Received: 28 July 2022 | Accepted: 15 October 2022

DOI: 10.1111/een.13203

ORIGINAL ARTICLE

Ecological Entomology

Experimental viral spillover can harm *Bombus terrestris* workers under field conditions

Tabea Streicher[1] | Anja Tehel[1] | Simon Tragust[1] | Robert J. Paxton[1,2]

[1]General Zoology, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

[2]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

Correspondence
Tabea Streicher, General Zoology, Institute for Biology, Martin Luther University Halle-Wittenberg, Hoher Weg 8, 06120 Halle (Saale), Germany.
Email: tabea.streicher@zoologie.uni-halle.de

Funding information
German Research Foundation

Associate Editor: Sophie Evison

Abstract

1. Deformed wing virus (DWV), notorious for its virulence in the western honey bee (*Apis mellifera)* when vectored by the ectoparasitic mite *Varroa destructor*, is also widespread among wild bumble bee species, presumably through spillover from honey bees. Experimental studies on the virulence of DWV in *Bombus* spp. have provided equivocal results and have until now been confined to bumble bees under laboratory conditions.
2. Here, we inoculated commercially reared *Bombus terrestris* workers with DWV-A through feeding or injection and introduced them into experimental colonies placed in the field, thus exposing them to the environment and associated stressors. We monitored the survival of inoculated worker bumble bees and quantified their viral load at 10 days post inoculation.
3. *Bombus terrestris* workers injected with DWV-A supported high viral loads and exhibited significantly reduced median survival compared to controls. Bumble bees inoculated by feeding had low or zero detectable viral loads while their mortality did not differ from the control group.
4. Our results demonstrate that, although DWV-A is pathogenic for commercial *B. terrestris*, the risks for individual fitness from spillover of DWV-A during foraging on shared flowers appear limited.
5. The findings of this experiment also highlight the necessity to address the potential context-dependence of virulence when evaluating the impact of a pathogen in an alternative host.

KEYWORDS
Bombus terrestris, context-dependent virulence, deformed wing virus

INTRODUCTION

Pathogen spillover, in which a pathogen is transmitted from a reservoir to an alternative host, is a major cause of disease outbreak in wildlife and domesticated animals (Daszak et al., 2000). In many cases of interspecific spillover, pathogenic agents are RNA viruses (Jones et al., 2008), renowned for their high rates of mutation that may facilitate adaptation to an alternative host (Holmes, 2009).

The positive single stranded RNA virus deformed wing virus (DWV) is notorious for its pathogenicity in the western honey bee (*Apis mellifera*) when vectored by the exotic ectoparasitic mite *Varroa destructor* (reviewed in Grozinger & Flenniken, 2019; Martin & Brettell, 2019).

It is found at high prevalence in populations of *A. mellifera* in the USA (Ryabov et al., 2017; Traynor et al., 2016) and elsewhere across the world (Paxton et al., 2022), and is one of the most ubiquitous viruses among communities of wild bee species (Jones et al., 2021; Nanetti et al., 2021; Tehel et al., 2016). Growing evidence supports the view that DWV spills over from managed honey bees into wild bee communities, especially bumble bees (*Bombus* spp.) (Alger et al., 2019; Fürst et al., 2014; Manley et al., 2019; McMahon et al., 2015; Tehel et al., 2022).

Even though DWV is well-studied in honey bees and known to replicate in alternative host species (Gusachenko et al., 2020; Radzevičiūtė et al., 2017; Tehel et al., 2020), its impact on bumble bee (*Bombus* spp.) fitness remains equivocal. Earlier laboratory studies have reported negative impacts of DWV on bumble bees, including deformed wings (Genersch et al., 2006) and reduced survival (Fürst et al., 2014; Graystock et al., 2016). In contrast, Tehel et al. (2020) found no impact of DWV on adult survival under benign laboratory conditions but a subtle shortening of lifespan following food deprivation.

Although robustly executed under controlled laboratory conditions, these initial virulence studies lacked an assessment of the influence of environmental stressors or ecological context which has the potential to modulate host–parasite interactions (Lazzaro & Little, 2009; Scholthof, 2007; Vale et al., 2011). Environmental temperature, for example, is known to profoundly affect invertebrate–parasite interactions, such as the degree of virulence that the host experiences (Lazzaro et al., 2008; Mitchell et al., 2005). Food availability and quality determine the host nutritional status and are known to alter the outcome of bee–parasite interactions (Alaux et al., 2010; Brown et al., 2000; DeGrandi-Hoffman & Chen, 2015; Dolezal & Toth, 2018), for example, a high quality and diverse diet allows honey bees to tolerate microsporidian or viral infections (Di Pasquale et al., 2013; Dolezal et al., 2019).

The bumble bee, *Bombus terrestris* (L.), is an eusocial Eurasian species (Hefetz & Grozinger, 2017; Lecocq et al., 2013) with an annual colony life cycle that lasts 2–4 months. Bumble bee colonies are exposed to an ensemble of natural and anthropogenic stressors such as fulfilling the colony's nutritional needs through foraging in an environment that is impacted by land-use change, agricultural intensification and climate change, affecting resource availability and potentially pollinator–pathogen networks (González-Varo et al., 2013; Proesmans et al., 2021). Bumble bee foraging under stressful conditions is crucial since a colony is often on the borderline of starvation (Heinrich, 1979). The overall condition of a host species has a strong effect on its capacity to sustain an efficient immune response or withstand an infection, which underscores the significant environmental context-dependence of host immune performance and probability and severity of an infection (Lazzaro & Little, 2009). It remains challenging to recreate the full complexity of abiotic and biotic variables affecting host–parasite interactions in a laboratory, however, a lack of field realism risks misjudging the virulence of a parasite in an alternative host when it is investigated under laboratory conditions alone.

