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Author for correspondence:

Tom Wenseleers

e-mail: tom.wenseleers@kuleuven.be

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Covert deformed wing virus infections have long-term deleterious effects on honeybee foraging and survival

Kristof Benaets¹, Anneleen Van Geystelen¹, Dries Cardoen¹, Lina De Smet², Dirk C. de Graaf², Liliane Schoofs³, Maarten H. D. Larmuseau^{1,4,5}, Laura E. Brettell⁶, Stephen J. Martin⁶ and Tom Wenseleers¹

¹Department of Biology, KU Leuven, Laboratory of Socio-ecology and Social Evolution, Leuven, Belgium

²Department of Biochemistry and Microbiology, UGent, Laboratory of Molecular Entomology and Bee Pathology, Gent, Belgium

³Department of Biology, KU Leuven, Research group of Functional Genomics and Proteomics, Leuven, Belgium

⁴Laboratory of Forensic Genetics and Molecular Archaeology, UZ Leuven, Leuven, Belgium

⁵Department of Imaging and Pathology, KU Leuven, Forensic Medicine, Leuven, Belgium

⁶School of Environment and Life Sciences, The University of Salford, Manchester, UK

TW, 0000-0002-1434-861X

Several studies have suggested that covert stressors can contribute to bee colony declines. Here we provide a novel case study and show using radio-frequency identification tracking technology that covert deformed wing virus (DWV) infections in adult honeybee workers seriously impact long-term foraging and survival under natural foraging conditions. In particular, our experiments show that adult workers injected with low doses of DWV experienced increased mortality rates, that DWV caused workers to start foraging at a premature age, and that the virus reduced the workers' total activity span as foragers. Altogether, these results demonstrate that covert DWV infections have strongly deleterious effects on honeybee foraging and survival. These results are consistent with previous studies that suggested DWV to be an important contributor to the ongoing bee declines in Europe and the USA. Overall, our study underlines the strong impact that covert pathogen infections can have on individual and group-level performance in bees.

1. Introduction

Over the past decades, serious declines in both wild and managed bee pollinators have been recorded in many parts of the world [1–5], thereby threatening the ecosystem services they provide. The underlying cause of the recent wave of honeybee colony losses has been subject to much debate and the current consensus is that multiple stressors likely contribute to these declines, including malnutrition owing to a lack of flower diversity, exposure to agrochemicals or the spread of emerging pathogens and parasites [2,5–10]. Pinpointing these stressors, however, can be hard, especially when their effect becomes obvious only over extended periods of time, such as following exposure to sublethal doses of pesticides [9,11] or after contracting some seemingly harmless 'covert' pathogen infections [12,13].

Among pathogens, recent studies have suggested that deformed wing virus (DWV) represents an important long-term stressor, as it has been statistically associated with both winter mortality and colony collapse in many studies [14–24]. DWV has a near global distribution and is the most widespread of the currently described viruses that infect honeybees, often affecting between 50% and 75% of all honeybee hives [25,26]. In addition, the virus can spill over to other bees [27,28], thereby posing an additional threat. DWV is named after the characteristic wing deformities that can arise when honeybees are infected in

the larval or pupal stage via the ectoparasitic mite vector *Varroa destructor* [29]. In this case, virus infections result in bloated abdomens, miscolouring and shortened lifespans [29]. Typically, however, infections take on a more ‘covert’ form, resulting in no visible morphological symptoms, especially when infection occurs in the adult stage or when mites carry only low virus titres. Nevertheless, the fact that colonies with covert infections can suffer from weakness, depopulation and sudden collapse [22] and that the presence of the virus has been linked with both winter mortality and colony collapse [14–24] suggests that DWV exerts a significant amount of long-term stress.

Indeed, several recent studies using controlled artificial infection of adult honeybee workers have shown a number of important effects of DWV, including the impairment of sensory responsiveness, associative olfactory learning and memory formation [30] (possibly linked to replication of the virus in the mushroom bodies and antennal lobes [31]), an accelerated pace of behavioural transition through their age-linked task allocation [32] and reduced lifespans of infected adult honeybees [25]. Furthermore, two studies in which natural variation in infection levels of DWV was combined with experimentally manipulated variation in infection levels with the microsporidian parasite *Nosema* showed that DWV-infected bees displayed shortened flight distances and flight durations in flight mill experiments [33], but no differences in orientation flight behaviour in a harmonic radar tracking set-up [34], whereas the reverse pattern was seen for *Nosema* [33,34]. As yet, however, effects of DWV on honeybee flight behaviour and foraging patterns have not yet been investigated using controlled infection set-ups.

