

Article

Associations Between Land Use, Climate, and Pathogen Prevalence in Honey Bee Colonies

Sabri Ala Eddine Zaidat ^{1,2}, Raied Abou Kubaa ^{3,4}, Giuseppe Cavallo ¹, Andrea Depalma ¹, Fabio Silvestre ⁵, Aymen Moghli ⁶, Antonio Petragallo ¹, Maria Saponari ³, Khaled Djelouah ^{1,*} and Giovanni Tamburini ²

¹ International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM-Bari), Via Ceglie 9, Valenzano, 70010 Bari, Italy; zaidatsabri@hotmail.com (S.A.E.Z.); cavallo@iamb.it (G.C.); depalma@iamb.it (A.D.); petragallo@iamb.it (A.P.)

² Department of Soil, Plant, and Food Sciences, University of Bari Aldo Moro, 70126 Bari, Italy; giovanni.tamburini@uniba.it

³ Institute for Sustainable Plant Protection, UOS Bari, National Research Council, 70126 Bari, Italy; raboukubaa@ucdavis.edu (R.A.K.); maria.saponari@ipsp.cnr.it (M.S.)

⁴ Department of Plant Pathology, University of California, Davis, CA 95616, USA

⁵ Regional Association of Apulian Beekeepers (A.R.A.P.), 73100 Lecce, Italy; fabio.silvestre.vet@gmail.com

⁶ Department of Ecology and Environment, Faculty of Nature and Life Sciences, University of Sétif 1-Ferhat Abbas, Sétif 19137, Algeria; moghliaymen@gmail.com

* Correspondence: djelouah@iamb.it

Abstract

Honey bees (*Apis mellifera*) are key pollinators in agricultural ecosystems that face increasing pressure from pathogens and environmental change. However, how these environmental factors interact remains incompletely understood. To assess associations between climate, landscape composition, and pathogen occurrence in real agroecosystems, we monitored honey bee colonies across 30 apiaries in southern Italy over two years, in summer and autumn. Molecular screening revealed widespread multi-pathogen exposure, with two viruses, Black Queen Cell Virus (BQCV) and Deformed Wing Virus (DWV), and gut trypanosomatid parasite (*Lotmaria passim*) being the most frequently detected. In contrast, *Nosema ceranae*, along with Bee Macula-like Virus (BeeMLV) and Acute Bee Paralysis Virus (ABPV), occurred at lower but still notable frequencies. Infections were generally more frequent in adult foragers than in in-hive bees and larvae, and overall pathogen occurrence tended to be higher in summer than in autumn. Higher humidity was associated with higher overall pathogen occurrence and coinfection levels, whereas higher temperature showed a weaker association with these outcomes. Associations between landscape composition and pathogen occurrence differed across pathogens: a higher proportion of semi-natural habitats was associated with lower viral occurrence, particularly BQCV and DWV; however, *N. ceranae* was more frequently detected under the same landscape conditions. In contrast, *L. passim* showed context-dependent responses, with landscape effects emerging only through interactions with humidity and temperature. Pathogen coinfections were more frequent under warm, humid conditions, although this pattern was partially buffered in landscapes richer in semi-natural habitats. Together, these results indicate that, within the studied apiaries, honey bee pathogen occurrence was associated with climate, season, and land use. These findings suggest that environmental context should be considered when interpreting honey bee health monitoring data in heterogeneous agricultural landscapes, with potential implications for apiary management.

Keywords: *Apis mellifera*; climate; landscape; *Nosema ceranae*; parasite and viruses



Academic Editor: Aleksandra Wilczyńska

Received: 26 May 2026

Revised: 26 June 2026

Accepted: 1 July 2026

Published: 3 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons](https://creativecommons.org/licenses/by/4.0/)

[Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Agricultural productivity relies greatly on wild and managed pollinators, which contribute an estimated 153 billion euros annually to crop production worldwide [1,2]. Among them, the honey bee (*Apis mellifera* L.) plays a crucial role in both agricultural and natural ecosystems, with pollination services valued at 22.8–57 billion euros per year [3,4]. However, honey bee populations have been experiencing elevated mortality since the early 2000s. Large-scale monitoring reports average losses of about 21% across several European and non-European countries, including 19.2% in Italy [5,6], while U.S. beekeepers experienced record losses of 55.6% in 2024–2025 [7]. This loss of honey bee colonies, often referred to as Colony Collapse Disorder, has been most frequently documented in North America and Europe, where large-scale unexplained colony losses with characteristic symptoms (e.g., sudden disappearance of adult worker bees) have been recorded since the mid-2000s [8,9]. It has been proposed that the decline is driven by multiple, often interacting factors, including pathogens, agricultural intensification, extreme weather, and suboptimal management, that collectively undermine colony health [10,11]. This complex convergence of biotic and abiotic pressures highlights the need for regionally adapted strategies to sustain honey bee populations.

The spread of pathogens represents a major threat to honey bee health [12–14]. Viral pathogens such as Deformed Wing Virus (DWV), Black Queen Cell Virus (BQCV), and Acute Bee Paralysis Virus (ABPV) undermine worker performance, queen development, and overall colony viability [15] and are frequently transmitted through vector-mediated routes, most notably by the ectoparasitic mite *Varroa destructor* [14]. Fungal infections, including *Nosema ceranae*, impair digestion and immunity in adult bees, while opportunistic fungi like *Aspergillus flavus* can induce brood disease under stressful conditions [16]. Bacterial pathogens such as *Peanibacillus larvae*, the agent of American foulbrood, further threaten larval survival. Crucially, these pathogens and parasites often occur simultaneously, compounding their effects on colonies [15], and their impact is further linked to environmental factors such as climate variability and landscape composition, which influence both the availability of nutritional resources and the risk of pesticide exposure. However, field studies that investigate the interactions among climate, landscape composition, and multi-pathogen dynamics in real landscapes remain relatively scarce.

Honey bee health and pathogen dynamics are strongly associated with climatic factors like temperature and humidity [17,18]. Extreme heat or cold episodes can overwhelm colony thermoregulation, slow or damage brood development, and weaken immune responses (e.g., disrupted brood homeostasis under heat stress) [19–21]. High humidity often favors microsporidian or fungal pathogens via better spore survival and transmission, as seen for *N. ceranae* [22]. Sudden drops or fluctuations in winter temperature impose energetic stress and increase mortality, especially in colonies already weakened by disease [23]. Because different pathogens respond differently to climatic conditions, determining how local climate is correlated with overall pathogen occurrence in bees remains a key research direction.

Understanding how land use shapes pathogen impacts on honey bees is another critical step towards supporting colony health in real agroecosystems [24,25]. Landscapes dominated by intensive agriculture or urban development often reduce the diversity and continuity of floral resources while simultaneously increasing pesticide exposure, leading to nutritional stress and weakened immunity, which directly affect bee nutrition and immune functions (habitat–disease relationship) [24,26]. In intensively managed regions, these stressors accumulate, leaving colonies with depleted lipid reserves and greater vulnerability to pathogens before overwintering, which heightens the risk of winter collapse [27,28]. Habitat simplification can also alter infection probability through changes in wild pollinator

communities (biodiversity–disease relationships) [29]. Landscapes characterized by abundant, diverse floral resources and low pesticide risk can enhance colony resilience, yet they may also increase opportunities for pathogen transmission by concentrating pollinators on abundant floral resources [12,29]. For example, monocultures of mass-flowering crops can elevate pathogen prevalence, reshaping foraging networks and increasing contact rates among pollinators [30,31]. The system is, hence, highly complex (i.e., the mechanisms linking landscape composition and disease dynamics can differ across pathogen and parasite species), underscoring the need for more field studies that disentangle these processes.

Here, we conducted a large-scale monitoring study of honey bee pathogens across 30 apiaries in southern Italy, a representative Mediterranean landscape, to study the association between climatic variables, landscape composition, seasonality, pathogen occurrence, and co-occurrence patterns. This Mediterranean region is characterized by marked seasonality and highly heterogeneous land use, making it particularly suitable for investigating how climate–landscape interactions shape multi-pathogen dynamics in real-world agroecosystems. The screening covered a relevant range of pathogenic threats, including major viruses (DWV, BQCV, ABPV, slow bee paralysis virus (SBPV), Bee Macula-like Virus (BeeMLV)), fungi (*N. ceranae* and *A. flavus*), bacteria (*P. larvae*), and parasites (*L. passim*). We expected pathogen occurrence to vary across climatic and landscape gradients, with warmer conditions favoring viral and parasitic pathogens through increased foraging activity and greater exposure to contaminated floral resources, while fungal pathogens were expected to be more frequent under the cool and moist conditions of autumn, which favor spore persistence and transmission. Landscapes dominated by semi-natural habitats were expected to be associated with lower viral prevalence, reflecting improved colony nutrition and reduced agrochemical exposure. We further predicted that pathogen responses would not be uniform across taxa, reflecting the distinct transmission routes and environmental sensitivities of viruses, fungi, and gut parasite. This integrative, multi-pathogen, and multi-year approach may contribute to understanding the complex relationships among environmental factors and honey bee health, supporting regionally adapted strategies for colony management in Mediterranean agroecosystems.

