npj | sustainable agriculture

Brief communication

https://doi.org/10.1038/s44264-024-00011-7

Green biomanufacturing of edible antiviral therapeutics for managed pollinators

Vincent A. Ricigliano ✉, Alexander McMenamin, Allyson Martin Ewert, Daniela Adjaye, Michael Simone-Finstrom & Victor P. Rainey

Managed bees are important agricultural pollinators threatened by declines linked to multi-host RNA viruses. Here, we developed a novel antiviral platform for bees using the edible photosynthetic cyanobacterium *Synechococcus elongatus* UTEX 2973. Cyanobacterial biomass was engineered to induce RNA interference (RNAi) immune responses when fed to bees. Treatments targeting deformed wing virus—a notorious pathogen—suppressed viral infection and improved survival in honey bees. This design presents a versatile and sustainable therapeutic that can be directly incorporated into supplemental feeds for managed pollinators to mitigate viruses and support global food security.

Pollination is an essential ecosystem service that maintains biodiversity and is the foundation of agricultural crop production. Most pollination is performed by bees, including widely managed honey bees and bumble bees as well as unmanaged wild bees[1,2]. Unprecedented levels of colony mortality are currently threatening beekeeping industries and global food security[3,4]. These losses are strongly linked to synergistic interactions between RNA viruses and parasitic mites[5,6]. Under global change, fast-evolving bee viruses are an increasing risk due to their propensity to shift hosts[7]. Antiviral treatments are urgently needed for managed bees, which could help maintain food production and reduce pathogen spillover to wild pollinators.

Bee antiviral immunity relies on RNAi, a conserved pathway triggered by double-stranded RNA (dsRNA) that targets and degrades similar RNA sequences[8]. dsRNA delivered by feeding, or recently by engineered gut bacteria, can trigger an RNAi response to knock down gene expression and inhibit the replication of RNA viruses[8–11]. However, these approaches are expensive and/or difficult to scale thus far[12,13].

Here, we used the edible cyanobacterium *Synechococcus elongatus* UTEX 2973 (henceforth referred to as S2973) to produce RNAi therapeutics for bees. Cyanobacteria are promising cellular factories for carbon-negative biomanufacturing due to their photosynthetic growth and easy scalability[14]. Cyanobacterial biomass has historically been part of human diets[15] and is nutritious to livestock animals[16], including honey bees[17–19]. We engineered S2973 to express recombinant dsRNAs that require no purification prior to delivery, are stable when mixed into feed, and protected during bee gastric transport. Next, we developed antiviral strains and tested their efficacy in virus-challenged honey bees.

Cyanobacteria have been used for the manufacture and oral delivery of protein therapeutics[20,21] but have not yet been developed for RNAi applications. We designed a set of modular genetic parts to engineer dsRNA production in S2973 (Supplemental Fig. 1). Parts consisted of two synthetic inverted promoters lacking ribosome binding sites that flank the target sequence for dsRNA production (Fig. 1a). A dsRNA expression cassette containing the yellow fluorescent protein (YFP) coding sequence was introduced into S2973 by homologous recombination with an integrative vector to generate strain S2973-YFP. Once genomic integration was confirmed using primers flanking the integration site (Fig. 1b), the strain was maintained up to 19 months without selection and dsRNA expression was periodically checked by RT-qPCR (Fig. 1c). dsRNA accumulation was stabilized by CRISPR-Cas9-mediated knockout of S2973's RNase III (Fig. 1c and Supplementary Figs. 3 and 4), a non-essential dsRNA-specific endonuclease.

Fresh S2973 biomass was harvested by centrifugation then mixed into a pollen-sugar paste for feeding bees (Fig. 1d and Supplementary Fig. 6.). This formulation approach is used by beekeepers to give colonies supplemental nutrition in the form of pollen patties[20,22]. dsRNA was stable in food for at least 7 days at 34 °C and 50% relative humidity, the internal conditions maintained by a honey bee colony (Fig. 1e, f). Diets were uniformly consumed and did not negatively impact bee survival (Supplementary Fig. 6d, e). Bees were fed vector control strain S2973-NR or strain S2973-YFP and then sampled over time. YFP RNA was detected in the abdomens of bees fed S2973-YFP for at least 11 days post-feeding but never in bees fed S2973-NR (Fig. 1g). Since caged bees do not defecate, this result may be due to undigested S2973 accumulation in the hindgut and not necessarily biologically active YFP dsRNA available to induce an RNAi response. On the other hand, reduction of YFP signal over the 11-day period indicates gradual digestion of S2973 biomass, which in a colony setting could act to time-release dsRNA into the gut lumen of young bees engaged in food consumption.