The aim of this study, therefore, was to determine the impact of DWV as a long-term stressor in honeybees, and test experimentally if inoculation of adult bees with the virus negatively affected honeybee foraging behaviour and performance. To this end, we used passively powered radiofrequency identification (RFID) transponder tags [9,11,35–40] as a key technology that enabled us to non-invasively monitor the long-term out-hive activity of honeybee workers that were or were not experimentally infected with the virus. Tracking out-hive activity is key in studies of the impact of pathogens on honeybee health, as the worker foraging force is responsible for all resource acquisition, and the foraging range, worker activity and the magnitude of resource influx are vital to colony growth and survival [41,42]. Previously, RFID technology has been successfully used to study sublethal effects of nutritional stress and pesticides on honeybee and bumblebee foraging behaviour [9,11,35–40]. Nevertheless, applications to the study of pathogen-induced stress on honeybee foraging behaviour are still rare, and are currently limited to one study on Israeli acute paralysis virus (IAPV) [43], which documented virus-induced differences in homing ability, but without taking full advantage of the technology to study the long-term impact on foraging behaviour, and one study that showed adverse effects of a microsporidian gut parasite, *Nosema apis*, on honeybee foraging and survival [44]. In addition, another tracking technology—harmonic radar—was recently used by one group to show that the emerging pathogen *Nosema ceranae* caused impaired homing behaviour in honeybees [45] and that *Nosema* infection also affected honeybee orientation flight behaviour [34]. The tracking method, however, was unable to reveal any correlation between DWV infection levels and orientation flight characteristic [34].

2. Material and methods

(a) Radiofrequency identification tracking set-up

Three three-frame observation hives with *Apis mellifera carnica* honeybees were installed at the laboratory’s apiary in Leuven, Belgium to serve as host colonies for RFID-tagged bees that were or were not experimentally infected with DWV (400 of each treatment condition per host colony, see below). Each host colony contained two frames of brood, one frame with stored pollen and honey, a queen and around 3000 host colony workers (figure 1). The colonies were placed indoors at room temperature and were connected to the outside via a single entrance tunnel to allow free foraging. The end of the tunnel was outfitted with two iID[®] MAJA 4.1 RFID reader modules placed in series and connected to a MAJA 4.1 host computer (Microsensus, Germany) to record and log the timing of all RFID-tagged honeybees leaving or entering the hive. By setting up the reader modules in a serial set-up, successive signals from both readers gave information regarding the direction of movement of the detected bees. The readers were separated from each other by a 4 cm wooden tunnel block to prevent interference between the readers (figure 1).

(b) Introduction of control and experimentally infected bees

In each of the host colonies, we introduced 400 DWV-negative control bees and 400 DWV infection-positive honeybees. This was done by allowing bees to emerge from a single donor colony that based on a prior screen was confirmed to be free of DWV as well as of any of the major known honeybee viruses or pathogens [46], injecting newly eclosed workers with appropriate treatment solutions, and introducing these bees into one of three host colonies (see electronic supplementary material Methods for details). All colonies in our apiary, including the donor and host colonies, were treated with Thymovar for *Varroa* control according to the manufacturer’s recommendations. The fact that only a single donor colony was used in our experiments was linked with the difficulty of finding a host colony that was free of the major known honeybee pathogens, but our experimental design partly compensated for this by incorporating replication across different host environments. Bees were allowed to emerge by placing brood frames of the donor colony in a MIR-253 incubator (Sanyo, Belgium) at 34°C and 60% humidity, after which newly eclosed workers were collected daily. Subsequently, 400 newly eclosed workers per treatment condition and host colony were injected with 3 µl of the appropriate treatment solution, using a 5 µl 26 s gauge Hamilton syringe inserted into the apical part of the thorax. Immediately afterwards, each of these bees were outfitted with a mic3[®] 64-bit read-only RFID transponder (Microsensus, Germany) by gluing the tag to the bee’s thorax using Kombi Turbo two-component glue (Bison, The Netherlands). The tags measured 2.0 × 1.7 × 0.5 mm, weighed less than 5 mg and transmitted at 13.56 MHz. The RFID codes of all experimentally manipulated workers, together with the treatment condition, host colony and time of introduction, were added to a transponder information database by reading each code using a iID[®] PENmini USB pen (Microsensus, Germany). Up to 50 tagged individuals subjected to one of the two treatment conditions were kept in separate 15 × 10 × 7 cm cages kept at 34°C and 60% humidity, and contained a 10 × 8 cm piece of honey-filled comb and water, to allow the bees to settle down before introducing them into the host colonies (figure 1). Before introduction, the cages were placed on top of the observation hives, separated only by a wire mesh, for a 30 min period to increase acceptance rates [47]. Each of the 400 workers per host colony and treatment condition were introduced over the course of a period of 5 days, and foraging