Here, we conducted an integrated laboratory and field colony experiment to control virus inoculation of bumble bee individuals while obtaining a more field-realistic view of the impact of DWV on this alternative host. We inoculated commercially sourced *Bombus terrestris* workers with DWV-A under controlled laboratory conditions, which we subsequently introduced into colonies placed in the field, thus exposing them to the environment and associated stressors. Virulence was estimated by monitoring the survival of inoculated bees, an important component of fitness, together with quantification of the viral load they carried. We tested if the mode of inoculation, oral versus injection, affects these parameters of virulence and if the environmental context, laboratory versus field, influences the viral load of infected bumble bee workers.

MATERIALS AND METHODS

Experimental overview

We maintained two commercial *B. terrestris* colonies inside the laboratory that served as source colonies for newly emerged, age controlled, bumble bee workers. To inoculate individual workers, we followed established methods (Tehel et al., 2020, 2022) using inoculation via feeding or injection. Inoculation via feeding was chosen to mimic the most likely route of virus spillover for bumble bees in the field via contaminated flowers (Burnham et al., 2021; McArt et al., 2014). In addition, we used inoculation via injection because it is a reliable method to establish an active infection (viral replication) with DWV in *B. terrestris* workers (Tehel et al., 2020).

Newly emerged workers taken from the two source colonies were assigned randomly to four treatment groups: viral injection, control injection, viral feeding and control feeding, as detailed below. After individual inoculation and marking, inoculated bumble bee workers were introduced into four commercial *B. terrestris* colonies that were located in the field to subject them to field realistic conditions. Introduction of inoculated bees was repeated over the course of 3 weeks to achieve a sufficient sample size (total $n = 36–41$ per treatment and per field colony). Each of the four field colonies received bumble bees from all four treatment groups (Table S5).

A subset of inoculated bumble bees of all four treatment groups was sampled from the field colonies at day 10 after inoculation to verify an active infection of the virus in inoculated bees and the absence of viral infection in control bees. Additionally, we incubated a subset of treated bumble bees in the laboratory, which were likewise analysed for their viral load 10 days post inoculation. This allowed us to compare the viral load in virus inoculated bumble bees under field conditions with the viral load of inoculated bumble bees under benign conditions in the laboratory.

Experimental set-up

Bombus terrestris colonies

In total, we used six commercial *Bombus terrestris* colonies (Koppert B.V., Berkel en Rodenrijs, The Netherlands). To check viral levels in

the supplied colonies, 10–12 adult bumble bee workers per colony were screened for six viral targets: DWV-A and -B (deformed wing virus), BQCV (black queen cell virus), SBPV (slow bee paralysis virus), SBV (sacbrood virus) and CBPV (chronic bee paralysis virus). The colonies were devoid of SBPV and CBPV while some showed a weak signal by real-time PCR (Cq > 35.5) for DWV-A, DWV-B, BQCV and SBV (Table S1).

Two of six colonies were designated 'source colonies' and were housed permanently in the laboratory at 28°C and 50–60% relative humidity with ad libitum 50% (w/v) sucrose solution. Every other day they were fed with defrosted pollen pellets (Imkerei Schachtner, Schardenberg, Austria), which had been previously tested for the absence of the same six common honey bee viruses (see Tehel et al., 2020). To control the age of bees, initial workers of the two source colonies were marked by cutting the tips of their wings, which permitted reliable identification of unmarked newly emerged bees. To account for the genetic makeup of the six colonies, we transferred brood once from the four designated field colonies into the two laboratory source colonies before the start of the experiment. Thus, bees newly emerging in the source colonies originated from all six colonies. By transferring brood to the source colonies, we also equalised the initial size of the designated field colonies, factoring in that we would continuously add inoculated bees to them over the following weeks. Field colonies were placed in the field at day nine upon their arrival.

Intracolony transmission of DWV-A among *B. terrestris* workers could compromise experimental infection groups. However, Tehel et al. (2022) have shown that this is likely an infrequent event, which allowed us to have virus and control treated bees together in each colony. The four field colonies were located on a grassy, lightly wooded hillside in front of our institute, which is located in a residential neighbourhood containing gardens and parks supplying floral resources (Appendix S1). Field colonies had no internal food or pollen source, which forced the workers to forage for the colony's resources.

Source of inoculum

As virus we used DWV-A because data from Tehel et al. (2020) indicate that it shortens the lifespan of *B. terrestris* workers under stressful food deprivation. The virus inoculum originated from infected honey bee pupae and was the same stock propagated and used in Tehel et al. (2020). The control solution consisted of a homogenate of three healthy honey bee pupae crushed in 0.05 M potassium phosphate buffer (pH 8.0) that was screened by qPCR and found to be devoid of DWV-A, DWV-B, BQCV, SBPV, SBV, CBPV and ABPV (acute bee paralysis virus).

Inoculation via feeding

Newly emerged bumble bee workers were starved for 4–5 h then individually presented with 10 µl sucrose solution (50% (w/v)) containing 10^9 genome equivalents (GEs) of DWV-A. The control group received 10 µl sucrose solution containing an equivalent volume of virus-free honey bee homogenate. Inoculations were monitored to ensure consumption within a maximum of 15 min. After inoculation, bumble bees were transferred to autoclaved metal cages (10 × 10 × 6 cm) in groups of 5–8 individuals containing the same treatment group, provided with ad libitum 50% (w/v) sucrose solution and held at 28°C. On the following day, treated workers were chilled for a few minutes to immobilise them, whereupon they were individually marked with numbered and coloured disks (Opalithplättchen®) which were attached to shaved thoraxes using super glue (UHU). After 3–4 h of recovery, inoculated and marked workers were introduced into field colonies.

