the field. Additionally, we found evidence to support increasing flower abundance and treating honeybees for their ectoparasite vector, *Varroa*, are both good strategies for significantly reducing transmission to the bee community.

### 3.1 | Experimental findings

To examine whether DWV could be picked up by bumblebees on contaminated flowers, we examined transmission directly via honeybee- and hand-inoculated flowers. In both hand-inoculated (HI) and honeybee-inoculated (HBI) treatments, 30% of bumblebees were found to be positive for DWV (95% CI: 12.2%, 65.2%). The $\log_{10}$ average loads (±*SE*) for positive individuals for HI and HBI were 5.07 ± 0.69 and 3.68 ± 0.23 respectively (Figure 2). In the random flowers control (RF), only one flower set out of 7 used was found to be contaminated with a small number of DWV copies ($\log_{10}$ load of 3.27). None of the bumblebees that foraged in the random flower control group picked up the virus. All handling control bees were uninfected. The average DWV loads on flowers used in the HI and HBI treatments were $10^{5.3}$ and $10^{5.4}$, respectively.

In the second set of experiments, we tested how DWV is picked up by bumblebees as a function of foraging time and constructed a dose curve. In the treatment group, there was a positive relationship between bumblebee foraging time and the amount of virus they picked up. Bees foraging for longer times picked up higher viral loads ($F_{1,29} = 8.96$, $p = 0.0056$, $R^2 = 0.21$). All controls were negative for DWV (Figure 3). The transfer of viral particles to flowers and pickup by foragers is evidence that flowers can facilitate transmission. However, to establish whether DWV remains detectable in the bee after transmission, we conducted an oral inoculation experiment. We orally inoculated bumblebees with 1, 3, 5 and 10 million genome copy doses. We found that as inoculation dose increased, both detected viral prevalence and load increased. When dosed with 1 million genome copies of isolated DWV, 40% of individuals tested positive for DWV (95% CI: 18.7%, 73.8%). The mean $\log_{10}$ viral loads (±*SE*) for positive individuals in this group was 4.33 ± 0.49. Levels increased in value with administered dosage for both prevalence ($\chi^2_1 = 7.58$, $p = 0.006$) and load ($F_{1,37} = 11.93$, $p = 0.001$; Figure 4).

In the third experiment, to examine whether infected bumblebees are able to deposit viruses on flowers, we conducted an experiment where infected bumblebees were allowed to forage on clean artificial