Next, we engineered S2973 to induce RNAi against deformed wing virus (DWV), a notorious pathogen linked to the deaths of millions of honey bee colonies worldwide[5,6]. Two dsRNA-expressing strains (S2973-DWV1 and S2973-DWV2) targeted different regions of the DWV genome (Fig. 2a). Adult bees were fed the formulated strains ad libitum for 4 days and then

USDA-ARS, Baton Rouge, LA, USA. ✉e-mail: Vincent.Ricigliano@USDA.gov

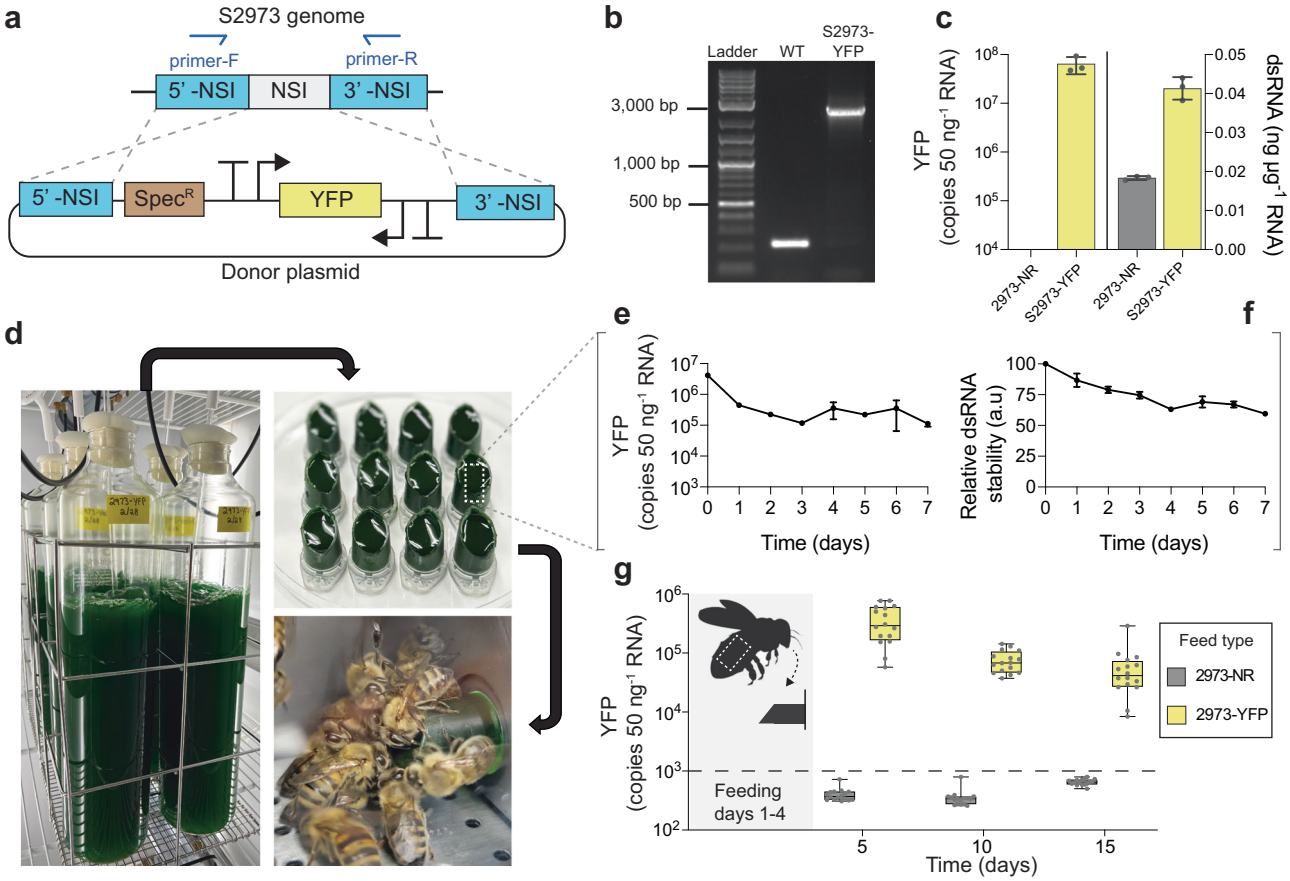

Fig. 1 | Engineering S2973 for in-feed delivery of dsRNA. a Integration of dsRNA expression cassettes into the S2973 genome. Cassettes produce target dsRNA with an inverted arrangement of two promoters and are flanked by sequences for homologous recombination at neutral site I (NSI). Primers used for PCR genotyping are noted. **b** Segregation analysis of a YFP dsRNA expression cassette. Wild type (WT) strain had a PCR product of 234 bp and no WT copy was present in strain S2973-YFP. **c** dsRNA production. Vector control strain S2973-NR did not express YFP RNA. dsRNA accumulation by S2973-YFP was twice that of endogenous background levels as measured by dsRNA ELISA. **d** Biomass production and feed formulation. Fresh S2973 biomass was mixed into a pollen-sugar paste for feeding bees. **e** YFP RNA stability and **f** relative dsRNA stability in formulated feeding treatments under colony conditions (34 °C and 50% relative humidity). **g** Detection of YFP RNA in bees fed S2973 diets and then sampled over time. Bee abdomens with guts intact were used for this analysis. YFP RNA was detected in bees fed S2973-YFP for at least 11 days after their last feeding ($n = 16$).

injected with DWV. This assay mimics the natural route of DWV transmission via parasitic mites feeding on bees[5]. Preliminary trials indicated that the S2973-DWV2 strain can limit virus replication and increase bee survival (Supplementary Fig. 8c–e). In a larger experiment, dsRNA strains significantly reduced DWV levels (Fig. 2b) and upregulated gene expression of *dicer*, the initiating step of the RNAi pathway (Fig. 2c). These results indicate immune modulation by dsRNA strains, but not by S2973-NR (Supplementary Fig. 9). Honey bees have a non-specific