Inoculation via injection

Newly emerged workers were chilled for a few minutes for immobilisation, followed by injection of 2×10^7 DWV-A genome equivalents in 2 µl potassium-phosphate-buffer (0.5 M, pH 8.0) laterally between the second and third sternite (Hamilton syringe, hypodermic needle outer diameter: 0.235 mm). Controls received a 2 µl injection of a virus-free honey bee homogenate. Immediately after the injection process, bumble bees were individually marked and then held for 24 h as described in 2.2.3, before they were introduced into the field colonies. Bees that did not survive 24 h post inoculation (≈10% of bees) were excluded from the experiment.

Field colony inspection

Inspection of field colonies harbouring the inoculated bumble bees took place daily, weather permitting, over a period of 40 days from June to July 2020. To increase the detection of inoculated workers inside a colony, colony exits were closed 1.5 h before inspection while entrances were left open, which allowed active foragers in the field to enter but not to exit the colony. Then the status, dead or alive, of all marked bumble bees inside the field colonies was recorded. Field colonies had a queen until the end of inspections, except for one colony where the queen died 3 days before observations were terminated.

Viral screening and quantification

Verification and quantification of DWV-A

To verify that the DWV-A inoculation of bumble bees resulted in an active infection and that control bees were uninfected, a subset of treated bees was individually analysed for viral titre (load) after 10 days post inoculation (d.p.i.). To do so, we kept eight bumble bees per treatment inside the laboratory and held them in autoclaved metal cages with ad libitum 50% (w/v) sucrose solution at 28°C. At 10 d.p.i., bees were freeze killed and stored at −80°C for viral quantification. To verify active DWV-A infection of treated bumble bees that had

been introduced into the field colonies, marked bumble bees ($n = 7–8$ per treatment) were collected from them at 10 d.p.i., freeze killed and stored at −80°C for viral quantification.

For quantification of viral load in frozen samples, bumble bees were individually crushed in 600/800 μl (size dependent) RLT- Buffer with 1% 2-mercaptoethanol using plastic pestles. RNA was extracted from 100 μl of supernatant using the RNeasy plant mini kit (Qiagen, without shredder columns, Appendix S1). Complementary DNA was synthesised from 800 ng of sample RNA using oligo(dT)$_{18}$ primer (Thermo Scientific) and M-MLV Reverse Transcriptase RNase (H-), Point Mutant (Promega), following manufacturer's instructions. Resulting cDNA was diluted 10-fold with nuclease-free dH_2O. Absolute quantification of DWV-A viral loads was done using DNA standard curves containing a serially diluted purified target PCR product. For cDNA quality control, honey bee *β-actin* was amplified as an internal reference gene. All samples were additionally screened for potentially contaminating DWV-B and BQCV, as these are among the most prevalent pollinator viruses (Alger et al., 2019; McMahon et al., 2015; Radzevičiūtė et al., 2017). Quantitative real-time PCRs (qPCR) were conducted in a Bio-Rad C1000 cycler using the SensiMix™ SYBR & Fluorescein kit (Bioline). Additional technical details and quality controls are given in the Appendix S1.

Data analyses

Inoculated *B. terrestris* workers that were not seen alive on at least one observation day inside the field colonies after their introduction were excluded from further analysis ($n = 73$ of 615 individually marked bees), because their mortality was likely caused by the inoculation process itself or initial rejection by colony members rather than the experimental treatment. After their removal, a total of $n = 131$ DWV-A injected bees, $n = 141$ control injected bees, $n = 132$ DWV-A fed bees and $n = 138$ control fed bees were included in the survival analysis (Table S5). Day of death was determined as the day after a worker was seen alive for the last time inside a colony.

All data analyses were carried out in R version 3.6.3 (R core team). To compare survival across treatment groups, we used a Cox proportional hazards model with the R package *coxme* (Therneau, 2020). Receiver colony identity and date of field colony introduction of inoculated bees were used as random factors. To assess if treatment was a significant predictor of survivorship, the model was compared to a null model (intercept only). Median survival of treated bees was estimated with the Survfit function in the *survival* package (Therneau et al., 2022).

Viral load of DWV-A injected bumblebees at 10 d.p.i. that were either introduced to the field colonies or maintained in the laboratory with ad libitum food was compared by log transforming titre data and analysing them in a linear model (LM). To test if location (field vs. laboratory) was a significant predictor of viral load, the model was again compared to a null model (intercept only) using a Likelihood-Ratio test (LR-test). Model assumptions (e.g. normality of residuals) were checked with the package *DHARMa* (Hartig, 2020).

RESULTS

Survival and viral load of bumble bee workers injected with DWV-A

Bombus terrestris workers that were inoculated with DWV-A via injection and introduced into the field colonies had a significantly reduced survival compared to the respective control injection group (Cox proportional hazard, LR-test: $\chi^2 = 5.768$, df $= 1$, $p = 0.016$). DWV-A injected bumble bees had a median survival of 11 days (95% CIs [10, 13]) in comparison to the respective control group of bumble bees with a median survival of 14 days (95% CIs [11, 16]). Differences in survival between DWV-A and control injection treatments became apparent around day 10 after colony introduction (Figure 1) and were consistent across colonies (Table S6).

Quantification of viral loads per bee 10 d.p.i. from a subset of inoculated bees by qPCR confirmed that DWV-A injection resulted in an active infection (viral replication) in both field and laboratory-maintained bumble bees, while field and laboratory control bumble bees were devoid of DWV-A (Figure 2). Inoculation via injection of 10^7 DWV-A genome equivalents (GEs) resulted in an average viral load of $4.54 \times 10^8 \pm 1.74 \times 10^8$ GEs per bee (mean ± SE) in DWV-A injected workers maintained in the laboratory while field maintained injected workers carried an approximate 4-fold higher viral load of 1.89×10^9 GEs $\pm 1.03 \times 10^9$ on average per bee (Figure 2). Differences in viral loads between field and laboratory-maintained bumble bees were not significant (LM, LR-test: $\chi^2 = -0.977$, df $= 1$, $p = 0.260$). DWV-A was not detected in control injected bumble bees. DWV-A and control injected bees were either free or had a minor background contamination of BQCV or DWV-B (Table S2).