2. Materials and Methods

2.1. Study Area, Sampling Sites, and Environmental Data

The survey was conducted across 30 apiaries in Apulia region, southern Italy, covering an area of approximately 19,500 km² (Figure 1). Apiaries were selected to encompass a wide range of environmental conditions and to ensure a spatially representative distribution across the study area (sites spaced an average of 50.4 ± 19.7 km). Apulia has approximately 1200 registered apiaries and more than 24,000 hives according to the official Apulia region beekeeping page, where the 30 selected apiaries were distributed among all major provinces of the region, including Bari (14%), BAT (10%), Brindisi (10%), Foggia (13%), Lecce (30%), and Taranto (23%). This distribution broadly reflects the regional distribution of honey bee colonies, with a higher sampling intensity in Lecce and Taranto, which together host the largest proportion of beehives in Apulia (approximately 28% and 39%, respectively). All sampled apiaries were stationary throughout the study period, thereby minimizing potential confounding effects associated with migratory beekeeping practices. The study was carried out over two years (2022 and 2023) to assess the sanitary status of honey bee colonies and the occurrence of targeted pathogens. The study region exhibits a predominantly Mediterranean climate, with marked regional variation. Coastal areas are characterized by hot, dry summers and mild winters, with mean annual temperatures ranging from 16 to 18 °C and with an average annual precipitation between 500 and 650 mm. In contrast, the inland and northern parts of the region tend to be slightly cooler

and wetter, occasionally reaching up to 700 mm of precipitation annually. The landscape is primarily agricultural, shaped by centuries of land use that integrates both extensive and intensive farming systems. Arable land is cultivated with annual crops, mainly cereals and legumes, while the main perennial crops include olive (*Olea europaea* L.), grapevine (*Vitis vinifera* L.), and a variety of fruit trees, such as almond (*Prunus dulcis* Mill.) and cherry (*Prunus avium* L.). Semi-natural habitats interspersed within this matrix include dry grasslands used for extensive grazing and remnant patches of Mediterranean woodland and shrubland dominated mainly by *Quercus pubescens* Willd. and *Pinus halepensis* Mill.

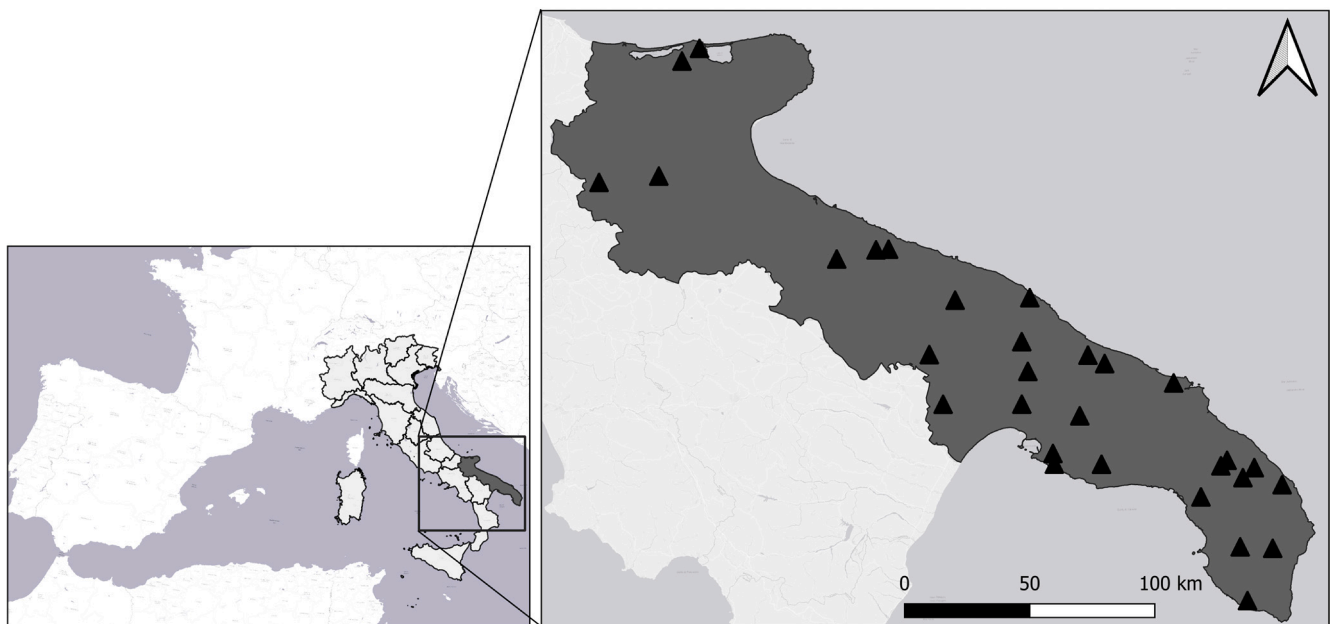


Figure 1. Surveyed sites across Apulia region, southern Italy.

Each site was characterized in terms of the landscape features surrounding the apiaries and the local climatic characteristics. To capture landscape structure, we delineated circular buffers with radii of 500, 1000, 1500, 2000, and 3000 m around each apiary, reflecting the typical foraging distances of *A. mellifera* [32]. We reclassified original land-use polygons into two main categories for the analysis: semi-natural habitats (including forests, shrublands, and grassland) and arable land (including annual and perennial crops). Land use within buffers was characterized based on regional land-use maps updated to 2011 (<https://pugliacon.regione.puglia.it/web/sit-puglia-sit> accessed on 3 March 2024), mapped at 1:5000 scale with a minimum mapping unit of 2500 m² and cross-validated with high-resolution satellite images (Google Earth Pro version 7.3.7.1155, Google Inc.©, 2017). Land-use categories were aggregated into arable land and semi-natural habitats to capture major gradients in management intensity and resource availability. While this approach simplifies landscape heterogeneity, it allows for a parsimonious and statistically robust assessment of broad associations with pathogen occurrence, given the structure of the dataset. Landscape analyses were performed in Quantum Geographic Information System version 3.30.3-’s-Hertogenbosch (QGIS Development Team, 2022). Across apiaries, the proportion of semi-natural habitats within the selected buffers ranged from 1 to 16%, while arable land ranged from 3 to 22%, indicating substantial variation in landscape composition across sampling sites.

Climatic data were obtained to capture differences among sites. For each apiary location, we extracted daily records of minimum, maximum, and mean air temperature (°C), cumulative precipitation (mm), and relative humidity from a historical meteorological database (<https://www.ilmeteo.it/> accessed on 28 June 2024). Annual averages were then

derived for each variable. Because several of these parameters were strongly correlated, we focused subsequent analyses on mean temperature and relative humidity, as they best represented site-level climatic variation and are known to influence colony thermoregulation, foraging activity, and pathogen transmission dynamics. Correlations among environmental variables are presented in Figure S1.

Beekeeping management was consistent across all apiaries. Colonies were treated simultaneously against *V. destructor* twice per year (in December and July) using commercial acaricides based on oxalic acid as an active ingredient applied through trickling, strips, and sublimation. Colonies were visually inspected twice per year during sampling, and no evident signs of infestation were observed. Queens were either reared within the colonies or sourced from local beekeepers, and replacement occurred in only four apiaries during the monitoring due to age or reduced performance.

2.2. Pathogen Survey

The sampling was conducted in 2022 and 2023 during both summer and autumn, for a total of four sampling rounds. In each apiary, samples were collected from three randomly selected hives (90 hives monitored in total), which remained the same throughout the entire monitoring period. During each survey, three sample types were obtained from every hive: foraging bees captured in front of the entrance, young in-hive bees, and larvae from brood combs. A total of 1080 samples comprising 16,200 individual bees (15 bees per sample) were collected, of which two thirds were adults and one third were larvae. The collected samples were transferred under refrigerated conditions to the laboratory at -80°C for further analysis. The panel of pathogens included in the present study was defined in collaboration with the Regional Association of Beekeepers of Apulia (ARAP) and reflected the surveillance priorities established at the beginning of the project.