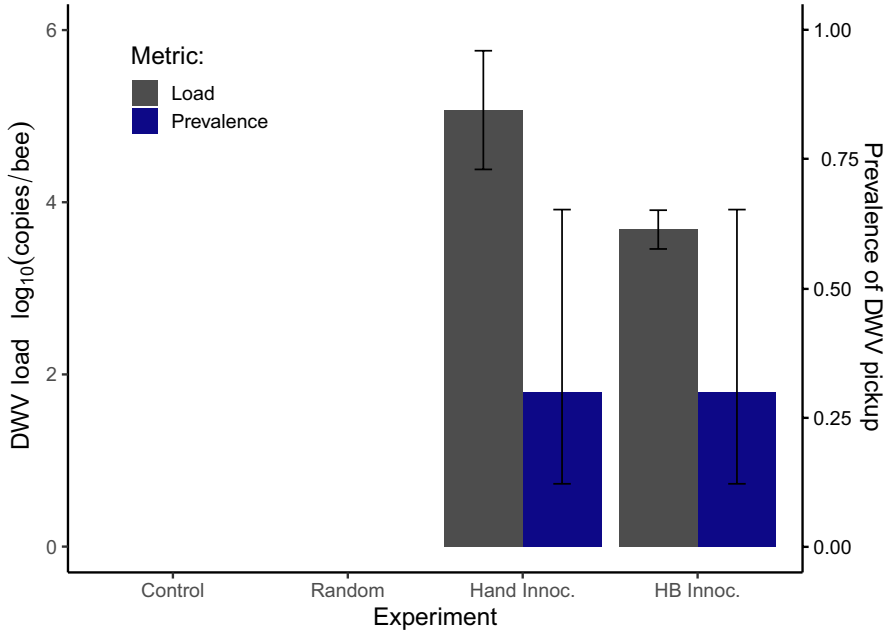


**FIGURE 2** The viral load on the left *y*-axis and prevalence on the right *y*-axis for bees that have been exposed to DWV on flowers. Control bees foraged on sterile sucrose solution on artificial flowers, 'Random' bees foraged on red clover haphazardly selected from the field, 'Hand innoc.' bees foraged on red clover that had been hand inoculated with a field-realistic dose of DWV (Alger, Burnham, Boncristiani, et al., 2019) and 'HB innoc.' bees foraged on red clover that had been exposed to infected honeybees. Error bars for prevalence represent the 95% confidence interval derived from the associated $\beta$ distribution's probability density function. Error bars for load represent standard errors

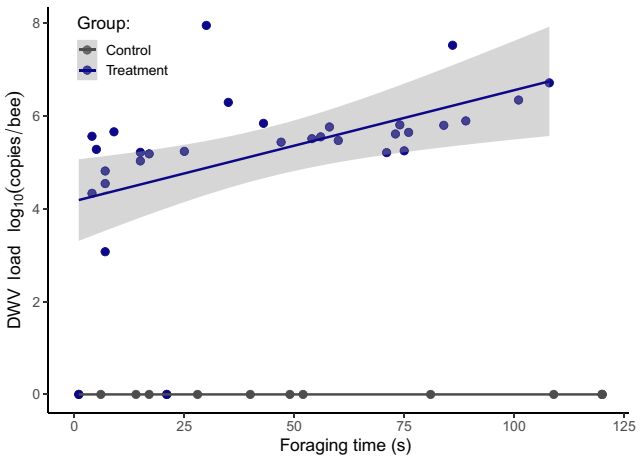


**FIGURE 3** The amount of virus acquired by a foraging bee as a function of foraging time. Blue dots represent individuals that foraged on inoculated artificial flowers while grey dots are control bees that foraged on sterile artificial flowers. Lines represent the line of best fit with shaded standard error. No bees that foraged on the control flowers were infected

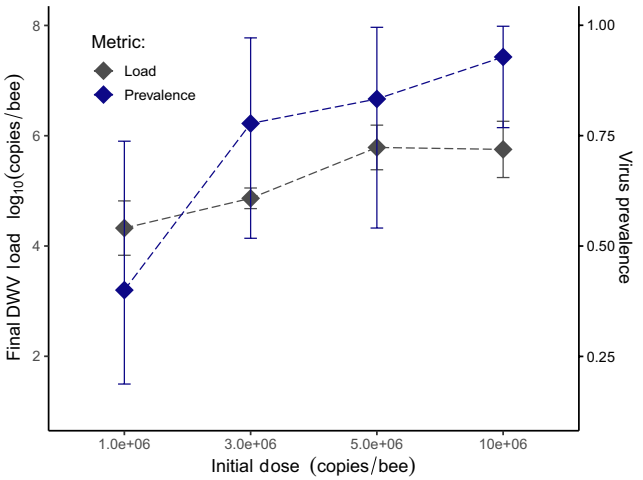


**FIGURE 4** The viral load on the left *y*-axis and prevalence on the right *y*-axis 3 days after being inoculated with a variable dosage of deformed wing virus (1, 3, 5 and 10 million genome copies). Load bars represent the 95% confidence interval derived from the associated $\beta$ distribution's probability density function. Prevalence bars represent standard errors

flowers. We found that infected bumblebees could deposit viruses when foraging on clean artificial flowers. One hundred percent of infected bumblebees deposited some level of DWV on their surface. However, we did not find a significant relationship between bumblebee viral load and load deposited on the flower ($F_{1,9} = 0.57$, $p = 0.47$; Figure S1).

## 3.2 | Model findings

We tested our model behaviour under a number of conditions to make predictions about the efficacy of possible control schemes by varying the number of foraging honeybees, the rate of within-apiary transmission and the density of flowers in the surrounding area.