dsRNA-mediated antiviral response[23], which may explain *dicer* upregulation and DWV reduction in bees fed S2973-YFP (Fig. 2b). Strain S2973-DWV2 had the strongest antiviral effects and kept bees alive the longest. After DWV injection, median survival was 19 days for bees fed S2973-DWV2 and 11 days for bees fed S2973-NR (Fig. 2d). Therefore, engineered S2973 treatments can induce sequence-specific antiviral RNAi against DWV.

In a honey bee colony, horizontal RNA flow is mediated by adult secretions and larval ingestion of jelly diets, which can trigger a persistent RNAi response[24]. Since exogenously applied dsRNA treatments could impact the bee lifecycle, we tested the effects of larval exposure to engineered S2973. Young larvae were reared in vitro (Fig. 2e, Supplementary Fig. 7) and then injected with DWV upon pupation. Bees reared on jelly diets containing S2973-DWV2 had significantly lower DWV levels (Fig. 2f) and developed fewer deformities relative to controls (Fig. 2g, h). In contrast to immune activation by S2973-YFP in adults, the non-specific dsRNA did not impact DWV levels in pupae. Nevertheless, DWV sequence-specific antiviral effects persist across bee life stages and can lessen characteristic DWV symptomology.

In summary, this work developed an effective antiviral platform for bees. The use of cyanobacteria as biofactories for edible therapeutics offers a highly scalable and sustainable approach to mitigate bee viruses and other pathogens. We previously showed that spirulina, a related cyanobacterium, is a viable feed additive for commercially managed honey bees involved in crop pollination[17]. Conceivably, beekeeping operations could use engineered feed to protect colonies against emerging pathogen threats or as a delivery system for gene therapies and nutritional modulations to improve bee health. Future research with appropriate biocontainment precautions is necessary to understand the impact of the design on entire colonies and how crop pollination efficiency may be affected. Several important questions remain to be answered such as persistence of antiviral effects, potential off-target effects, and a benefit-cost analysis of extended immune activation in bees.