2.3. Nucleic Acid Extraction and Molecular Analysis

For nucleic acid extraction from adult bees, five individuals from the same hive/sampling round were retrieved from the storage. Using a sterile scalpel, the heads were removed to minimize interference from ocular and cephalic tissues [33]. While DWV and ABPV may localize in head tissues, they are also detected in other body tissues [34,35]. The bodies were pooled in a 2 mL microcentrifuge tube for subsequent processing. For larvae, three individuals were retrieved from storage, and the same procedure was applied.

Each processed sample was homogenized in sterilized mortars with liquid nitrogen. From the same sample, 0.2 g of the resulting powdered tissue was mixed with 1 mL of CTAB buffer (Promega, Madison, WI, USA) for DNA extraction and 0.2 g with homogenization buffer (Promega, USA) for RNA extraction. The homogenates were transferred in a 2 mL microcentrifuge tube and incubated for 10 min at 70°C , followed by 5 min at 4°C . Subsequently, after centrifugation, 300 μL of the resulting supernatant was added from each extract (DNA and RNA) to the cartridge provided in the automated kit (Promega, USA). Maxwell[®] RSC PureFood GMO and Authentication Kit and Maxwell[®] RSC RNA Kits (Promega, Madison, WI, USA) were used for DNA and RNA extraction, respectively, in accordance with the manufacturer's protocol of the automated nucleic acid extraction Maxwell RSC 48 instrument (Promega, USA). The quality and concentration of the RNA and DNA were checked by using a NanoDrop spectrophotometer (ThermoFisher Scientific, Milan, Italy). Then, the samples were stored at -20°C .

Reverse transcription (RT) was performed for all the RNA extracts by using 15 μL from each sample (250 ng/ μL), mixed with 2 μL of hexamer random primers (Roche Diagnostics, Rotkreuz, Switzerland) (0.5 $\mu\text{g}/\mu\text{L}$), 2.5 μL of 10 mM dNTPs, and 10 μL RNase-free water. The mix was denatured at 70°C for 5 min, followed by fast cooling in ice. Synthesis of

cDNA was performed by adding 10 µL M-MLV Buffer 5× (50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM MgCl₂), 2.5 µL of 0.1 M DTT, and 200 units of Moloney Murine Leukemia virus (M-MLV) reverse transcriptase enzyme (Invitrogen, Carlsbad, CA, Italy) in a final volume of 50 µL. The mix was incubated at 37 °C for 60 min and then 70 °C for 10 min, and finally stored at −20 °C.

To detect the presence of BQCV, DWV, BeeMLV, and ABPV, real-time PCR was conducted using the specific primers described in Table S1. The mix consisted of 5.5 µL of 2× POWERup SYBR green (ThermoFisher Milan, Italy), 0.3 µL of each primer (10 mM), and 1 µL of cDNA in a final volume of 11 µL. Reactions were carried out in 96-well PCR plates using a CFX96 Touch Real-time PCR Detection System (Bio-Rad, Hercules, CA, USA). The thermal cycling conditions were as follows: 2 min at 50 °C, 2 min at 95 °C and 40 cycles of 95 °C for 15 s and 58–60 °C (depending on the primer set) for 1 min, and then extension at 72 °C for 20 s. The specificity of all the primer pairs was confirmed by melting curve analysis: initial denaturation for 5 min at 95 °C, cooling to 55 °C, and melting from 55 °C to 95 °C with a 0.5 °C transition rate every 10 s.

SBPV screening was performed by conventional RT-PCR. The RT-PCR mix consisted of 12.5 µL 2× DreamTaq Green PCR Master Mix (ThermoFisher Scientific, Waltham, MA, USA), 0.4 µL of each primer (Table S1), and 2.5 µL of cDNA in a total volume of 25 µL. After amplification, PCR product was visualized in agarose gel (1.2%).

Although absolute quantification was not the primary aim of this study, honey bee housekeeping gene RPL32 was used as an internal control to verify nucleic acid integrity, successful reverse transcription, and the absence of PCR inhibition, thereby ensuring the reliability of negative results for RNA viruses.

The detection of the other pathogens, i.e., *N. ceranae*, *A. flavus*, *P. larvae*, and *L. passim*, was done in qPCR reactions using the DNA samples and specific primer pairs reported in Table S1. The reaction mix contained 2× POWERup SYBR green Master Mix (ThermoFisher Scientific), 0.25 µM of each forward and reverse primer, and 100 ng per reaction of DNA in a final volume of 11 µL. The thermal cycling conditions were those recommended by the manufacturer for standard cycling mode; annealing temperatures are reported in Table S1. Reactions were carried out in 96 wells of PCR plates, in a CFX96 Touch Real-time PCR Detection System (Bio-Rad). To confirm the specificity assay, at the end of each run, melt curves were analyzed from 65 to 95 °C.

All reactions included no-template controls (NTCs) to check for contamination and negative controls (pathogen-free samples), while positive controls consisted of previously confirmed positive samples from the National Center of research (CNR-IPSP, Bari) and the university of Bari (UNIBA). Each sample was analyzed in technical duplicates to ensure reproducibility. A cycle threshold (Ct) cut-off was used to define positive detection, following thresholds established in pathogen screening studies. Samples showing inconsistent amplification or non-specific melt curves were excluded from further analyses.

2.4. Statistical Analyses

To investigate associations between landscape composition, climatic conditions, and honey bee pathogen occurrence, we adopted a stepwise analytical approach progressing from broad temporal patterns to pathogen-specific environmental responses. Analyses were conducted at progressively higher levels of aggregation, from individual samples within hives to the apiary × season level, to improve model residuals and ensure that each question was addressed at the appropriate ecological scale. This aggregation improves model stability and aligns the analysis with site-level environmental predictors, but it reduces within-hive variability, which should be considered when interpreting the results.

Site-level climatic conditions were characterized using annual averages to capture spatial gradients across the study area, rather than conditions at the time of sampling, which would largely recapitulate the summer–autumn contrast already accounted for by season as a fixed factor. Year was initially included as a fixed factor, but it did not affect model outcomes, and so it was excluded for parsimony.

First, we explored general temporal variation in pathogen occurrence across all samples. We ran a generalized linear mixed-effects model (binomial distribution) with pathogen occurrence (presence/absence) as the response variable. Fixed predictors included pathogen identity (categorical, multiple levels), sample type (foragers, in-hive bees, and larvae), and season (summer vs. autumn), as well as the interactions between pathogen identity and sample type, and between pathogen identity and season. Apiary and hive identity were included as nested random effects to account for repeated sampling within apiaries. This model allowed us to assess overall differences in detection probability among pathogens, sample types, and seasons while controlling for hierarchical sampling structure.

Second, we investigated the role of landscape and climate in shaping pathogen prevalence, with a particular focus on pathogen type (viruses, parasite, and fungi). To improve model residuals and because our interest was not in interannual fluctuations, data were averaged at the apiary $\times$ season level. Linear mixed-effects models were then fitted with standardized pathogen occurrence as the response. Fixed predictors included pathogen type (categorical, three levels: viruses, parasite, and fungi), landscape composition (continuous, % cover of semi-natural habitat or arable land within buffers of 500–3000 m), mean temperature, mean relative humidity, and season. Correlation analyses (Figure S1) and VIF assessment indicated no significant relationships among the environmental predictors included in the models, supporting their use as independent explanatory variables. Interactions were tested between pathogen type and environmental variables, and between pathogen type and season. Apiary ID was retained as a random factor. Because semi-natural habitat and arable cover were strongly correlated at multiple scales, we ran parallel models including each landscape predictor separately. The most informative spatial scale was selected based on Akaike Information Criterion (AIC) comparison (Table S2).

Third, to better understand pathogen-specific responses, we fitted separate models for the main pathogens detected (DWV, BQCV, BeeMLV, *N. ceranae*, ABPV, and *L. passim*). In these models, pathogen occurrence was modeled against the most informative landscape predictor (semi-natural habitat or arable cover at the selected scale), mean temperature, mean relative humidity, and season, while accounting for random variation among apiaries. Interaction terms between landscape composition and climatic predictors, and between landscape composition and season, were included. BeeMLV and ABPV occurrence data were square root-transformed to meet model assumptions of normality and homoscedasticity.