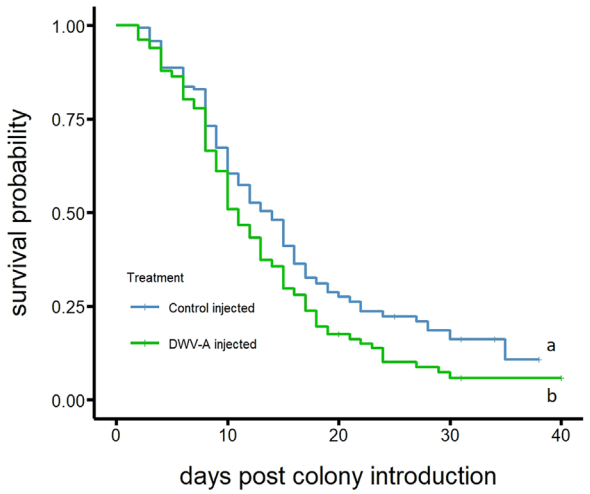

FIGURE 1 Survival (Kaplan–Meier curves) illustrating probability of survival of *Bombus terrestris* workers inoculated by injection with 10^7 DWV-A genome equivalents ($n = 131$, green) or the respective control solution ($n = 141$, blue) in days after introduction to one of four experimental field colonies. DWV-A injected bees died faster than control injected bees (different lower case letters, $p < 0.05$)

Ecological Entomology

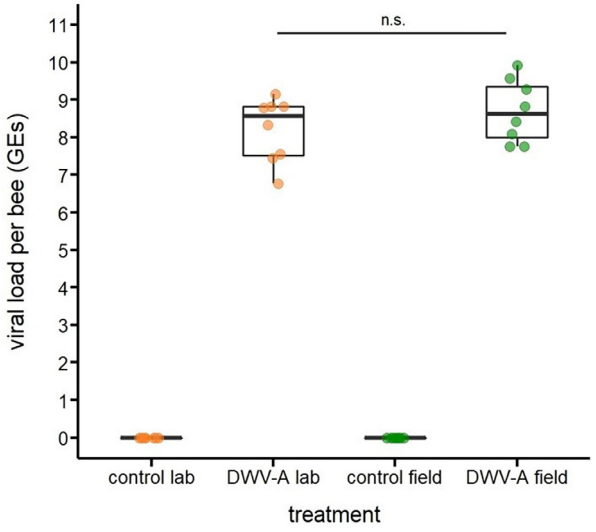

FIGURE 2 Viral load (in genome equivalents: GEs per bee) of *Bombus terrestris* workers inoculated by injection with DWV-A or the respective control solution ($n = 8$ bees per treatment and location bees were maintained in). The acronym lab indicates inoculated bees that were maintained in the laboratory under benign conditions while field indicates inoculated bees that were maintained in experimental field colonies under natural conditions

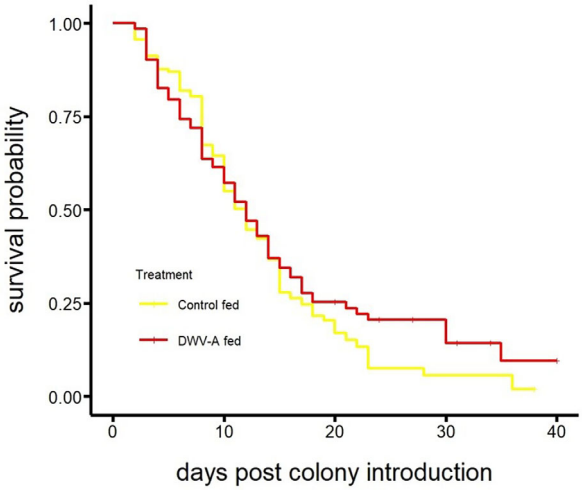

FIGURE 3 Survival (Kaplan–Meier curves) illustrating probability of survival of *Bombus terrestris* workers orally inoculated with 10^9 DWV-A genome equivalents ($n = 132$, red) or the respective control solution ($n = 138$, yellow) in days after introduction to one of four experimental field colonies. Differences in survival between treatments were not significant

Survival of bumble bee workers orally inoculated with DWV-A

Bombus terrestris workers that were inoculated orally through feeding with 10^9 DWV-A GEs or the respective virus-free control solution did not differ in their survival in the field (Figure 3). Bumble bees from both treatment groups had a median survival of 12 days (95% CIs [10, 14]; Table S7).

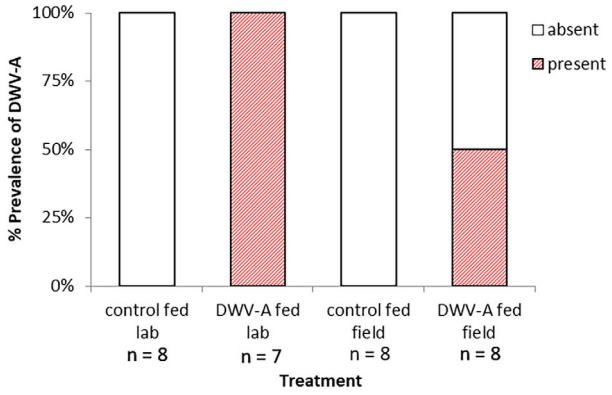

FIGURE 4 Viral prevalence in *B. terrestris* workers orally inoculated with 10^9 DWV-A genome equivalents or the respective control solution 10 days post inoculation. Colour shading of bars indicates the absence (white) or presence (red) of a positive signal for DWV-A in qPCRs. The acronym lab stands for inoculated bees that were maintained in the laboratory under benign conditions while the acronym field indicates inoculated bees that were maintained in experimental field colonies under natural conditions

qPCR of workers orally exposed to DWV-A gave a positive signal for DWV-A in 100% of analysed bumble bees that were fed with DWV-A and maintained under laboratory conditions and in 50% of workers that were fed with DWV-A and housed in field colonies (Figure 4). However, viral targets at 10 d.p.i. were too low for reliable absolute quantification by qPCR (Appendix S1). All analysed control-fed bumble bees, either lab or field maintained, were devoid of DWV-A. DWV-A and control fed bees were either free or had a minor background contamination of BQCV or DWV-B (Table S3).