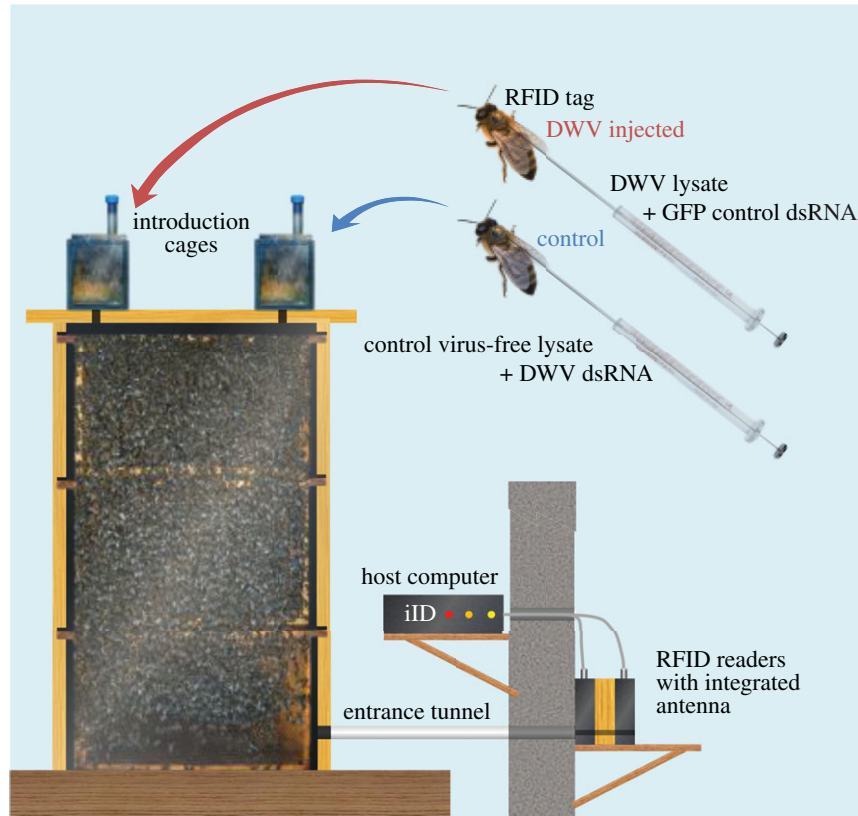


Figure 1. Experimental set-up. Observation hives were installed indoors with two RFID readers at the hive entrance to detect and log RFID-tagged bees entering or leaving the hive. The two RFID readers modules, connected to the host computer, were placed in series to determine the walking direction of detected bees. Tagged bees which were or were not experimentally infected with deformed wing virus were introduced into the host colony via separate introduction cages shown at the top ($n = 400$ bees per treatment and host colony). (Online version in colour.)

behaviour was monitored up to 40 days after introduction, in August–September 2012 for replicate host colonies A and B and September–October 2012 for host colony C.

(c) Controlled infection

In order to obtain two groups of adult age-matched bees that were or were not infected with DWV, we injected bees with lysate of honeybees that were either infected with DWV (and none of the other common honeybee viruses) or with that of honeybees that were confirmed to be virus-free (for details, see electronic supplementary material). To reduce the likelihood of horizontal transmission from the DWV infected to the group of uninfected bees [48], we also added a double-stranded RNA treatment to our DWV-negative lysate [49,50] to try to keep those bees DWV-free, and added a control GFP-dsRNA treatment in the DWV positive lysate to control for the possible effects of foreign dsRNA injection. The amount of DWV injected was estimated at 1.2×10^4 – 4.6×10^5 DWV copies per bee, and was aimed at mimicking infection loads reported for bees with covert infections (1.4×10^3 – 2.4×10^9 copies per bee, [51]). Based on Illumina ultra-high throughput sequencing, the DWV strain used for inoculation was determined to belong to the type B DWV master variant [52], which has recently been found to be an emergent, slightly more virulent strain of the DWV virus [25] that is currently also the most common strain in Britain [25] (for details, see the electronic supplementary material).