Figure 5 (left panel) shows the steady state of the proportion of infected bumblebees under various values of $N_H$, the number of honeybees in the system, and $\beta$, the within-apiary transmission rate. A reduction in $\beta$ can drastically reduce bumblebee infection and leads to eradication when lowered sufficiently while a reduction in $N_H$ has a more consistent impact but only leads to virus eradication at extremely low number of honeybees. As seen in the right panel of Figure 5, a more interesting relationship is found between $\beta$ and $N_F$, the number of flowers in the area. Lowering $\beta$ has little positive

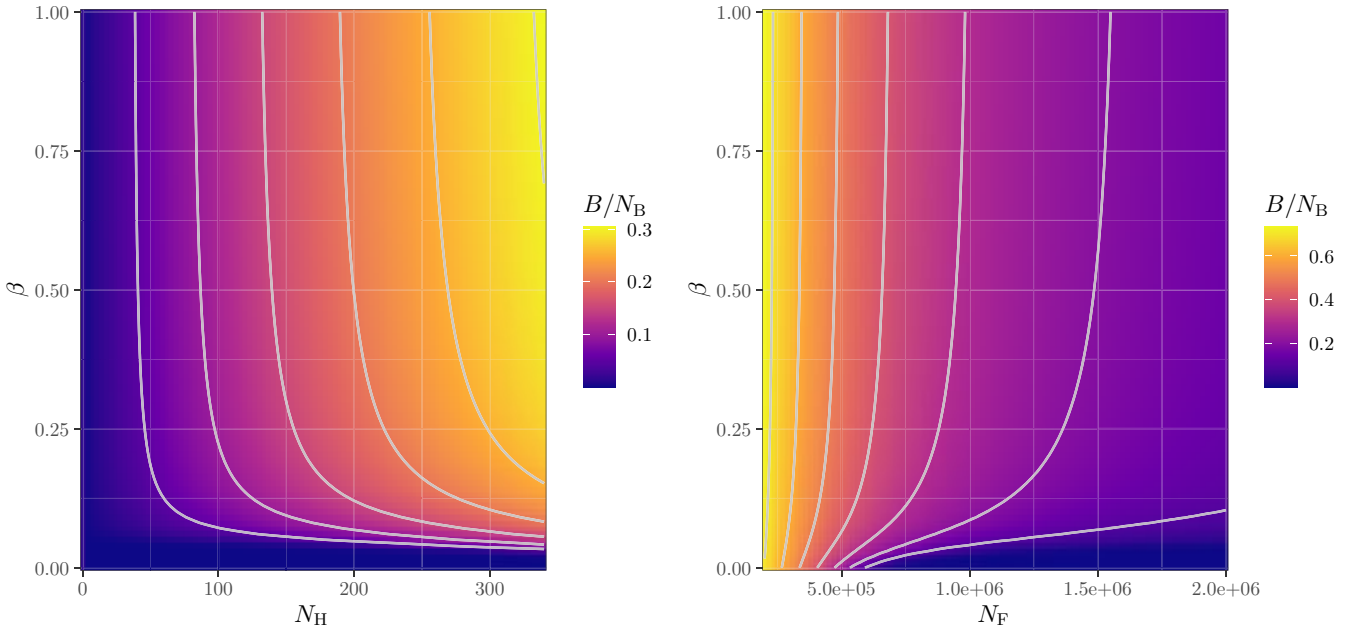


**FIGURE 5** Model simulations under different parameter combinations. Heatmap and contours show the proportion of infected bumblebees after model convergence. On the *y*-axes, we show within-apiary transmission ($\beta$). This is shown as a function of the number of honeybees in system ($N_H$) in the left panel, and number of flowers ($N_F$) in the right panel. Cool colours represent low bumblebee infection, while hotter colours represent higher bumblebee infection

impact for low $N_F$, compared to the reduction caused by increasing $N_F$ alone. Together, lowering $\beta$ while increasing the number of flowers $N_F$ appears to be the most efficient pathway towards eradication of the virus from the bumblebee population.