DISCUSSION

Spillover of DWV from *A. mellifera* to *Bombus* spp. has frequently been inferred from field-collected bees (Fürst et al., 2014; Manley et al., 2019; McMahon et al., 2015), yet we know little about the consequences of cross-species transmission of DWV for bumble bee fitness components in the field. Here we show that the survival of commercial *B. terrestris* workers in colonies in the field was reduced when bees were inoculated by injection with DWV-A, indicating that once infected DWV-A represents a potential risk for the health of this alternative host. The mode of infection is, however, an important determinant of that risk being realised as oral inoculation of DWV-A by feeding *B. terrestris* did not similarly translate into reduced survival.

The reduction in median survival by 3 days in our injection treatment reveals the context- dependent virulence of DWV-A when infecting free-flying *B. terrestris* workers, since no significant effect of the same viral inoculum was observed on worker survival in a previous virulence study when bumble bees were held in apparently benign

laboratory conditions (Tehel et al., 2020). Other studies on virulence in bumble bee-pathogen systems have shown context-dependent virulence, with an increased mortality only for bees that were exposed to an additional stressor, such as a period of starvation (Brown et al., 2000; Manley et al., 2017). The contradictory results of previous laboratory experiments inoculating *B. terrestris* with DWV (Fürst et al., 2014; Graystock et al., 2016; Tehel et al., 2020) may well lie in the idiosyncrasies of specific experimental paradigms (benign/stressful) to which bees were exposed.

The most plausible mechanism for viral spillover between pollinator species is that viruses are acquired orally during foraging on flowers (McArt et al., 2014). We found that *B. terrestris* workers that were inoculated with DWV-A by feeding did not exhibit a reduced lifespan compared to the control group when exposed to field-realistic conditions. It might be concluded that DWV-A spillover via floral sharing causes little impact on survival for commercial *B. terrestris* workers in the field. It should, however, be noted that a single viral oral inoculation of young bumble bees does not fully mimic natural conditions. It is likely that foraging bumble bees encounter virus-contaminated flowers throughout their lifetimes, increasing the chances for repeated virus uptake and replication (Burnham et al., 2021). Additionally, the immune defence mechanisms of *B. terrestris* workers senesce (Doums et al., 2002) such that worker bees might become more susceptible to infection with advancing age. Additional field-realistic experiments involving repeated viral inoculation of workers of different ages will help to understand the age- and infection-frequency dependence of bumble bee viral susceptibility.

Quantification of DWV-A at 10 d.p.i. in bumble bees inoculated orally revealed that workers carried only low or zero detectable viral loads of DWV-A. Why half of the orally inoculated *B. terrestris* workers that experienced field-realistic conditions in our experiment were seemingly devoid of DWV-A is unclear. The bumble bee immune response may have been able to keep an infection at bay or eventually clear the administered virus when consumed orally. Alternatively, inoculated workers may have excreted the administered virus without becoming infected, as when they are infested with the honey bee Microsporidia *Nosema ceranae* (Gisder et al., 2020). Infectivity studies on bumble bees indicate a dose-dependency to achieve high rates of viral infection and viral loads when inoculation is via the oral route in the laboratory (Burnham et al., 2021; Doublet et al., 2015; Gusachenko et al., 2020; Wang et al., 2018). Orally administered virus has access primarily to the digestive tract, where it has to overcome local immune defence mechanisms and potentially interfering host microbiota to establish an infection and before it can spread to other tissue. The host gut microbiota in particular, which itself is interlinked with host nutrition and other environmental factors, might play a substantial role in viral defence mechanisms in bees (Dosch et al., 2021) and deserves greater research attention (González & Elena, 2021). In contrast, viral injection directly into the haemocoel provides systemic access to host tissue as a consequence of the host's open circulatory system, facilitating viral spread to permissive cells, which might explain the higher infectivity achieved by this route of infection (Tehel et al., 2020; Wang et al., 2018).

The mechanism behind the reduced survival of DWV-A injected bumble bees in the field remains to be identified. Activation of the immune system and sustaining an immune response require host resources that cannot be maintained simultaneously with other energetically costly tasks (Moret & Schmid-Hempel, 2000). Additionally, the growth of a parasite deprives the host of resources (Ebert et al., 2004; Hall et al., 2009), setting a conflict or trade-off over resource utilisation that is probably dependent upon resource availability for the host (Vale et al., 2011). Bumble bees are generally dependent on high levels of energy input from nectar and pollen to maintain themselves and their colony (Goulson, 2009; Heinrich, 1979). A forager that is energy depleted due to an infection might therefore become more prone to predation during foraging flights or, like honey bees infected with the Microsporidia *Nosema ceranae*, face greater difficulty in returning to the nest from a foraging flight (Wolf et al., 2016).