Fourth, we analyzed patterns of co-occurrence between pathogens in two complementary ways. First, pairwise correlations among pathogens were calculated using Pearson's correlation coefficients. Analyses were performed separately for summer and autumn datasets to highlight seasonal patterns of pathogen co-occurrence. Second, to assess how environmental factors influenced coinfection, we quantified the number of different pathogens detected per hive. This measure of multi-infection pressure was modeled against landscape composition (as above), mean temperature, mean relative humidity, and season, including interaction terms between landscape and both climate and season. This two-step approach allowed us to identify non-random pathogen associations while also testing how seasonality and environmental drivers shape the extent of coinfection within colonies. For all models, residual distribution, overdispersion, and spatial autocorrelation were assessed using the DHARMA package (version 0.5.0) [36], and multicollinearity was

checked with variance inflation factors (all VIF < 2) following [37], indicating that landscape and climatic variables did not represent a single underlying spatial gradient, supporting their joint inclusion in the models.

The absence of spatial autocorrelation in model residuals (Moran's $I = -0.016$, $p = 0.76$) indicates that no residual spatial structure remained after model fitting, although this does not imply independence between landscape and climatic gradients. To avoid overparameterization and ensure model interpretability, we restricted interactions to biologically meaningful two-way terms, primarily those involving pathogen identity/type and environmental variables, which represented the central focus of the study. Other higher-order or weakly supported interactions were excluded to maintain parsimony and follow recommendations for hypothesis-driven model construction. All analyses were performed in R (R Core Team 2022, version 4.2.2) using the glmmTMB package (version 1.1.14) [38] for generalized linear mixed-effects models.

3. Results

3.1. Pathogen Detection and Overall Occurrence

Across two years in both seasons of monitoring, six of the nine surveyed pathogens were consistently detected, while *P. larvae* and *A. flavus* were never observed, and SBPV was only rarely detected (five samples) and hence not included in the analyses. The most prevalent pathogens were BQCV, DWV, and *L. passim*, which were detected in most colonies, whereas BeeMLV, *N. ceranae*, and ABPV occurred at lower frequencies (Figure 2a). Overall occurrence patterns were consistent but not uniform: occurrence was generally highest in adult foragers, intermediate in in-hive adults, and lowest in larvae, and it tended to be higher in summer than in autumn; however, these gradients varied by pathogen (Figure 2a,c) and by season (Figure 2b).

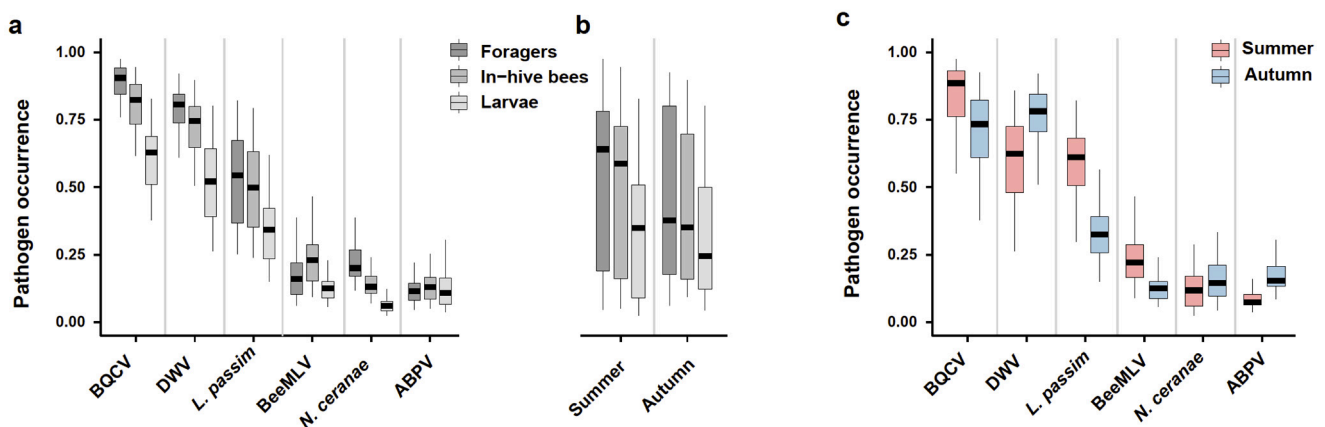


Figure 2. (a) Probability of pathogen occurrence among sample types (foragers, in-hive bees, and larvae) across pathogens. (b) Seasonal differences in predicted pathogen occurrence by sample type. (c) Seasonal variation in pathogen occurrence across pathogens. Predictions are derived from generalized linear mixed models fitted to pathogen presence/absence data (binomial GLMMs). Boxplots show interquartile range, medians, and whiskers.

3.2. Seasonal and Environmental Patterns of Pathogen Occurrence

Parasite occurrence was highly correlated to season (Table 1) and more frequent in summer; however, viruses and fungi did not show clear seasonal trends (Table S3, Figure 3a). Pathogen occurrence also exhibited variation across environmental and landscape composition, showing a slight positive correlation with humidity (Figure 3b), whereas temperature showed no detectable effect within the observed range. Viral occurrence corresponded to higher proportions of arable land within a 3 km radius, whereas fungal occurrence was

observed more frequently under lower arable cover; parasite occurrence showed little variation across the same landscape gradient, with only a slight positive pattern (Figure 3c).

Table 1. Results of the best fitting linear mixed-effects models testing the effects of seasonality (summer vs. autumn), landscape composition (% semi-natural habitat, SNH, or arable land), and climate (mean temperature and mean humidity) on overall pathogen occurrence (model 1), standardized pathogen occurrence (model 2), number of co-occurring pathogens (model 3), and pathogen-specific occurrence (models 4–9). Other fixed factors included were pathogen species (BQCV, DWV, *Lotmaria passim*, BeeMLV, *Nosema ceranae*, ABPV) and sample type (forager, in-hive bees, larvae) in model 1, and pathogen type (virus, fungi, parasite) in model 2.

Variable	Chisq	df	p-Value
(1) Pathogen occurrence n = 180			
Pathogen species	1356.324	5	<0.001
Sample type	168.01	2	<0.001
Season	9.99	1	<0.001
Pathogen species × sample type	56.11	10	<0.001
Pathogen species × season	167.33	5	<0.001
Sample type × season	4.76	2	0.092
(2) Standardized pathogen occurrence n = 180			
Pathogen type	0.00	2	1.000
Arable (3000 m)	0.54	1	0.816
Mean temperature	3.10	1	0.078
Mean humidity	17.29	1	<0.001
Season	6.20	1	0.127
Pathogen type × arable (3000 m)	15.76	2	<0.001
Pathogen type × mean temperature	0.98	2	0.612
Pathogen type × mean humidity	0.11	2	0.943
Pathogen type × season	27.82	2	<0.001
(3) Number of co-occurring pathogens n = 60			
SNH (1000 m)	1.46	1	0.227
Season	8.49	1	0.004
Mean temperature	3.57	1	0.059
Mean humidity	14.92	1	<0.001
SNH (1000 m) × season	0.07	1	0.796
SNH (1000 m) × mean temperature	8.41	1	0.004
SNH (1000 m) × mean humidity	3.87	1	0.049
(4) BQCV n = 60			
SNH3000	6.95	1	0.008
Season	11.70	1	<0.001
Mean temperature	0.67	1	0.410
Mean humidity	0.12	1	0.720
SNH (3000 m) × season	0	1	0.930
SNH (3000 m) × mean temperature	0.01	1	0.900
SNH (3000 m) × mean humidity	7.25	1	0.007
(5) DWV n = 60			
Arable (3000 m)	6.41	1	0.011
Season	14.86	1	<0.001
Mean temperature	0.35	1	0.550
Mean humidity	6.04	1	0.013
Arable (3000 m) × season	6.15	1	0.013
Arable (3000 m) × mean temperature	0.87	1	0.350
Arable (3000 m) × mean humidity	1.93	1	0.160

Table 1. Cont.

Variable	Chisq	df	p-Value
(6) <i>L. passim</i> n = 60			
SNH3000	0.68	1	0.409
Season	50.06	1	<0.001
Mean temperature	0.36	1	0.540
Mean humidity	7.99	1	0.005
SNH (3000 m) × season	1.59	1	0.200
SNH (3000 m) × mean temperature	0.02	1	0.870
SNH (3000 m) × mean humidity	6.57	1	0.010
(7) <i>BeeMLV</i> n = 60			
SNH (1000 m)	1.52	1	0.210
Season	14.56	1	<0.001
Mean temperature	1.40	1	0.230
Mean humidity	0.78	1	0.370
SNH (1000 m) × season	4.50	1	0.030
SNH (1000 m) × mean	0.61	1	0.430
SNH (1000 m) × mean humidity	1.15	1	0.280
(8) <i>N. ceranae</i> n = 60			
SNH (500 m)	2.84	1	0.092
Season	2.19	1	0.139
Mean temperature	0.17	1	0.682
Mean humidity	2.84	1	0.092
SNH (500 m) × season	5.89	1	0.015
SNH (500 m) × mean temperature	9.55	1	0.002
SNH (500 m) × mean humidity	1.05	1	0.300
(9) ABPV n = 60			
SNH (1000 m)	0.01	1	0.900
Season	7.21	1	0.007
Mean temperature	1.19	1	0.270
Mean humidity	3.33	1	0.067
SNH (1000 m) × season	0.05	1	0.820
SNH (1000 m) × mean temperature	4.23	1	0.039
SNH (1000 m) × mean humidity	7.67	1	0.005

Significant predictors ($p < 0.05$) are highlighted in bold. Gray background indicates separate fitted models.