## 4 | DISCUSSION

Our previous results from field observations and experiments hinted at the role of flowers in virus transmission. The current work now offers a rigorous experimental demonstration of this mechanism in a controlled laboratory setting. We identify foraging by infected honeybees and shared floral resources as two key parameters governing virus pickup in bumblebees. By isolating key aspects of this transmission route, we were able to identify and test individual mechanisms which were not well understood. Here, we demonstrated that bumblebees can pick up DWV from visiting honeybee contaminated flowers. Viruses can be detected in high loads in bumblebees days after exposure and infected bumblebees, in turn, can deposit viruses on flowers. Our finding closes the cycle in this hypothesized transmission route and corroborates our previous findings and that of others (Adler et al., 2018; Alger, Burnham, Boncristiani, et al., 2019; Alger, Burnham, & Brody, 2019; Truitt et al., 2019) that flowers act as fomites in the diseases shared among bee species. Our mechanistic demonstration of these steps along the route is strong evidence supporting the likelihood of its occurring in nature.

We found that foraging time is an important variable. Longer foraging times lead to higher virus acquisition. It should be noted that some bees in this experiment picked up more copies of WV than were thought to have been put on the flower. This is likely due to variance in pipetting and the RT-qPCR process rather than replication as bees were sacrificed immediately after foraging in these trials. It is possible that bees visiting flowers quickly do not pick up sufficient particles to become infected, we found that only 1 million genome copies of DWV was enough for 30% of bees to test positive for DWV 3 days later. We found $10^{5.5}$ copies can be picked up from field-realistically contaminated flowers in as little as 10 s, which is well within realistic foraging times for highly rewarding flowers (Heinrich, 1976). It seems a very probable route given that our previous work found that 30% of flowers near honeybee apiaries had some level of DWV detectable via qPCR and that bumblebees can forage on between 10 and 30 flowers per minute (Heinrich, 1976).

We found that it is not just honeybees that contribute to this route. Ours is the first study to show that orally inoculated bumblebees can deposit DWV on floral surfaces too after 72 hr of consuming only sterile sucrose. Understanding that bumblebees contribute to overall transmission influenced the construction of our model and is important information for future work in this system. More work is needed to quantify the role infected bumblebees add to the system and whether or not the levels of infection we see in nature depend upon bumblebee deposition.

The transmission experiments, along with previous field observations, informed the design of a plant–pollinator virus transmission model, which featured the exchange of DWV between commercial and wild bees through flowers alongside within-apiary transmission primarily via *Varroa* for commercial honeybees. These coupled processes accounted for the large difference in DWV prevalence between honey and bumblebees observed in the field. After fixing

several parameters according to a typical system of foraging wild and commercial bees, our model had two free parameters which, after model fitting, can serve as rough predictions of two quantities which are difficult to measure experimentally. First, our fitting procedure suggests a typical infected bee deposits a meaningful (i.e. $\geq 10^6$ copies) load of DWV on just four flowers per day. This may suggest that while deposition events on flowers near apiaries are common during the thousands of flowers a foraging bee visits each day, depositions of high viral loads ($10^6$ or greater) may be much more rare. Although we do know flowers can harbour high viral loads in the field (Alger, Burnham, Boncristiani, et al., 2019), in our bumblebee deposition experiments the average load deposited was only around 1,000 genome copies (Figure S2). Second, we found support for a very high rate of within-apiary transmission, which is consistent with field observations of higher DWV levels in honeybees (Manley et al., 2019). Together, these two parameters suggest a picture of moderate spillover of DWV between pollinator communities, exacerbated by accelerated disease spread within honeybees through *Varroa*.

Our model shares a number of similarities with another recently proposed model (Truitt et al., 2019), as well as other ODE models for multi-vector diseases (Turner et al., 2013). Most notably, our model includes bee-to-bee transmission in honeybees, a well-documented route of transmission mediated in part by the parasitic *Varroa* mite. While inclusion of this process was effective in explaining the observed difference in DWV prevalence in honeybees and bumblebees, we cannot rule out this heterogeneity is due to other unmeasured factors, such as the differences in foraging patterns or immune response of wild and commercial bees.

Several findings from our model have real-world implications for strategies to mitigate interspecies transmission. The simulation results showed that increasing floral density led to a reduction in transmission, whereby DWV prevalence was reduced in bumblebees due to an overall decreasing concentration of flowers harbouring viral particles. While dilution effects are typically described as decreases in transmission due to increases in host diversity (Keesing et al., 2010), increasing flower density in our model resulted in a similar effect. This suggests that increasing pollinator-friendly plantings in areas that are shared by honeybees and wild pollinators would be beneficial. This is a readily adoptable practice, which will benefit pollinators in the area if planted responsibly.