To validate the effects of the two treatment solutions, 150 additional eclosed workers were injected with each treatment solution, paint-marked and introduced into a fourth observation. Every 4 days, 20 individuals of each treatment were sampled from this colony and subjected to MLPA analysis to determine their DWV infection state [46]. These analyses confirmed the establishment of stable DWV infections in the DWV-lysate-inoculated bees,

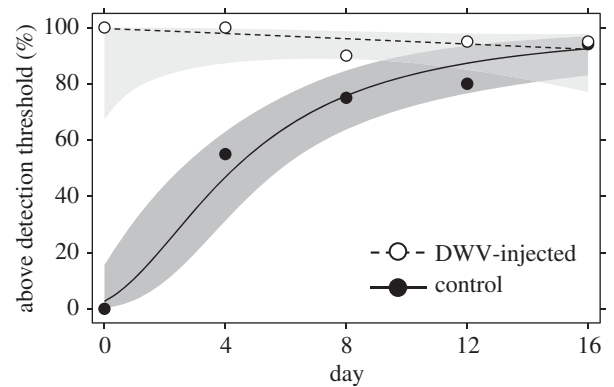


Figure 2. Treatment validation results. Evolution of the proportion of infected individuals over time in control and DWV treatment groups, based on MLPA analysis [46] of four daily sample sets of 20 individuals each from both treatments (fits and 95% CIs based on binomial GLM with treatment, $\log(x + 1)$ transformed duration after introduction into the host colony and their interaction included as independent variables).

even if there was evidence that some control bees became infected during the later stages of the experiment as well (figure 2), though possibly at lower levels (see electronic supplementary material for a full discussion of the methods and results of these analyses).

(d) Data analysis

Raw RFID tracking data were analysed with the Track-A-Forager Java application [53], which filters out rapid-succession scans of the same scanner, labels ingoing and outgoing flights by tagged workers, and corrects occasional errors in the data, including the possible occurrence of missed scans (for details see electronic

supplementary material). To compare the foraging behaviour of DWV-infected and control bees, we quantified the number of trips performed by each individual, trip duration and the proportion of the introduced workers of each treatment group which survived up to foraging age, to gauge differential early-life mortality. In addition, we measured the age at onset of foraging, defined as the age of each individual at their first reconstructed trip, the foragers' life expectancy, measured as the age of each individual at their last scan, and forager activity span, i.e. the time difference in days between the first and last registered scan of each bee. We should note that our experiment could not distinguish between a DWV-induced reduction in direct mortality and a DWV-induced mortality owing to indirect effects, e.g. caused by a decrease in homing ability or an increase in the susceptibility to predation or other environmental stressors. Details of all statistical analyses performed are given in electronic supplemental material and the R script included on the Dryad repository.

3. Results

Visual analysis of foraging activity over the course of our experiment revealed clear disparities in the age at onset of foraging, forager life expectancy and forager activity span between the DWV-infected and control bees (figure 3). In addition, bees that survived to foraging age showed a clear deviation from the 50 : 50 ratio at which they were first introduced (figure 3), with control bees evidently having much better chances to survive to foraging age than DWV-infected ones. To thoroughly examine each of these effects as well as to look for other possible effects of DWV on foraging behaviour, we conducted a number of detailed statistical analyses. In particular, we tested for significant effects of DWV on the probability that bees would survive to foraging age, the onset of foraging, forager life expectancy and activity span as well as the number and trip duration of foraging trips carried out by individuals that survived to foraging age.