Methods

Bacterial strains and growth

E. coli DH5α was grown at 37 °C in LB media supplemented with the following antibiotics when necessary: carbenicillin (100 µg ml^{-1}), kanamycin (50 µg ml^{-1}), and spectinomycin (50 µg ml^{-1}). *Synechococcus elongatus* UTEX 2973 (S2973) was grown in BG-11 agar media under continuous white light (300 µmole photons m^{-2} s^{-1}) at 38 °C. Spectinomycin

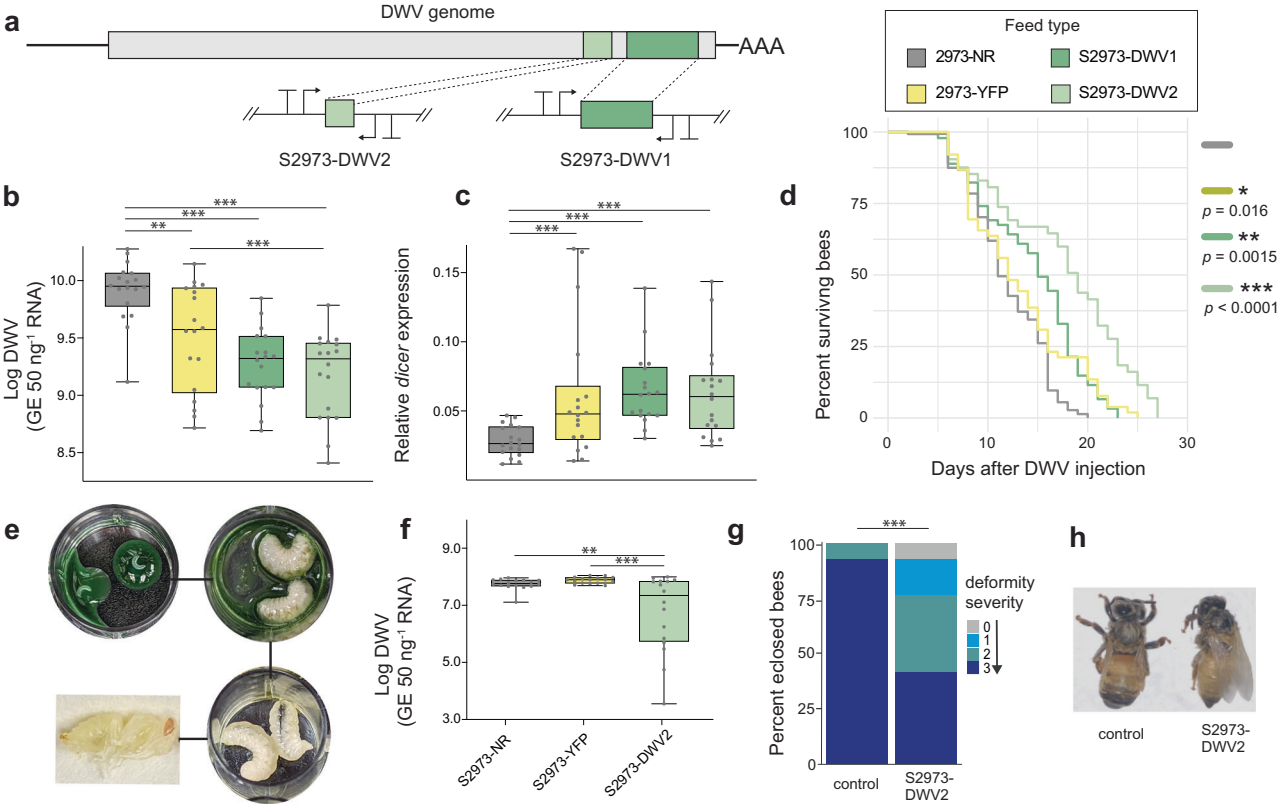

Fig. 2 | Suppression of deformed wing virus in honey bees. a Construct design for antiviral strains S2973-DWV1 and S2973-DWV2. **b** Viral load and **c** relative *dicer* expression in adult bees 72 h after DWV injection ($n = 18$). **d** Survival curves of adult bees following DWV injection. Bees fed dsRNA strains showed increased survival relative to bees fed S2973-NR. Bees fed S2973-DWV2 lived the longest (median survival = 19 days, total $N = 549$ bees). **e** Treatment persistence across life stages. Larvae were reared on jelly diets±S2973 then injected with DWV at pupation. **f** Viral loads in pupae 72 h after DWV injection ($n = 16$). **g** Reduced DWV symptomology in bees reared on S2973-DWV2 compared to a jelly-only control diet and **h** representative phenotypes of each treatment group. Bees reared on S2973-DWV2 showed little to no wing deformities. Linear models were used to assess pairwise differences in **b**, **c**, **f** and **g** with a Benjamini-Hochberg FDR correction for multiple comparisons. Differential survival **d** was assessed by Cox Proportional Hazards model. Asterisks denote statistical significance ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

(25 μg ml^{-1}) or kanamycin (50 μg ml^{-1}) were added to the media when necessary for selection. S2973 cultures were grown in 20–100 ml of liquid BG-11 medium to prepare strains for transformation or as seed cultures. Primary cultures were grown in 3 L of BG-11 medium with sterile, ambient aeration.

Design of dsRNA expression cassettes

Parts for dsRNA expression in S2973 were designed for compatibility with the assembly library and syntax of the CyanoGate cloning suite for engineering cyanobacteria[25]. We designed two new DNA parts: (1) an upstream terminator followed by a J23119 promoter without a ribosome binding site and (2) an inverted J23119 promoter, also without a ribosome binding site, followed by a flanking terminator sequence (Supplementary Fig. 1). dsRNA target sequences with BsaI sites and compatible overhangs were designed in silico and synthesized by Integrated DNA Technologies. Promoter and target parts were combined with an acceptor vector by BsaI assembly to generate dsRNA expression cassettes (Supplementary Fig. 2). All plasmids used in this work are listed in Supplementary Table 1.