We also found that sufficient reduction in the rate of within-apiary transmission could be quite beneficial to bumblebees, an attractive possibility since keeping honeybees healthy though managing for *Varroa* will not only benefit beekeepers but also the native bee communities with whom they share the environment. However, this control strategy should be introduced with the understanding that a reduction in bumblebee infection appears only when within-apiary transmission is brought very low.

This improved understanding of how DWV can be passed between bee species presents several areas for future research. Although other RNA bee viruses may have characteristics that differ from DWV, the idea that one RNA virus can be picked up from shared flowers gives strong evidence that others may do the same. Future studies should address additional viruses. As we found in Alger, Burnham, and Brody (2019) that different viruses may be deposited differentially based on floral species, future work should examine how different viruses are shed (oral, faecal, etc.) and how floral morphology influences this transmission. The location of DWV on the flowers is yet unknown as only whole flower extractions have been conducted. Future studies should examine the nectaries and petals of flowers separately for multiple viruses. Our empirical and modelling results, which establish that bee disease spreads through the use of shared flowers, force critical analysis of the pros and cons of providing 'bee friendly' habitat. It also underscores the importance of improving honeybee management practices to mitigate the spread of DWV and other flower-mediated parasites and diseases.

In this study, we mainly examine the likelihood of this route occurring in a mechanistic sense. Thus, results are framed in terms of bees depositing virus and picking them up. However, we did find evidence that orally inoculated bumblebees retained high levels of DWV 3 days after infection and were able to shed viruses on floral surfaces after that period. Though a recent paper found that it was unlikely that orally inoculated bumblebees would develop replicating infections, dismissing the floral transmission route (Susachenko et al., 2020), we disagree with the authors in this conclusion. Though we did not test for replication directly in this study, we and others have found that a significant proportion of DWV-positive bumblebees have active replicating infections (Alger, Burnham, Boncristiani, et al., 2019; Fürst et al., 2014; Manley et al., 2019). Other studies have examined oral inoculation of viruses in bumblebees and found active replication for DWV (Fürst et al., 2014; Tehel et al., 2020) and other RNA viruses (Manley et al., 2017; Meeus et al., 2014). We look forward to additional studies that examine oral inoculation of viruses in bumblebees and the conditions necessary to result in replication.

An integrated approach to studying virus transmission and dynamics in this non-human animal system is vital to maximizing our understanding and minimizing its threat. Informing laboratory experiments from field surveys and realistically parameterizing models via these experiments allows for more interpretative results. Maintaining the feedback loop between observation, experimentation and epidemiological modelling ensures that our evidence is complementary and thus stronger for it. In recent years, the scientific community has embraced the benefits of interdisciplinary collaboration. Studying disease from many angles, and with the insights of all constituents involved is important now more than ever in our changing world. We applaud this approach and urge beekeepers, naturalists and scientists from all backgrounds to come together to ensure we have healthy honeybees and safe wild pollinators that guarantee a functioning agricultural system and healthy ecosystems.

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## AUTHORS' CONTRIBUTIONS

P.A.B. designed and conducted the experiments, processed the samples, analysed the data and contributed to the mean field model; S.A.A. aided in the conceptualization, design and execution of the experiments; H.B. isolated and purified the deformed wing virus; B.C. lead the mean field model design and simulation; L.H.-D. assisted in constructing the model; A.K.B. provided critical feedback on experimental design and edited the manuscript. In addition, all authors reviewed and contributed to the written portion of the manuscript.

## DATA AVAILABILITY STATEMENT

Data available via the Zenodo Digital Repository https://doi.org/10.5281/zenodo.4923152 (Burnham et al., 2021).

## ORCID

*Phillip Alexander Burnham* https://orcid.org/0000-0001-8233-3391
*Samantha A. Alger* https://orcid.org/0000-0001-7787-1312

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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# Flowers as dirty doorknobs: Deformed wing virus transmitted between Apis mellifera and Bombus impatiens through shared flowers

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