Survival is considered the most important fitness parameter of an individual worker bumble bee (Doums et al., 2002), yet how survival of an individual affects overall colony fitness of this eusocial insect remains to be established. Colony resource input depends on forager activity and foraging conditions so that a reduced worker life-span, as we observed for DWV-A injected workers, would increase the risk for an energetic-shortfall, causing negative consequence for colony performance (Cartar & Dill, 1991; Plowright & Pendrel, 1977; Sutcliffe & Plowright, 1990). Furthermore, a lower resource inflow reduces colony growth, a crucial and time-sensitive parameter that is directly linked to colony reproductive success (Crone & Williams, 2016; Pelletier & McNeil, 2003). Hence, shortened worker survival could lower overall colony fitness and therefore the individual's inclusive fitness, although this remains to be tested experimentally.

Although host mortality is a parameter commonly used as a measure of a parasite's virulence, a caveat of our study is that, by measuring survival alone, we may have missed other important impacts of viral infection on the performance of host bumble bee workers and colony fitness. For example, honey bees experimentally injected with DWV exhibit an accelerated temporal polyethism schedule and reduced foraging activity (Benaets et al., 2017; Natsopoulou et al., 2016). Coupling our experimental setup with RFID-tagged workers (Hall et al., 2021; Russell et al., 2017) would help to address this point by revealing how viral infection creates sub-lethal impacts on bumble bee foraging behaviour or flight activity. Furthermore, though our experimental design, in which all treatment groups were mixed in the same colony, allowed us to estimate survival independent of the colony's health status or resource input, it obviously did not allow us to measure colony-level impacts of experimental infection. Future studies should therefore focus on colony level impacts of viral infection,

preferably in environments varying in resource availability, to infer the costs imposed by DWV on bumble bee colonies. Finally, our study investigated commercial *B. terrestris*, a cohort of bumble bees that may have been selected for tolerance or resistance to DWV infection because of more than three decades of artificial rearing in close proximity to honey bees (Velthuis & van Doorn, 2006). We sorely need measures of viral impact on other bumble bee species – wild and managed (Osterman et al., 2021) – to evaluate the role of spillover in population decline.

AUTHOR CONTRIBUTIONS

T.S.: conceptualisation, experimental and molecular work, data analysis and interpretation, writing- original draft, review and editing. A.T.: conceptualisation, experimental work, data interpretation, writing-review and editing. S.T.: conceptualisation, data analysis and interpretation, writing-review and editing. R.J.P.: conceptualisation, data interpretation, funding acquisition, project administration, resources, supervision, writing-review and editing.

ACKNOWLEDGEMENTS

We thank Christopher Wild for his help and experience during the preparation of the bumble bee colonies prior to the experiment, Adam Vanbergen, the editor and the anonymous referees for their helpful comments on the manuscript. Open Access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

This research was funded by the DFG Pa632/10–1 and Pa632/12–1 (428721090) BiodivERsA VOODOO project (Viral eco-evolutionary dynamics of wild and domestic pollinators under global change, https://voodoo-project.eu/).

CONFLICT OF INTEREST

The authors have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.21269664.

ORCID

Tabea Streicher https://orcid.org/0000-0003-1315-7042
Anja Tehel https://orcid.org/0000-0002-2667-7306
Simon Tragust https://orcid.org/0000-0002-3675-9583
Robert J. Paxton https://orcid.org/0000-0003-2517-1351

REFERENCES

Alaux, C., Ducloz, F., Crauser, D. & Conte, Y.L. (2010) Diet effects on honeybee immunocompetence. *Biology Letters*, 6, 562–565.
Alger, S.A., Burnham, P.A., Boncristiani, H.F. & Brody, A.K. (2019) RNA virus spillover from managed honeybees *(Apis mellifera)* to wild bumblebees *(Bombus spp.)*. *PLoS One*, 14, e0217822.
Benaets, K., Van Geystelen, A., Cardoen, D., De Smet, L., De Graaf, D.C., Schoofs, L. et al. (2017) Covert deformed wing virus infections have long-term deleterious effects on honeybee foraging and survival. *Proceedings of the Royal Society B: Biological Sciences*, 284, 20162149.
Brown, M.J.F., Loosli, R. & Schmid-Hempel, P. (2000) Condition-dependent expression of virulence in a trypanosome infecting bumblebees. *Oikos*, 91, 421–427.
Burnham, P.A., Alger, S.A., Case, B., Boncristiani, H., Hébert-Dufresne, L. & Brody, A.K. (2021) Flowers as dirty doorknobs: deformed wing virus transmitted between *Apis mellifera* and *Bombus impatiens* through shared flowers. *Journal of Applied Ecology*, 58, 2065–2074.
Cartar, R.V. & Dill, L.M. (1991) Costs of energy shortfall for bumble bee colonies: predation, social parasitism, and brood development. *The Canadian Entomologist*, 123, 283–293.
Crone, E.E. & Williams, N.M. (2016) Bumble bee colony dynamics: quantifying the importance of land use and floral resources for colony growth and queen production. *Ecology Letters*, 19, 460–468.
Daszak, P., Cunningham, A.A. & Hyatt, A.D. (2000) Emerging infectious diseases of wildlife- threats to biodiversity and human health. *Science*, 287, 443–449.
DeGrandi-Hoffman, G. & Chen, Y. (2015) Nutrition, immunity and viral infections in honey bees. *Current Opinion in Insect Science*, 10, 170–176.
Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L.P., Decourtye, A., Kretzschmar, A. et al. (2013) Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter? *PLoS One*, 8, e72016.
Dolezal, A.G., Carrillo-Tripp, J., Judd, T.M., Miller, W.A., Bonning, B.C. & Toth, A.L. (2019) Interacting stressors matter: diet quality and virus infection in honeybee health. *Royal Society Open Science*, 6, 181803.
Dolezal, A.G. & Toth, A.L. (2018) Feedbacks between nutrition and disease in honey bee health. *Current Opinion in Insect Science*, 26, 114–119.
Dosch, C., Manigk, A., Streicher, T., Tehel, A., Paxton, R.J. & Tragust, S. (2021) The gut microbiota can provide viral tolerance in the honey bee. *Microorganisms*, 9, 871.
Doublet, V., Labarussias, M., de Miranda, J.R., Moritz, R.F.A. & Paxton, R.J. (2015) Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. *Environmental Microbiology*, 17, 969–983.
Doums, C., Moret, Y., Benelli, E. & Schmid-Hempel, P. (2002) Senescence of immune defence in *Bombus* workers. *Ecological Entomology*, 27, 138–144.
Ebert, D., Carius, H.J., Little, T. & Decaestecker, E. (2004) The evolution of virulence when parasites cause host castration and gigantism. *American Naturalist*, 164, S19–S32.
Fürst, M.A., McMahon, D.P., Osborne, J.L., Paxton, R.J. & Brown, M.J.F. (2014) Disease associations between honeybees and bumblebees as a threat to wild pollinators. *Nature*, 506, 364–366.
Genersch, E., Yue, C., Fries, I. & de Miranda, J.R. (2006) Detection of deformed wing virus, a honey bee viral pathogen, in bumble bees (*Bombus terrestris* and *Bombus pascuorum*) with wing deformities. *Journal of Invertebrate Pathology*, 91, 61–63.
Gisder, S., Horchler, L., Pieper, F., Schüler, V., Šima, P. & Genersch, E. (2020) Rapid gastrointestinal passage may protect *Bombus terrestris* from becoming a true host for *Nosema ceranae*. *Applied and Environmental Microbiology*, 86, e00629–e00620.
González, R. & Elena, S.F. (2021) The interplay between the host microbiome and pathogenic viral infections. *MBio*, 12, e02496–e02421.
González-Varo, J.P., Biesmeijer, J.C., Bommarco, R., Potts, S.G., Schweiger, O., Smith, H.G. et al. (2013) Combined effects of global change pressures on animal-mediated pollination. *Trends in Ecology and Evolution*, 28, 524–530.
Goulson, D. (2009) *Bumblebees: behaviour, ecology, and conservation*. Oxford, UK: Oxford University Press.