Stable transformation of S2973

Integrative constructs consisted of a RSF1010 origin-containing vector backbone with a dsRNA expression cassette, spectinomycin resistance cassette, and flanking sequences for homologous recombination at neutral site 1 in the S2973 genome (Fig. 1a). Parts were combined by BbsI assembly (Supplementary Fig. 2). Constructs were introduced into S2973 by conjugation with *E. coli* DH5α harboring cargo and mobilization plasmids as previously described[25]. Segregation of the cargo plasmid was achieved by repeated subcultures in nonselective BG11 liquid media. Spectinomycin resistant colonies were screened by PCR using primers for integration at neutral site 1 and for segregation of the cargo plasmid.

CRISPR-Cas9 modification of S2973

An expression cassette for dCas9 with a C-terminal protein degradation tag was combined with single guide RNA (gRNA) expression cassette targeting the S2973 RNase III gene (Supplementary Fig. 3). For assembly of the gRNA expression cassette, we targeted a 22 bp region of the RNase III coding strand with an adjoining 3′ protospacer adjacent motif. The complete vector containing the dCas9 expression cassette, gRNA expression cassette, and gRNA scaffold was generated by BbsI assembly and introduced into 2973 by conjugation. This modification led to efficient dsRNA production (Supplementary Fig. 4). Thus, a RNase III deletion background was used for all dsRNA strains.

dsRNA production by S2973

For absolute quantification of YFP RNA copies, primer targets were cloned into a plasmid to serve as a standard for RT-qPCR. dsRNA production was quantified by a dsRNA antibody-based colorimetric sandwich ELISA assay (Novus Biologicals) following manufacturer's instructions. This assay selectively detects dsRNA with little to no cross-reactivity with other nucleic acid species. Briefly, the K1 (IgG2a) antibody was used to coat polystyrene microwell plates to capture dsRNAs and the K2 (IgM) antibody was used as the downstream detector antibody. Assays were developed for colorimetric detection with a horseradish peroxidase linked anti-mouse secondary antibody and absorbance was read at a test wavelength of 450 nm and an off-peak reference at 650 nm. A linear standard curve was prepared for each assay with poly(I:C) in a 3-fold series from 25 ng to 0.01 ng/well.

Interpolation from the standard curve was used to estimate test sample dsRNA quantities based on the reference wavelength-corrected absorbance of the sample.