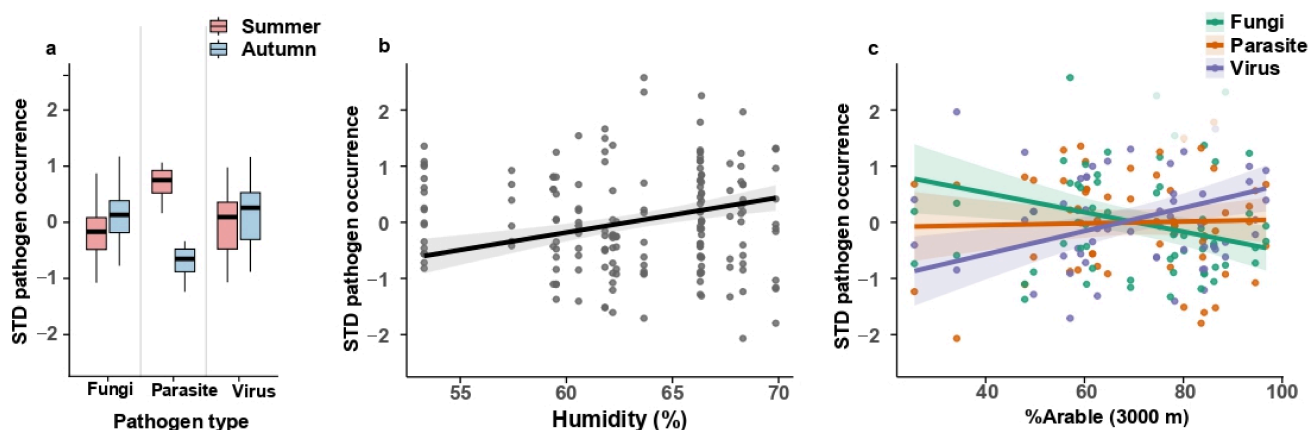


Figure 3. (a) Standardized (STD) pathogen occurrence for fungal, parasitic, and viral groups between summer and autumn. (b) Relationship between mean relative humidity (%) and overall STD pathogen occurrence. (c) Effect of arable land cover within 3 km on STD pathogen occurrence for fungi, parasite, and viruses. Points indicate model residuals, and lines represent model predictions, with shaded areas representing 95% confidence intervals.

3.3. Pathogen-Specific Environmental Responses

Pathogens displayed species-specific and interactive responses to environmental and seasonal factors, with landscape composition often associated with the effects of climatic

predictors (Figure 4). BQCV occurrence declined with increasing semi-natural habitat cover, and its prevalence was highest where both SNH cover and humidity were low (SNH $\times$ humidity interaction; Figure 4a). DWV prevalence was higher with agricultural land cover and humidity, with a significant arable land $\times$ season interaction showing stronger associations in summer and constant high prevalence in autumn (Figure 4b). BeeMLV showed a strong seasonal variation: no clear habitat associations emerged in summer, but in autumn, its prevalence rose in landscapes dominated by semi-natural habitats (SNH $\times$ season; Figure 4c). ABPV was associated with humidity and temperature, in interaction with landscape complexity (SNH $\times$ humidity, SNH $\times$ temperature; Figure 4d,e). *N. ceranae* occurrence was highly correlated with temperature but only in complex landscapes (SNH $\times$ temperature; Figure 4e). Also, in summer, its prevalence increased with the cover of semi-natural habitats in the landscape at 500 m (SNH500 $\times$ season; Figure 4g). Finally, *L. passim* prevalence was higher with humidity, especially where semi-natural habitats were scarce (SNH $\times$ humidity; Figure 4h).

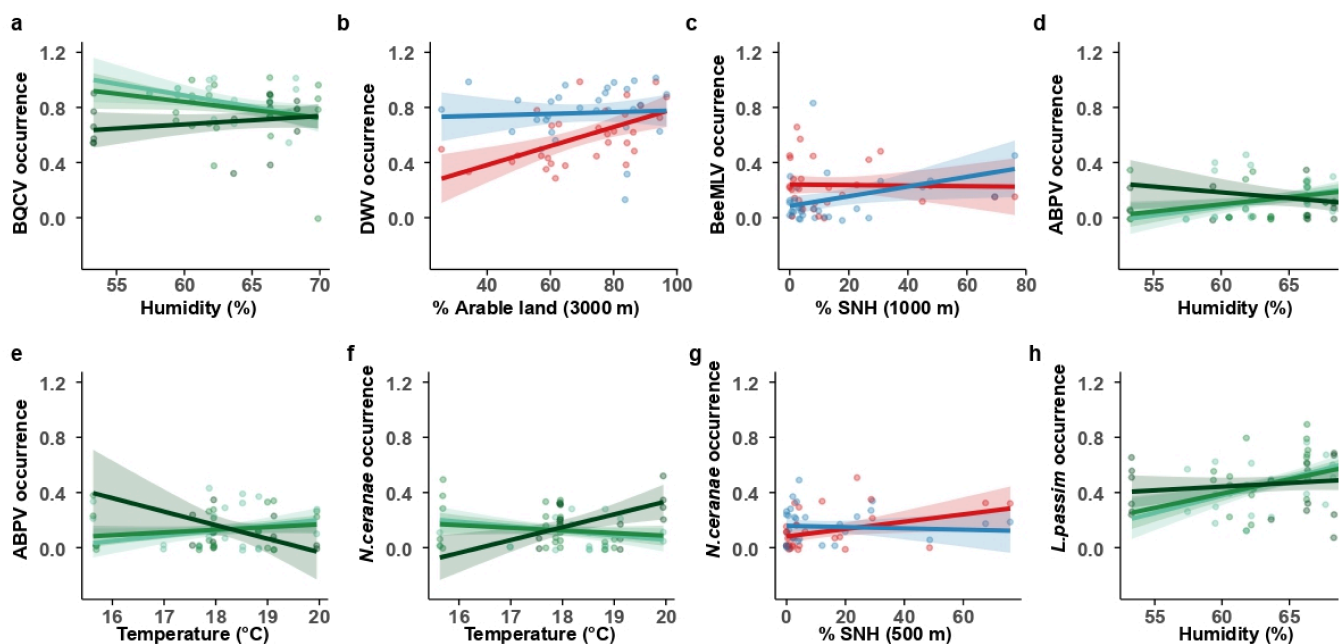


Figure 4. Impact of environmental variables on pathogen occurrence: (a) BQCV, (b) DWV, (c) BeeMLV, (d,e) ABPV, (f,g) *Nosema ceranae*, and (h) *Lotmaria passim*. Red and blue lines represent summer and autumn, respectively, while green lines indicate gradients in semi-natural habitat (SNH) cover (low = light green, mid = medium green, high = dark green) across spatial scales (500 m, 1000 m, and 3000 m). Points indicate model residuals and lines represent predicted relationships from fitted models, with shaded areas indicating 95% confidence intervals.

3.4. Pathogen Co-Occurrence

The correlation analysis revealed clear seasonal shifts in pathogen associations (Figure S2). In summer, significant co-occurrence was limited to a positive correlation between ABPV and *L. passim*. In autumn, additional correlations emerged, including a positive association between DWV and BQCV and a negative association between ABPV and *N. ceranae*.

The number of co-occurring pathogens varied with season, climatic conditions, and semi-natural habitat cover (Figure S3). Pathogen co-occurrence was generally higher in summer than in autumn, and it increased with humidity and temperatures. However, semi-natural habitats buffered these climatic effects: the positive effect of temperature and humidity on pathogen co-occurrence was reduced as SNH cover increased (SNH $\times$ temperature, SNH $\times$ humidity; Figure S3a,b).