(a) Effect of deformed wing virus on the likelihood that bees would survive to foraging age

The biased representation of control bees among bees that started to forage relative to the 50 : 50 ratio at which they were first introduced (figure 3) indicates that the DWV-inoculated bees experienced greater mortality early on in their life. This is confirmed by the fact that a significantly greater proportion of the DWV-inoculated bees died before making it to foraging age than control bees in each of the three host colonies (277/400 versus 244/400, 324/400 versus 266/400 and 279/400 versus 175/400; binomial colony \times treatment full factorial GLM, $z = 2.44$ and $p = 0.015$, $z = 4.61$ and $p = 4 \times 10^{-6}$, $z = 7.33$ and $p = 2 \times 10^{-13}$; figure 4a). We should note that this mortality also includes baseline mortality linked to occasional rejection of tagged bees by the host colony. To control for this baseline mortality, we also calculated the relative odds that bees would survive to foraging age. This showed that DWV-inoculated bees on average had 2.1 (s.e. 0.19) times lower odds to make it to foraging age than control bees.

(b) Effect of deformed wing virus on age at onset of foraging, life expectancy and activity span

We also found significant differences between the DWV-infected and non-infected treatments in the observed age at

onset of foraging. In particular, bees that were inoculated with DWV and which survived until foraging age starting to forage 2.31 days earlier (s.e. 0.73), on average, than bees inoculated with the control solution (figure 4b; full factorial colony \times treatment ANOVA, main effect of treatment: $p = 7 \times 10^{-9}$; LR $\chi^2_1 = 33.63$, sample sizes as shown in figure 3a). In addition, infected foragers had a life expectancy that was reduced by 4.74 days on average (s.e. 0.20) compared with control bees (figure 4c; ANOVA, main effect of treatment: $p = 0.01$; LR $\chi^2_1 = 28.7$) and a total activity span that was reduced by 2.60 days (s.e. 0.79) compared with control foragers (figure 4d; ANOVA, main effect of treatment: $p = 0.0003$; LR $\chi^2_1 = 13.26$; sample sizes for both are as given in figure 3a). Hence, DWV-infected bees had a reduced life expectancy and activity span also after they started to forage, even though the onset of foraging occurred 2.3 days earlier in the DWV-infected group than in the control group. Overall, the onset of foraging (at 12–17 days) was somewhat earlier and forager life expectancy shorter (11–26 days) than that observed in mature hives (onset at *ca* three weeks and forager life expectancy of *ca* six weeks [47,54]). Nevertheless, both figures were comparable to those found in other studies that used a comparable observation hive set-up (e.g. onset of foraging at *ca* 10 days and forager life expectancy of 14–40 days [55]). This discrepancy may be linked with stress induced by the RFID tags or the injection itself, as stress in honeybees is known to induce precocious foraging and shorten worker lifespan [32,56]. Even so, we expect our conclusions to be robust as our treatment effects are all measured relative to the control under identical conditions.

(c) Effect of deformed wing virus on number of foraging trips or trip duration

Although DWV infection could in principle also have affected the number of foraging trips and the duration of the trips carried out by individuals that survived to foraging age [33], our statistical analyses revealed that there was no strong evidence for this. For example, the effect of treatment on the number of trips performed was inconsistent and different across the replicate host colonies used (quasi-Poisson GLM, effects of treatment in colonies A, B and C: A. $z = 7.55$ and $p = 1 \times 10^{-7}$, B. $z = -2.49$ and $p = 0.04$; C. $z = -1.38$, $p = 0.42$). On average, across all three colonies, workers made 5.46 trips per bee per day (s.e. 0.50) in the DWV-inoculated group versus 5.85 trips per bee per day (s.e. 0.80) in the control (sample sizes as in figure 3a), which are figures consistent with those given in other studies [47,54]. Nevertheless, given that DWV infection strongly reduced the chances for workers to survive to foraging age and that DWV infection reduced the life expectancy and total activity span of foragers (cf. results above), it is clear that DWV still had a strongly negative overall effect on the net number of trips and amount of foraging performed. Similarly, the effect on trip duration was not consistent across host colonies, as DWV-inoculated bees made significantly longer trips than control bees in colony A (gamma GLMM, Tukey post-hoc test, 1.86 h versus 1.22 h, s.e. 0.17 and 0.08, $p = 0.0004$), whereas there was no significant effect on trip duration in colony C (2.89 h versus 2.50, s.e. 0.41 and 0.47, $p = 0.90$), and an opposite trend in colony B than in colony A (1.62 h versus 1.99 h, s.e. 0.12 and 0.27, $p = 0.39$). Inclusion of time or worker age as explicit linear or polynomial terms, either in interaction with treatment or not, did not