Antiviral S2973 strains

We designed dsRNA expression constructs targeting deformed wing virus (DWV) based on an infectious cDNA clone of DWV-A (GenBank# MG831200, Supplementary Fig 5.) Gene synthesis of target sequences (Supplementary Table 2) was performed by Integrated DNA Technologies and BsaI sites were added to facilitate cloning into dsRNA expression cassettes. Assembly of dsRNA cassettes and integrative vectors were carried out as described above. The constructs were introduced into S2973 by conjugation to generate strains S2973-DWV1 and S2973-DWV2 (Fig. 2a).

Adult honey bees and feeding setup

Honey bees (*Apis mellifera)* were acquired from colonies of the Ricigliano Lab at the USDA-ARS Honey Bee Lab in Baton Rouge, LA, USA. Newly emerged workers (<24 h old) from three different colonies were obtained by incubating sealed brood combs overnight at 34 °C and 50% relative humidity. Bees were randomly assigned to feeding treatment cages (50-60 bees per cage and 3-4 cage replicates per treatment). All cages were provided *ad libitum* access to drip feeders containing 50% (w/v) sucrose solution. For feeding treatments, fresh S2973 biomass was harvested by repeated centrifugation (5 min at 18,213 × *g*). Diets were formulated by mixing 1 g of biomass, 2.5 g of pollen, and approximately 1 ml of sugar syrup into a thick paste. All diets were prepared with the same batch of mixed corbicular pollen that was freshly collected from returning forager bees using hive entrance-mounted pollen traps and frozen at −80 °C until used. This recipe was sufficient to feed four cage replicates each 1 g of formulated diet paste loaded into modified 1.5 ml microcentrifuge tubes (Supplementary Fig. 6a). Feeding tubes were replenished daily with 1 g of freshly prepared diet over a four-day period. Dead bees were counted and removed from the cages daily. Diets treatments were uniformly consumed (Supplementary Fig. 6b–d) and did not negatively impact bee survival (Supplementary Fig. 6e).

Honey bee in vitro larval rearing

Larvae were reared in the laboratory following standard protocols and using a royal jelly-containing base diet[26]. On day 1, first-instar larvae from two different colonies were grafted into pools of base diet in sterile 24-well plates and maintained in darkness at 34 °C. On day 2, larvae were transferred to 30 µL aliquots of base diet or S2973-containing base diets in fresh 24-well plates (Supplementary Fig. 7a). S2973 biomass was added to the base diet at a concentration of 40 mg ml^{-1}. Larvae received an additional 30 µL, 40 µL, and 50 µL of freshly prepared treatment diet on days 5,6 and 7, respectively. Dead larvae, identified as lacking peristalsis and respiration activity or as appearing generally discolored, sunken, or deflated, were removed daily. S2973 diets had minimal impact on larval survival to pupation (Supplementary Fig. 7b)

Antiviral assays with DWV

An infectious clone of DWV-A[27] was used as the source of viral inoculum. Crude virus particles were propagated by inoculating pupae and harvesting by filter purification[28]. DWV was quantified in each preparation by RT-qPCR (Supplementary Fig. 8a) and a standardized dose of viral genome equivalents (GE) was used to inject bees. For antiviral assays in adult bees, cages were setup and fed S2973-containing diets as described above. After 4 days of *ad libitum* feeding, bees were anesthetized with CO_2 and virus was injected using an UltraMicroPump with an SYSMicro4 controller (World Precision Instruments) with an infusion flow rate of 1.5 µl/s. For each injection, a dose of 10^6 viral genome copies was administered using a 30G needle (12 degree bevel) inserted between the 2nd and 3rd abdominal tergites (Supplementary Fig. 8b). Bees were sampled 72 hours post injection for absolute quantification of virus levels by RT-qPCR using a DWV plasmid standard curve. We present three experimental repeats demonstrating antiviral efficacy in adult bees (Fig. 2b–d, Supplementary Fig. 8c–e). For antiviral assays in pupae, larvae were reared on jelly diets containing S2973 as described in above. Prepupae were transferred to filter papers in petri dishes and white-eyed pupae were injected with 10^4 viral genome copies. Pupae were sampled 72 h post injection for quantification of virus levels by RT-qPCR. For scoring deformity severity, DWV-injected pupae were allowed to eclose as adults and then wing deformity was scored on a scale from 0 (no deformity) to 3 (severe wing deformity). We present three experimental repeats demonstrating antiviral efficacy in pupae (Fig. 2f, g, h and Supplementary Fig. 10).