4. Discussion

Our findings show that honey bee pathogen occurrence in Mediterranean agroecosystems varied markedly with climate, landscape, and season, with responses that differ across pathogen groups. Although multi-pathogen exposure was common and generally higher in sites characterized by humid conditions, individual pathogens differed markedly in their environmental associations: viruses were more prevalent in agriculturally dominated landscapes, *N. ceranae* occurrence was higher in structurally complex environments under warm conditions, and the gut parasite *L. passim* displayed highly context-dependent patterns shaped by humidity, landscape composition, and season. These contrasting responses reflect the heterogeneous ecology and transmission routes of bee pathogens and reinforce that environmental drivers rarely act uniformly across taxa [39–41]. Overall, our results highlight the complexity of multi-pathogen dynamics in real agroecosystems and suggest that climate, landscape composition, and season should be considered jointly when interpreting honey bee health monitoring data [12,42,43].

The detection of six pathogens across nearly all apiaries confirms that multi-pathogen exposure is widespread in Mediterranean honey bee colonies. The absence of *P. larvae* and *A. flavus*, together with the rarity of SBPV, may reflect effective sanitary practices, low regional prevalence, or environmental conditions that limit the establishment of these pathogens. In contrast, the high occurrence of BQCV, DWV, and *L. passim* suggests that these pathogens appear well adapted to persist within colonies and spread efficiently through common transmission routes such as trophallaxis, shared floral resources, or, particularly for viruses, varroa-mediated transmission [44–46]. The consistently higher infection levels in adult foragers compared to in-hive bees and larvae likely reflect greater exposure to contaminated flowers, hive-to-hive drift, and environmental stressors encountered during foraging [47]. Seasonal differences, with generally higher occurrence in summer than in autumn, may result from larger colony populations and intensified foraging activity in summer, creating more opportunities for transmission, whereas declining colony size and reduced worker turnover in autumn may limit pathogen spread [16,48]. Pathogen-specific deviations from these general patterns highlight the diverse ecological strategies and transmission modes underlying honey bee disease dynamics.

Viral pathogens showed little seasonal variation in our study, consistent with the idea that many honey bee viruses persist within colonies year-round and can be maintained through continuous transmission routes, including those mediated by *V. destructor* mites [49]. This temporal stability contrasts with the strong seasonality observed for fungal and protozoan pathogens and underscores the chronic nature of viral infections in contemporary apiaries, which are known to exert long-term sublethal effects independently of season [41]. Despite their stability across seasons, viral prevalence was strongly linked to landscape and environmental conditions. DWV occurrence was positively associated with agricultural land cover and humidity, particularly in summer, suggesting that land-use-related stressors may intensify viral transmission or reduce host resilience. Such stressors may include reduced nutritional diversity in simplified landscapes, greater forager aggregation on limited floral resources, or increased exposure to agrochemicals, all of which can compromise immune function or amplify the effects of Varroa infestation [26,50–52]. BQCV occurrence was negatively associated with semi-natural habitat cover and was lowest in humid, structurally complex landscapes, consistent with the idea that resource-rich environments may enhance colony nutritional status and thereby improve antiviral defenses [26,50]. The tendency for simplified, agriculture-dominated landscapes to exhibit higher viral prevalence aligns with evidence that intensively managed environments amplify pathogen pressures in pollinators. In contrast, landscapes with a greater share of semi-natural habitats often buffer colonies against viral infection while

simultaneously supporting more abundant and richer wild pollinator communities [53,54]. BeeMLV and ABPV, which occurred at lower frequencies, also displayed environmentally modulated patterns: BeeMLV prevalence increased in autumn within landscapes dominated by semi-natural habitats, while ABPV responded to interactions between humidity, temperature, and habitat composition. Although less common, these viruses may therefore be sensitive to climatic fluctuations or subtle aspects of habitat quality that influence colony stress, foraging behavior, or contact rates. Overall, our results highlight that viral disease risk emerges from the interplay between land use, climatic context, and colony-level transmission processes, reinforcing the importance of environmental setting when managing honey bee health.

Fungal infections by *N. ceranae* were more frequent in autumn, consistent with the buildup of infections under cooler, humid conditions that favor spore persistence [55]. In our study, *N. ceranae* prevalence was positively associated with semi-natural habitat cover, particularly under warm temperatures. Semi-natural habitats tend in fact to provide cooler, more humid microclimates and support denser, more diverse floral resources, conditions that might enhance *N. ceranae* spore persistence and increase oral exposure during foraging. These resource-rich environments may also sustain larger and more active colonies, inadvertently promoting within-hive transmission of this gut pathogen. The positive association between *N. ceranae* occurrence, temperature, and semi-natural habitats suggests that complex, resource-rich environments may promote microclimatic or behavioral conditions that enhance transmission of this gut pathogen.

The parasite *L. passim* was markedly more prevalent in summer, consistent with the expectation that transmission peaks during warm months when foraging activity and exposure to contaminated floral resources are highest [56]. Their decline in autumn likely reflects reduced foraging intensity and environmental conditions less favorable for fecal–oral transmission pathways [57]. Climatic effects were context-dependent: parasite occurrence generally increased with humidity, but this pattern emerged only in simplified landscapes; this response suggests that resource-rich landscapes might disperse foraging and reduce contact-based transmission under humid conditions.

Patterns of pathogen co-occurrence showed seasonal and environmental modulation. While no virus–fungus associations were detected in summer, autumn was characterized by a negative ABPV–*N. ceranae* association and a positive DWV–BQCV correlation, suggesting that the structure of multi-pathogen assemblages within colonies varies with seasonal changes in colony conditions. Warmer and more humid conditions favored pathogen co-occurrence, consistent with the idea that optimum environmental conditions enhance overall pathogen activity and transmission opportunities. Importantly, semi-natural habitats seemed to buffer these climatic effects, reducing the extent to which temperature and humidity amplified coinfection risk. Environmental conditions influenced not only the prevalence of individual pathogens but also the structure of multi-pathogen assemblages within colonies.

Several limitations warrant careful consideration when interpreting the present findings. Owing to the observational design of the study, the reported relationships should be regarded as associations rather than evidence of causality between environmental factors and pathogen occurrence. Another limitation of this study is that the pathogen panel was established according to the regional surveillance priorities defined at the start of the project and did not include Chronic Bee Paralysis Virus (CBPV). Given the increasing epidemiological relevance and marked seasonal occurrence of this virus, future investigations incorporating this pathogen may provide additional insights into associations between environmental conditions and honey bee pathogen occurrence. Furthermore, the reliance on presence–absence data, rather than quantitative estimates of pathogen abundance, may

have constrained the ability to detect heterogeneity in infection intensity among colonies and to evaluate potential dose–response patterns. Finally, because the study was conducted in a single Mediterranean region, the broader applicability of the results to other regions characterized by distinct climatic conditions, land-use regimes, or beekeeping practices remains uncertain.

5. Conclusions

Overall, our results show that honey bee disease prevalence in Mediterranean agroecosystems emerges from the interaction of climate, season, and landscape structure, with pathogens responding in distinct and sometimes contrasting ways. The scale and configuration of semi-natural habitats played a central but nuanced role, buffering some pathogens, particularly viruses, while in other cases creating microclimatic or foraging conditions that favored fungal transmission. These patterns underscore that pathogen occurrence depends on the broad environmental context in which colonies operate. From an applied perspective, our findings suggest that landscape diversification and the conservation of semi-natural habitats may help mitigate specific pathogen pressures, although their effects are not uniformly beneficial across pathogen groups. This complexity highlights the need for integrated, context-aware management strategies that consider the interacting influences of climate, land use, and colony phenology when aiming to support honey bee health in real-world landscapes.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agriculture16131459/s1>, Table S1: List of the primer pairs used for the detection of bee viruses in RT-qPCR and RT-PCR. Table S2: Results of model selection for choosing landscape predictor and the appropriate scale for the different models that were run, including the cover of semi-natural habitat and arable land at different scales (500, 1000, 2000, 3000). The most informative spatial scale was selected based on the lowest AIC score for each landscape predictor in bold. Table S3: Pairwise seasonal contrasts in pathogen occurrence by pathogen type. Figure S1. Distribution of apiaries along environmental gradients and pairwise relationships among landscape and climatic predictors. SNH: semi-natural habitat. Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Figure S2: Correlation matrices illustrating pathogen co-occurrence patterns in honey bees during summer and autumn. Blue and red indicate positive and negative correlations, respectively, with color shading denoting the strength of each association. Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Figure S3: Interactive effects of semi-natural habitat cover (SNH, 1000 m buffer) and (a) mean temperature and (b) mean relative humidity on the number of co-occurring pathogens. Shaded areas represent 95% confidence intervals. References [58–64] are cited in Supplementary Materials.