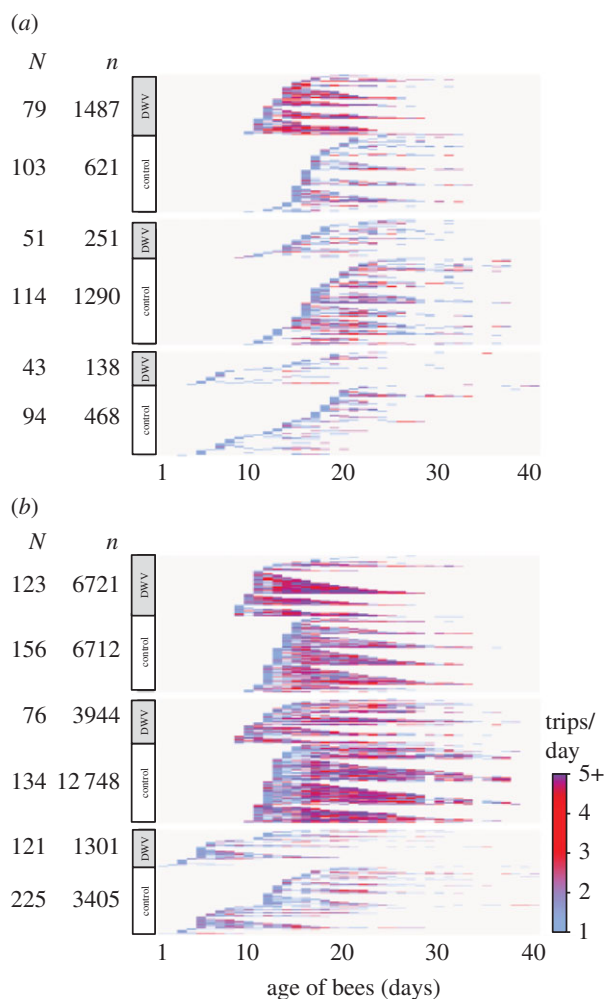


Figure 3. Total foraging activity of DWV-infected and control bees. The daily foraging activity over the course of the experiment is shown based on both the reconstructed foraging trips (a) and the unfiltered RFID scans (b) for colonies A (top), B (middle) and C (bottom) (N = total number of unique bees detected per treatment and host colony, n = total number of reconstructed foraging trips or scan events across all tracked bees). Individuals, represented as rows in the diagram, are sorted by treatment, age at onset of foraging and total activity span. The overrepresentation of control bees among observed foragers demonstrates that control workers had higher odds to survive to foraging age, and visual analysis of the data also indicate disparities between DWV and control bees in the age at onset of foraging, forager life expectancy and activity span (cf. figure 4). (Online version in colour.)

improve the explanatory power of any of the fitted models, and hence such analyses were not further pursued.

4. Discussion

Overall, our results demonstrate that DWV infections have strongly deleterious effects on adult honey bees, with both mortality rates, and—to a lesser extent—-foraging behaviour being clearly affected. In particular, DWV-infected bees started to forage at an earlier age and showed reduced lifespans and total activity spans than control bees. Finally, next-gen sequencing demonstrated that the DWV strain we used for inoculation belonged to the type B DWB master variant [52], which has recently been found to be an emergent, more virulent strain of the DWV virus [25] that currently appears to be the most common strain in Britain [25]. The fact that our DWV lysate