Bee RNA isolation and RT-qPCR

Bee samples were collected into 2 ml bead beater tubes (MP Biomedicals) and stored at −80 °C prior to processing. Samples were homogenized in 600 µL of Maxwell® simplyRNA homogenization solution (Promega). RNA extractions were carried out according to the Maxwell® RSC simplyRNA tissue kit protocol (Promega). cDNA synthesis was carried out using 1 µg of DNAase-treated total RNA and QuantiTect Reverse Transcription Kits (Qiagen) according to the manufacture's protocols. RT-qPCR was performed using the primers listed in Supplementary Table 3. The reactions were carried out using Luna® Universal qPCR Master Mix (New England Biolabs) in triplicate on a CFX96 Real-Time PCR Detection System (Bio-rad). Plasmid standards were used for absolute quantification of DWV genome copies and YFP. Relative expression levels were calculated based on standardized Ct values (Δ Ct) using honeybee *β-actin* for normalization.

Statistical analyses

All statistical analyses were performed in R version 4.1.2. Differences in group means were assessed by linear regression using the base R *stats* package. Pairwise analyses of group means were assessed using the *emmeans* package[5] with a Bonferroni multiple comparisons correction with an FDR of 5%. Model fit was visually evaluated with quantile-quantile plots. All fixed effect structures were determined *a priori* with no model selection techniques applied for model refinement. Differences in group survival were measured by constructing Cox Proportional Hazards models using the the *survival* package[6].

Received: 14 January 2024; Accepted: 27 February 2024;
Published online: 19 March 2024

References

1. Bartomeus, I. et al. Contribution of insect pollinators to crop yield and quality varies with agricultural intensification. *PeerJ* **2**, e328 (2014).
2. Klein, A.-M. et al. Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B: Biol. Sci.* **274**, 303–313 (2007).
3. Potts, S. G. et al. Global pollinator declines: trends, impacts and drivers. *Trends Ecol. Evol.* **25**, 345–353 (2010).
4. Bruckner, S. et al. A national survey of managed honey bee colony losses in the USA: results from the Bee Informed Partnership for 2017–18, 2018–19, and 2019–20. *J. Apicult. Res.* **62**, 429–443 (2023).
5. Traynor, K. S. et al. Varroa destructor: a complex parasite, crippling honey bees worldwide. *Trends Parasitol.* **36**, 592–606 (2020).
6. McMahon, D. P. et al. Elevated virulence of an emerging viral genotype as a driver of honeybee loss. *Proc. R. Soc. B: Biol. Sci.* **283**, 20160811 (2016).
7. Proesmans, W. et al. Pathways for novel epidemiology: plant–pollinator–pathogen networks and global change. *Trends Ecol. Evol.* **36**, 623–636 (2021).
8. McMenamin, A. J., Daughenbaugh, K. F., Parekh, F., Pizzorno, M. C. & Flenniken, M. L. Honey bee and bumble bee antiviral defense. *Viruses* **10**, 395 (2018).
9. Hunter, W. et al. Large-scale field application of RNAi technology reducing Israeli acute paralysis virus disease in honey bees (Apis mellifera, Hymenoptera: Apidae). *PLoS Pathogens* **6**, e1001160 (2010).