Graystock, P., Meeus, I., Smagghe, G., Goulson, D. & Hughes, W.O.H. (2016) The effects of single and mixed infections of *Apicystis bombi* and deformed wing virus in *Bombus terrestris*. *Parasitology*, 143, 358–365.

Grozinger, C.M. & Flenniken, M.L. (2019) Bee viruses: ecology, pathogenicity, and impacts. *Annual Review of Entomology*, 64, 205–226.

Gusachenko, O.N., Woodford, L. & Cumming, K.B. (2020) Evidence for and against deformed wing virus spillover from honey bees to bumble bees: a reverse genetic analysis. *Scientific Reports*, 10, 16847.

Hall, K., Robert, T., Gaston, K.J. & Hempel de Ibarra, N. (2021) Onset of morning activity in bumblebee foragers under natural low light conditions. *Ecology and Evolution*, 11, 6536–6545.

Hall, S.R., Simonis, J.L., Nisbet, R.M., Tessier, A.J. & Cáceres, C.E. (2009) Resource ecology of virulence in a planktonic host-parasite system: an explanation using dynamic energy budgets. *American Naturalist*, 174, 149–162.

Hartig, F. (2020). DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.3, 3.

Hefetz, A. & Grozinger, C.M. (2017) Hormonal regulation of behavioral and phenotypic plasticity in bumblebees. In: Hormones, brain and behaviour, Vol. 2 *Non-mammalian hormone-behavior systems*. Amsterdam, NL: Elsevier/Academic Press, pp. 453–464.

Heinrich, B. (1979) *Bumblebee economics*. Cambridge, MA: Harvard University Press.

Holmes, E.C. (2009) *The evolution and emergence of RNA viruses*. New York, NY: Oxford University Press.

Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L. et al. (2008) Global trends in emerging infectious diseases. *Nature*, 451, 990–993.

Jones, L.J., Ford, R.P., Schilder, R.J. & López-Uribe, M.M. (2021) Honey bee viruses are highly prevalent but at low intensities in wild pollinators of cucurbit agroecosystems. *Journal of Invertebrate Pathology*, 185, 107667.

Lazzaro, B.P., Flores, H.A., Lorigan, J.G. & Yourth, C.P. (2008) Genotype-by-environment interactions and adaptation to local temperature affect immunity and fecundity in *Drosophila melanogaster*. *PLoS Pathogens*, 4, e1000025.

Lazzaro, B.P. & Little, T.J. (2009) Immunity in a variable world. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364, 15–26.

Lecocq, T., Vereecken, N.J., Michez, D., Dellicour, S., Lhomme, P., Valterová, I. et al. (2013) Patterns of genetic and reproductive traits differentiation in mainland vs. corsican populations of bumblebees. *PLoS One*, 8, e65642.

Manley, R., Boots, M. & Wilfert, L. (2017) Condition-dependent virulence of slow bee paralysis virus in *Bombus terrestris*: are the impacts of honeybee viruses in wild pollinators underestimated? *Oecologia*, 184, 305–315.

Manley, R., Temperton, B., Doyle, T., Gates, D., Hedges, S., Boots, M. et al. (2019) Knock-on community impacts of a novel vector: spillover of emerging DWV-B from *Varroa*-infested honeybees to wild bumblebees. *Ecology Letters*, 22, 1306–1315.

Martin, S.J. & Brettell, L.E. (2019) Deformed wing virus in honeybees and other insects. *Annual Review of Virology*, 6, 49–69.

McArt, S.H., Koch, H., Irwin, R.E. & Adler, L.S. (2014) Arranging the bouquet of disease: floral traits and the transmission of plant and animal pathogens. *Ecology Letters*, 17, 624–636.