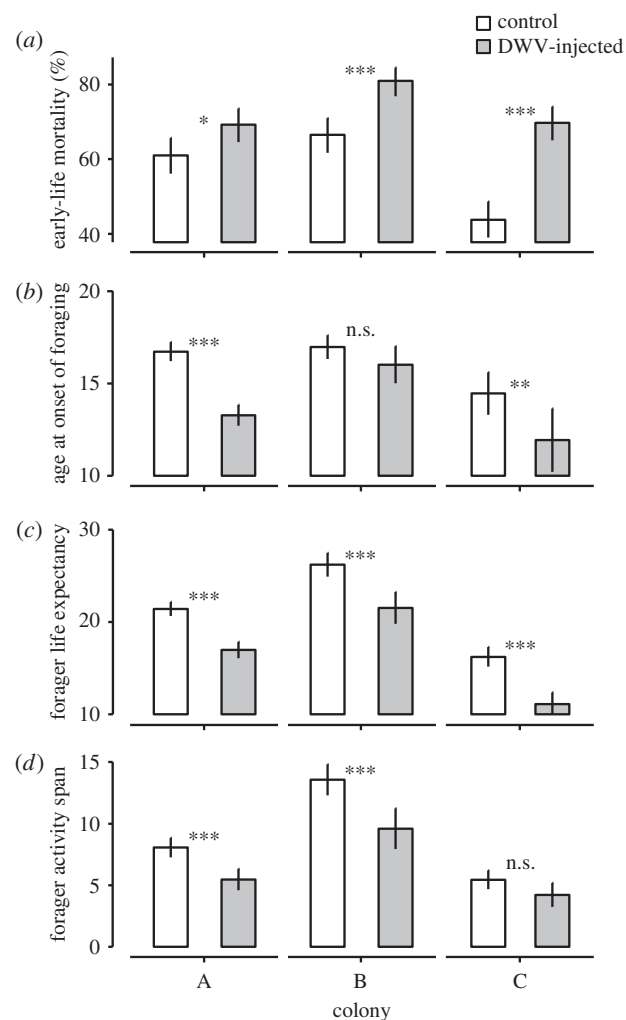


Figure 4. Effect of DWV infection on honeybee foraging and survival. Out of the 400 bees that were tagged per treatment and host colony, a significantly smaller proportion survived to foraging age (being detected at least once by the RFID scanners) in the DWV injected group than in the control group (panel a, binomial full factorial GLM, overall $p < 2 \times 10^{-16}$, LR $\chi^2_1 = 71.89$; means and 95% C.L.s are shown and significance levels per colony based on Tukey post-hoc Wald z tests shown by asterisks). In addition, honeybees that were artificially infected with DWV and which survived to foraging age started to forage significantly earlier than uninfected control bees (panel b, two-way full factorial ANOVA, $p = 7 \times 10^{-19}$, LR $\chi^2_1 = 33.63$; means and 95% C.L.s are shown and significance levels per colony based on Tukey post-hoc Wald z tests shown by asterisks; total number of tracked bees as in figure 3a), and DWV-infected foragers had a significantly reduced life expectancy (defined as age at last detection, panel c; ANOVA, $p = 0.01$, LR $\chi^2_1 = 28.7$) and activity span (defined as age at last foraging trip minus age at first foraging trip, panel d; ANOVA, $p = 0.0003$, LR $\chi^2_1 = 13.26$) (means and 95% C.L.s are shown and significance levels per colony based on Tukey post-hoc Wald z tests shown by asterisks; total number of tracked bees and foraging trips as in figure 3a).

was prepared from a randomly selected sample of bees with overt DWV infection symptoms suggest that this strain is now also common in Continental Europe.

Our finding that DWV-inoculated workers started foraging at an earlier age and experienced so-called precocious foraging was in line with expectation, as previous studies have also found that unhealthy or stressed honeybee workers start to perform risky foraging tasks at an earlier age compared with healthy individuals. For example, *Nosema* [13,57,58], sacbrood virus [59] and *Varroa* [60,61] have all been found to induce

precocious foraging in honeybees. From an ultimate perspective, diseased or health compromised workers have been suggested to benefit from starting to foraging earlier as a way to protect other individuals inside the nest from getting infected [58] or to make the most of their reduced lifetime [56]. In addition, it would be possible that the disease agent itself benefited from an earlier onset of foraging if this promoted its horizontal transmission to other host colonies [62]. Indeed, in the case of DWV, a direct influence on the behaviour of its host is not unlikely, given that DWV particles have previously been found in the mushroom bodies—a key higher brain centre of these insects [31] (but see [34] for a study where no behavioural effects were found). Irrespective of these possible adaptive causes, it is clear that precocious foraging would have a major effect on colony well-being, as premature foraging partially depletes the nurse bee population [63] and disrupts various activities inside the hive [64–66], and rapid behavioural maturation has been shown to strongly accelerate the failure of stressed honeybee colonies [63].