10. Leonard, S. P. et al. Engineered symbionts activate honey bee immunity and limit pathogens. *Science* **367**, 573–576 (2020).
11. Xue, Q., Avila dos Santos, É., Smagghe, G., Zotti, M. J. & Taning, N. T. C. Engineering strategies for insect viruses and microbes for dsRNA production and delivery to insects for targeted gene silencing. *Entomol. Gen.* **43**, 31–53 (2023).
12. Kunte, N., McGraw, E., Bell, S., Held, D. & Avila, L. A. Prospects, challenges and current status of RNAi through insect feeding. *Pest Manag. Sci.* **76**, 26–41 (2020).
13. Motta, E. V., Powell, J. E., Leonard, S. P. & Moran, N. A. Prospects for probiotics in social bees. *Philos. Trans. R. Soc. B* **377**, 20210156 (2022).
14. Luan, G. & Lu, X. Tailoring cyanobacterial cell factory for improved industrial properties. *Biotechnol. Adv.* **36**, 430–442 (2018).
15. Grosshagauer, S., Kraemer, K. & Somoza, V. The true value of Spirulina. *J. Agric. Food Chem.* **68**, 4109–4115 (2020).
16. Madeira, M. S. et al. Microalgae as feed ingredients for livestock production and meat quality: a review. *Livestock Sci.* **205**, 111–121 (2017).
17. McMenamin, A., Weiss, M., Meikle, W. & Ricigliano, V. Efficacy of a Microalgal Feed Additive in Commercial Honey Bee Colonies Used for Crop Pollination. *ACS Agric. Sci. Technol.* https://doi.org/10.1021/acsagscitech.3c00082 (2023).
18. Ricigliano, V. A. et al. Honey bee proteome responses to plant and cyanobacteria (blue-green algae) diets. *ACS Food Sci. Technol.* **1**, 17–26 (2020).
19. Ricigliano, V. A. & Simone-Finstrom, M. Nutritional and prebiotic efficacy of the microalga Arthrospira platensis (spirulina) in honey bees. *Apidologie* **51**, 898–910 (2020).
20. Jester, B. W. et al. Development of spirulina for the manufacture and oral delivery of protein therapeutics. *Nat. Biotechnol.* **40**, 956–964 (2022).
21. Betterle, N., Hidalgo Martinez, D. & Melis, A. Cyanobacterial production of biopharmaceutical and biotherapeutic proteins. *Front. Plant Sci.* **11**, 237 (2020).
22. Noordyke, E. R. & Ellis, J. D. Reviewing the efficacy of pollen substitutes as a management tool for improving the health and productivity of western honey bee (Apis mellifera) colonies. *Front. Sustain. Food Syst.* **5**, 772897 (2021).
23. Flenniken, M. L. & Andino, R. Non-specific dsRNA-mediated antiviral response in the honey bee. *PLoS ONE* **8**, e77263 (2013).
24. Maori, E. et al. A transmissible RNA pathway in honey bees. *Cell Rep.* **27**, 1949–1959 (2019).
25. Vasudevan, R. et al. CyanoGate: a modular cloning suite for engineering cyanobacteria based on the plant MoClo syntax. *Plant Physiol.* **180**, 39–55 (2019).
26. Crailsheim, K. et al. Standard methods for artificial rearing of Apis mellifera larvae. *J. Apicult. Res.* **52**, 1–16 (2013).
27. Ryabov, E. V. et al. Dynamic evolution in the key honey bee pathogen deformed wing virus: Novel insights into virulence and competition using reverse genetics. *PLoS Biol.* **17**, e3000502 (2019).
28. De Miranda, J. R. et al. Standard methods for virus research in Apis mellifera. *J. Apicult. Res.* **52**, 1–56 (2013).

Acknowledgements

We acknowledge members of the Ricigliano Lab for technical assistance and stimulating discussions. This work was funded by USDA-ARS research plan 501-2022-050 017 and Agriculture and Food Research Initiative Grant no. 2021-67013-33556 from the USDA National Institute of Food and Agriculture. The USDA is an equal opportunity employer and provider. We thank two anonymous reviewers for comments that improved the manuscript.

Author contributions

Conceptualization: V.A.R., A.M., M.S.; data curation: V.A.R., A.M., A.M.E., D.A.; formal analysis, V.A.R., A.M., funding acquisition: V.A.R.; investigation: V.A.R., A.M., A.M.E., D.A., M.S., V.P.R.; methodology: V.R., A.M., A.M.E., D.A., M.S.; project administration: V.R.; resources: V.R.; supervision: V.R., A.M., M.S.; visualization: V.R., A.M.; roles/writing—original draft: V.R.; writing—review & editing: V.R., A.M., A.M.E., M.S.

Competing interests

Vincent Ricigliano and Michael-Simone Finstrom have filed a patent application (18/175,394) on the use of engineered microalgae to improve bee health.

Additional information

Supplementary information The online version contains

supplementary material available at https://doi.org/10.1038/s44264-024-00011-7.

Correspondence and requests for materials should be addressed to Vincent A. Ricigliano.