McMahon, D.P., Fürst, M.A., Caspar, J., Theodorou, P., Brown, M.J.F. & Paxton, R.J. (2015) A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. *Journal of Animal Ecology*, 84, 615–624.

Mitchell, S.E., Rogers, E.S., Little, T.J. & Read, A.F. (2005) Host-parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. *Evolution*, 59, 70–80.

Moret, Y. & Schmid-Hempel, P. (2000) Survival for immunity: the price of immune system activation for bumblebee workers. *Science*, 290, 1166–1168.

Nanetti, A., Bortolotti, L. & Cilia, G. (2021) Pathogens spillover from honey bees to other arthropods. *Pathogens*, 10, 1044.

Natsopoulou, M.E., McMahon, D.P. & Paxton, R.J. (2016) Parasites modulate within-colony activity and accelerate the temporal polyethism schedule of a social insect, the honey bee. *Behavioral Ecology and Sociobiology*, 70, 1019–1031.

Osterman, J., Aizen, M.A., Biesmeijer, J.C., Bosch, J., Howlett, B.G., Inouye, D.W. et al. (2021) Global trends in the number and diversity of managed pollinator species. *Agriculture, Ecosystems and Environment*, 322, 107653.

Paxton, R.J., Schäfer, M.O., Nazzi, F., Zanni, V., Annoscia, D., Marroni, F. et al. (2022) Epidemiology of a major honey bee pathogen, deformed wing virus: worldwide replacement of genotype a by genotype B. *International Journal for Parasitology: Parasites and Wildlife*, 18, 157–171.

Pelletier, L. & McNeil, J.N. (2003) The effect of food supplementation on reproductive success in bumblebee field colonies. *Oikos*, 103, 688–694.

Plowright, R.C. & Pendrel, B.A. (1977) Larval growth in bumble bees (hymenoptera: Apidae). *The Canadian Entomologist*, 109, 967–973.

Proesmans, W., Albrecht, M., Gajda, A., Neumann, P., Paxton, R.J., Pioz, M. et al. (2021) Pathways for novel epidemiology: plant–pollinator–pathogen networks and global change. *Trends in Ecology and Evolution*, 36, 623–636.

Radzevičiūtė, R., Theodorou, P., Husemann, M., Japoshvili, G., Kirkitadze, G., Zhusupbaeva, A. et al. (2017) Replication of honey bee-associated RNA viruses across multiple bee species in apple orchards of Georgia, Germany and Kyrgyzstan. *Journal of Invertebrate Pathology*, 146, 14–23.

Russell, A.L., Morrison, S.J., Moschonas, E.H. & Papaj, D.R. (2017) Patterns of pollen and nectar foraging specialization by bumblebees over multiple timescales using RFID. *Scientific Reports*, 7, 42448.

Ryabov, E.V., Childers, A.K., Chen, Y., Madella, S., Nessa, A., VanEngelsdorp, D. et al. (2017) Recent spread of Varroa destructor virus-1, a honey bee pathogen, in the United States. *Scientific Reports*, 7, 1–10.

Scholthof, K.B.G. (2007) The disease triangle: pathogens, the environment and society. *Nature Reviews Microbiology*, 5, 152–156.

Sutcliffe, G.H. & Plowright, R.C. (1990) The effects of pollen availability of development time in the bumble bee *Bombus terricola* K. (hymenoptera: Apidae). *Canadian Journal of Zoology*, 68, 1120–1123.

Tehel, A., Brown, M.J.F. & Paxton, R.J. (2016) Impact of managed honey bee viruses on wild bees. *Current Opinion in Virology*, 19, 16–22.

Tehel, A., Streicher, T. & Tragust, S. (2020) Experimental infection of bumblebees with honeybee-associated viruses: no direct fitness costs but potential future threats to novel wild bee hosts. *Royal Society Open Science*, 7, 200480.

Tehel, A., Streicher, T., Tragust, S. & Paxton, R.J. (2022) Experimental cross species transmission of a major viral pathogen in bees is predominantly from honeybees to bumblebees. *Proceedings of the Royal Society B: Biological Sciences*, 289, 20212255.

Therneau, T. M. (2020). Mixed effects cox models. R package coxme version 2.2-16.

Therneau, T.M., Lumley, T., Atkinson, E. & Crowson, C. (2022) Survival analysis. R package survival version 3.3-1.

Traynor, K.S., Rennich, K., Forsgren, E., Rose, R., Pettis, J., Kunkel, G. et al. (2016) Multiyear survey targeting disease incidence in US honey bees. *Apidologie*, 47, 325–347.

Vale, P.F., Wilson, A.J., Best, A., Boots, M. & Little, T.J. (2011) Epidemiological, evolutionary, and coevolutionary implications of context-dependent parasitism. *American Naturalist*, 177, 510–521.

Velthuis, H.H.W. & van Doorn, A. (2006) A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination*. *Apidologie*, 37, 421–451.

Wang, H., Meeus, I., Piot, N. & Smagghe, G. (2018) Systemic Israeli acute paralysis virus (IAPV) infection in bumblebees (*Bombus terrestris*) through feeding and injection. *Journal of Invertebrate Pathology*, 151, 158–164.

Wolf, S., Nicholls, E., Reynolds, A.M., Wells, P., Lim, K.S., Paxton, R.J. et al. (2016) Optimal search patterns in honeybee orientation flights are robust against emerging infectious diseases. *Scientific Reports*, 6, 1–10.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1 Supporting information.

How to cite this article: Streicher, T., Tehel, A., Tragust, S. & Paxton, R.J. (2023) Experimental viral spillover can harm *Bombus terrestris* workers under field conditions. *Ecological Entomology*, 48(1), 81–89. Available from: https://doi.org/10.1111/een.13203