Author Contributions: Conceptualization, K.D., R.A.K., G.C. and F.S.; methodology, R.A.K., S.A.E.Z. and G.C.; software, S.A.E.Z. and G.T.; validation, S.A.E.Z., R.A.K. and K.D.; formal analysis, S.A.E.Z., G.T. and A.M.; investigation, S.A.E.Z., G.C., F.S., A.D. and A.P.; resources, K.D. and M.S.; data curation, S.A.E.Z. and A.M.; writing—original draft preparation, S.A.E.Z. and G.T.; writing—review and editing, S.A.E.Z., G.T., R.A.K., A.M. and K.D.; visualization, S.A.E.Z., R.A.K. and G.T.; supervision, K.D. and G.T.; project administration, K.D., G.T. and M.S.; funding acquisition, K.D. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the Ministero dell’Agricoltura, della Sovranità Alimentare e delle Foreste (MASAF), grant no. J69J21012950007, and the APC was funded by the Department of Soil, Plant, and Food Sciences, University of Bari Aldo Moro.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article and Supplementary Materials. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to thank the beekeepers from the Associazione Regionale Apicoltori Pugliesi (A.R.A.P.) from whom samples were collected.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- Gallai, N.; Salles, J.-M.; Vaissiere, B.E. Évaluation de La Contribution Économique Du Service de La Pollinisation à l’agriculture Européenne. *Bull. Tech. Apic.* **2009**, *36*, 110–116.
- Vanbergen, A.J.; The Insect Pollinators Initiative. Threats to an Ecosystem Service: Pressures on Pollinators. *Front. Ecol. Environ.* **2013**, *11*, 251–259. [[CrossRef](#)]
- Hung, K.-L.J.; Kingston, J.M.; Albrecht, M.; Holway, D.A.; Kohn, J.R. The Worldwide Importance of Honey Bees as Pollinators in Natural Habitats. *Proc. R. Soc. B Biol. Sci.* **2018**, *285*, 20172140. [[CrossRef](#)] [[PubMed](#)]
- Khalifa, S.A.M.; Elshafiey, E.H.; Shetaia, A.A.; El-Wahed, A.A.A.; Algethami, A.F.; Musharraf, S.G.; AlAjmi, M.F.; Zhao, C.; Masry, S.H.D.; Abdel-Daim, M.M.; et al. Overview of Bee Pollination and Its Economic Value for Crop Production. *Insects* **2021**, *12*, 688. [[CrossRef](#)] [[PubMed](#)]
- Brodtschneider, R.; Gray, A.; Adjlane, N.; Ballis, A.; Brusbardis, V.; Charrière, J.-D.; Chlebo, R.; Coffey, M.F.; Dahle, B.; de Graaf, D.C.; et al. Multi-Country Loss Rates of Honey Bee Colonies during Winter 2016/2017 from the COLOSS Survey. *J. Apic. Res.* **2018**, *57*, 452–457. [[CrossRef](#)]
- Requier, F.; Leyton, M.S.; Morales, C.L.; Garibaldi, L.A.; Giacobino, A.; Porrini, M.P.; Rosso-Londoño, J.M.; Velarde, R.A.; Aignasse, A.; Aldea-Sánchez, P.; et al. First Large-Scale Study Reveals Important Losses of Managed Honey Bee and Stingless Bee Colonies in Latin America. *Sci. Rep.* **2024**, *14*, 10079. [[CrossRef](#)] [[PubMed](#)]
- Giacobino, A.; Steinhauer, N.; Brunner, S.; Garcia-Andersen, N.; Aurell, D.; Bruckner, S.; Rogers, S.; Williams, G. *Preliminary Results from the 2024–2025 US Beekeeping Survey: Honey Bee Colony Loss and Management*; Apiary Inspectors of America: Sterling, ND, USA, 2025.
- Dainat, B.; vanEngelsdorp, D.; Neumann, P. Colony Collapse Disorder in Europe. *Environ. Microbiol. Rep.* **2012**, *4*, 123–125. [[CrossRef](#)] [[PubMed](#)]
- VanEngelsdorp, D.; Evans, J.D.; Saegerman, C.; Mullin, C.; Haubruge, E.; Nguyen, B.K.; Frazier, M.; Frazier, J.; Cox-Foster, D.; Chen, Y. Colony Collapse Disorder: A Descriptive Study. *PLoS ONE* **2009**, *4*, e6481. [[CrossRef](#)] [[PubMed](#)]
- Barroso, P.; Reza-Varzandi, A.; Sardo, A.; Pesavento, A.; Allais, L.; Zanet, S.; Ferroglia, E. Impact of Intensive Agriculture and Pathogens on Honeybee (*Apis mellifera*) Colony Strength in Northwestern Italy. *Environ. Pollut.* **2025**, *367*, 125571. [[CrossRef](#)] [[PubMed](#)]
- Hristov, P.; Shumkova, R.; Palova, N.; Neov, B. Factors Associated with Honey Bee Colony Losses: A Mini-Review. *Vet. Sci.* **2020**, *7*, 166. [[CrossRef](#)] [[PubMed](#)]
- Babin, A.; Schurr, F.; Delannoy, S.; Fach, P.; Huyen Ton Nu Nguyet, M.; Bougeard, S.; de Miranda, J.R.; Rundlöf, M.; Wintermantel, D.; Albrecht, M.; et al. Distribution of Infectious and Parasitic Agents among Three Sentinel Bee Species across European Agricultural Landscapes. *Sci. Rep.* **2024**, *14*, 3524. [[CrossRef](#)] [[PubMed](#)]
- Cilia, G.; Forzan, M. Editorial: Insights into Bee Diseases and Bee Health. *Front. Cell. Infect. Microbiol.* **2022**, *12*, 993440. [[CrossRef](#)] [[PubMed](#)]
- Nekoei, S.; Rezvan, M.; Khamesipour, F.; Mayack, C.; Molento, M.B.; Revainera, P.D. A Systematic Review of Honey Bee (*Apis mellifera*, Linnaeus, 1758) Infections and Available Treatment Options. *Vet. Med. Sci.* **2023**, *9*, 1848–1860. [[CrossRef](#)] [[PubMed](#)]
- Deutsch, K.R.; Graham, J.R.; Boncristiani, H.F.; Bustamante, T.; Mortensen, A.N.; Schmehl, D.R.; Wedde, A.E.; Lopez, D.L.; Evans, J.D.; Ellis, J.D. Widespread Distribution of Honey Bee-Associated Pathogens in Native Bees and Wasps: Trends in Pathogen Prevalence and Co-Occurrence. *J. Invertebr. Pathol.* **2023**, *200*, 107973. [[CrossRef](#)] [[PubMed](#)]
- Kunat-Budzyńska, M.; Łabuć, E.; Ptaszyńska, A.A. Seasonal Detection of Pathogens in Honeybees Kept in Natural and Laboratory Conditions. *Parasitol. Int.* **2025**, *104*, 102978. [[CrossRef](#)] [[PubMed](#)]
- Ciglencečki, U.J.; Ocepek, M.P.; Jenčič, V.; Toplak, I. Seasonal Variations of Four Honey Bee Viruses in Pupae, Hive and Forager Bees of Carniolan Gray Bee (*Apis mellifera* Carnica). *Slov. Vet. Res.* **2014**, *51*, 131–140.
- Olgun, T.; Everhart, S.E.; Anderson, T.; Wu-Smart, J. Comparative Analysis of Viruses in Four Bee Species Collected from Agricultural, Urban, and Natural Landscapes. *PLoS ONE* **2020**, *15*, e0234431. [[CrossRef](#)] [[PubMed](#)]
- Jhavar, J.; Davidson, J.D.; Weidenmüller, A.; Wild, B.; Dormagen, D.M.; Landgraf, T.; Couzin, I.D.; Smith, M.L. How Honeybees Respond to Heat Stress from the Individual to Colony Level. *J. R. Soc. Interface* **2023**, *20*, 20230290. [[CrossRef](#)] [[PubMed](#)]
- Lin, Z.; Shen, S.; Wang, K.; Ji, T. Biotic and Abiotic Stresses on Honeybee Health. *Integr. Zool.* **2024**, *19*, 442–457. [[CrossRef](#)] [[PubMed](#)]
- Stabentheiner, A.; Kovac, H.; Mandl, M.; Käfer, H. Coping with the Cold and Fighting the Heat: Thermal Homeostasis of a Superorganism, the Honeybee Colony. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* **2021**, *207*, 337–351. [[CrossRef](#)] [[PubMed](#)]