The strong evidence we found for a DWV-induced effect on mortality patterns and long-term survival was more unexpected. Traditionally, secondary DWV infections in adult workers are regarded as ‘covert’ and largely asymptomatic [29,67,68], but this proposition is clearly challenged by our findings, which document very clear and significant long-term effects of the virus. Although increased mortality has been documented in bees that display overt DWV infection symptoms and crippled wings [69–71], similar mortality in bees that acquired the virus in the adult stage has been demonstrated only recently in experimentally caged and non-foraging bees [25]. Our results now show that this mortality effect continues after the onset of foraging, and that the virus therefore acts as a long-term stressor on honeybee health and survival. DWV-induced mortality could have several causes. Given that DWV have been shown to occur in the honeybee brain, including in the mushroom bodies [31,62,72], which are involved in learning and memory, and that DWV infections have been shown to induce learning deficits [30], it is possible that increased mortality is caused by impaired orientation capabilities or predator avoidance or that it makes them more susceptible to other environmental stressors. Indeed, the DWV Kakugo strain has earlier been found to be associated with increased aggression and risk-taking behaviour [73,74]. Alternatively, it is possible that DWV directly results in increased mortality, e.g. owing to costly upregulation of the host’s immune system [75]. However, given the well-documented effect on direct, early-life mortality, both in our study and that of McMahon *et al.* [25], we consider a direct mortality effect most likely. Furthermore, and regardless of the underlying causes, it is clear that the early disappearance of DWV-infected bees and their significantly shorter activity spans would have strongly deleterious effects on the total amount of pollen and nectar foraging performed by infected colonies. Additionally, a shorter activity span of workers would also cause fewer workers to engage in discovering novel food patches, thereby impacting the flow of information and causing further synergistic costs to global colony health [47].

The fact that in our RFID data, DWV inoculation did not affect trip duration or the number of trips performed by DWV-infected foragers went against the conclusions of [33], who concluded that DWV infection but not *Nosema ceranae* reduced average trip duration. As there was significant

variation in the impact of the virus across our three replicate host environments, however, it is possible that the same effect would still have been found with a larger number of replicate donor and/or host colonies. Given that our donor bees all came from a single, rare uninfected colony, we had *a priori* not expected any large variation in the impact of the virus. Possible reasons for this variation could be linked with seasonal factors, variation in the genetic compatibility with the host colonies, or subtle differences in the performance or health of the host colonies, such as the possible presence of *Nosema* among the host workers, which we did not explicitly look at, but which is known to cause precocious foraging and affect longevity, activity and out-of-hive performance of honeybees [13,45,58,76,77]. Alternatively, it is possible that the variation in DWV impact is linked to some of the control bees having become infected during the later stages of our experiment, which our treatment validation results suggest may have been the case (though likely at lower levels, figure 2), and that the speed at which this occurred differed across host colonies. These results also suggest that a single dsRNA injection was not sufficient to fully protect bees for extended periods of time, and that continued oral administration would have to be used for effective long-term control via RNA interference [49,50,78]. Despite this variation in the effect of the virus, however, it was clear that overall, DWV had a strongly deleterious effect across all three colonies, with significant effects on early-life mortality (figure 4a), forager life expectancy (figure 4c) and forager activity span (figure 4d) in three replicate host colony environments, and significant effects on the onset of foraging in two out of three host colonies (figure 4b). These findings are consistent with studies showing that DWV is among the most important predictors implicated in honeybee colony declines in both Europe [14,16,17,24] and the USA [18] and hence an important contributory factor to the current pollination crisis.

Overall, our results highlight the impact of long-term stressors on bee health and survival, thereby reinforcing the conclusions of several recent tracking studies that have studied stress-induced changes in bees caused by either pathogens [33,43–45], nutritional stress [36] or pesticide exposure [9,11,35,37–40]. We hope that in the future, these approaches may continue to be used to further our understanding of the factors involved in the ongoing pollinator declines [1–5] and how they interact with each other in exerting long-term stress [2,5–10].

Data accessibility. All raw data files and code used in analyses are available in Dryad: <http://dx.doi.org/10.5061/dryad.fm0r1>. Illumina reads and the full genome sequence of the DWV inoculate are available from the Sequence Read Archive and GenBank (accession nos. PRJNA336281 and KX783225).

Authors’ contributions. K.B., D.C., D.C.d.G., L.S. and T.W. conceived and designed the work; K.B., D.C. and L.D.S. performed the experiments; K.B., A.V.G. and T.W. analysed the data; L.D., D.C.d.G., L.D.S., L.B., S.J.M. and T.W. contributed reagents, materials, or analysis tools and K.B., M.H.D.L., L.B., S.J.M. and T.W. wrote the paper. All authors gave final approval for publication.

Competing interests. We have no competing interests.

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