22. Tosun, O.; Yaman, M. The Effects of Temperature and Humidity around the Beehives on the Distribution of Nosema Ceranae, and Also Geographical and Seasonal Activity of the Infection in the Eastern Black Sea Region of Turkey. *J. Environ. Sci. Eng. B* **2016**, *5*, 513–522. [\[CrossRef\]](#)
23. Smoliński, S.; Glazaczow, A. Causal Network Linking Honey Bee (*Apis mellifera*) Winter Mortality to Temperature Variations and Varroa Mite Density. *Sci. Total Environ.* **2024**, *954*, 176245. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Degrandi-Hoffman, G.; Chen, Y.; Watkins Dejong, E.; Chambers, M.L.; Hidalgo, G. Effects of Oral Exposure to Fungicides on Honey Bee Nutrition and Virus Levels. *J. Econ. Entomol.* **2015**, *108*, 2518–2528. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Dolezal, A.G.; Carrillo-Tripp, J.; Miller, W.A.; Bonning, B.C.; Toth, A.L. Intensively Cultivated Landscape and Varroa Mite Infestation Are Associated with Reduced Honey Bee Nutritional State. *PLoS ONE* **2016**, *11*, e0153531. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Cohen, H.; Ponisio, L.C.; Russell, K.A.; Philpott, S.M.; McFrederick, Q.S. Floral Resources Shape Parasite and Pathogen Dynamics in Bees Facing Urbanization. *Mol. Ecol.* **2022**, *31*, 2157–2171. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Döke, M.A.; Frazier, M.; Grozinger, C.M. Overwintering Honey Bees: Biology and Management. *Curr. Opin. Insect Sci.* **2015**, *10*, 185–193. [\[CrossRef\]](#) [\[PubMed\]](#)
28. van Dooremalen, C.; Gerritsen, L.; Cornelissen, B.; van der Steen, J.J.M.; van Langevelde, F.; Blacquiére, T. Winter Survival of Individual Honey Bees and Honey Bee Colonies Depends on Level of *Varroa destructor* Infestation. *PLoS ONE* **2012**, *7*, e36285. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Fearon, M.L.; Wood, C.L.; Tibbetts, E.A. Habitat Quality Influences Pollinator Pathogen Prevalence through Both Habitat–Disease and Biodiversity–Disease Pathways. *Ecology* **2023**, *104*, e3933. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Piot, N.; Schweiger, O.; Meeus, I.; Yañez, O.; Straub, L.; Villamar-Bouza, L.; De la Rúa, P.; Jara, L.; Ruiz, C.; Malmström, M.; et al. Honey Bees and Climate Explain Viral Prevalence in Wild Bee Communities on a Continental Scale. *Sci. Rep.* **2022**, *12*, 1904. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Tuerlings, T.; Buydens, L.; Smagghe, G.; Piot, N. The Impact of Mass-Flowering Crops on Bee Pathogen Dynamics. *Int. J. Parasitol. Parasites Wildl.* **2022**, *18*, 135–147. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Beekman, M.; Ratnieks, F.L.W. Long-Range Foraging by the Honey-Bee, *Apis mellifera* L. *Funct. Ecol.* **2000**, *14*, 490–496. [\[CrossRef\]](#)
33. Boncristiani, H.; Li, J.; Evans, J.D.; Pettis, J.; Chen, Y. Scientific Note on PCR Inhibitors in the Compound Eyes of Honey Bees, *Apis mellifera*. *Apidologie* **2011**, *42*, 457–460. [\[CrossRef\]](#)
34. Lanzi, G.; De Miranda, J.R.; Boniotti, M.B.; Cameron, C.E.; Lavazza, A.; Capucci, L.; Camazine, S.M.; Rossi, C. Molecular and Biological Characterization of Deformed Wing Virus of Honeybees (*Apis mellifera* L.). *J. Virol.* **2006**, *80*, 4998–5009. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Gratton, E.M.; McNeil, D.J.; Sawyer, R.; Martinello, A.; Grozinger, C.M.; Hines, H.M. The Role of Landscape Factors in Shaping Bumble Bee Pathogen Loads across Regions of the Eastern Nearctic. *Insect Conserv. Divers.* **2024**, *17*, 1098–1112. [\[CrossRef\]](#)
36. Hartig, F. DHARMA: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. R Package Version 0.4.6. 2022. Available online: <https://cran.r-project.org/web/packages/DHARMA/vignettes/DHARMA.html> (accessed on 20 September 2023).
37. Dormann, C.F.; Elith, J.; Bacher, S.; Buchmann, C.; Carl, G.; Carré, G.; Marquéz, J.R.G.; Gruber, B.; Lafourcade, B.; Leitão, P.J.; et al. Collinearity: A Review of Methods to Deal with It and a Simulation Study Evaluating Their Performance. *Ecography* **2013**, *36*, 27–46. [\[CrossRef\]](#)
38. Brooks, M.; Kristensen, K.; van Benthem, K.; Magnusson, A.; Berg, C.; Nielsen, A.; Skaug, H.; Mächler, M.; Bolker, B. glmmTMB Balances Speed and Flexibility Among Packages for Zero-Inflated Generalized Linear Mixed Modeling. *R J.* **2017**, *9*, 378. [\[CrossRef\]](#)
39. Fürst, M.A.; McMahon, D.P.; Osborne, J.L.; Paxton, R.J.; Brown, M.J.F. Disease Associations between Honeybees and Bumblebees as a Threat to Wild Pollinators. *Nature* **2014**, *506*, 364–366. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Manley, R.; Boots, M.; Wilfert, L. REVIEW: Emerging Viral Disease Risk to Pollinating Insects: Ecological, Evolutionary and Anthropogenic Factors. *J. Appl. Ecol.* **2015**, *52*, 331–340. [\[CrossRef\]](#) [\[PubMed\]](#)
41. McMenamin, A.J.; Brutscher, L.M.; Glenn, W.; Flenniken, M.L. Abiotic and Biotic Factors Affecting the Replication and Pathogenicity of Bee Viruses. *Curr. Opin. Insect Sci.* **2016**, *16*, 14–21. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Doublet, V.; Poeschl, Y.; Gogol-Döring, A.; Alaux, C.; Annoscia, D.; Aurori, C.; Barribeau, S.M.; Bedoya-Reina, O.C.; Brown, M.J.F.; Bull, J.C.; et al. Unity in Defence: Honeybee Workers Exhibit Conserved Molecular Responses to Diverse Pathogens. *BMC Genom.* **2017**, *18*, 207. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Tiritelli, R.; Flaminio, S.; Zavatta, L.; Ranalli, R.; Giovanetti, M.; Grasso, D.A.; Leonardi, S.; Bonforte, M.; Boni, C.B.; Cargnus, E.; et al. Ecological and Social Factors Influence Interspecific Pathogens Occurrence among Bees. *Sci. Rep.* **2024**, *14*, 5136. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Al Naggar, Y.; Paxton, R.J. Mode of Transmission Determines the Virulence of Black Queen Cell Virus in Adult Honey Bees, Posing a Future Threat to Bees and Apiculture. *Viruses* **2020**, *12*, 535. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Wolmuth-Gordon, H.S.; Brown, M.J.F. Transmission of a Bumblebee Parasite Is Robust despite Parasite Exposure to Extreme Temperatures. *Ecol. Evol.* **2023**, *13*, e10379. [\[CrossRef\]](#) [\[PubMed\]](#)

46. Woodford, L.; Christie, C.R.; Campbell, E.M.; Budge, G.E.; Bowman, A.S.; Evans, D.J. Quantitative and Qualitative Changes in the Deformed Wing Virus Population in Honey Bees Associated with the Introduction or Removal of *Varroa destructor*. *Viruses* **2022**, *14*, 1597. [[CrossRef](#)] [[PubMed](#)]
47. Dalmon, A.; Diévar, V.; Thomasson, M.; Fouque, R.; Vaissière, B.E.; Guilbaud, L.; Le Conte, Y.; Henry, M. Possible Spillover of Pathogens between Bee Communities Foraging on the Same Floral Resource. *Insects* **2021**, *12*, 122. [[CrossRef](#)] [[PubMed](#)]
48. Van Herzele, C.; Coppens, S.; Vereecke, N.; Theuns, S.; de Graaf, D.C.; Nauwynck, H. New Insights into Honey Bee Viral and Bacterial Seasonal Infection Patterns Using Third-Generation Nanopore Sequencing on Honey Bee Haemolymph. *Vet. Res.* **2024**, *55*, 118. [[CrossRef](#)] [[PubMed](#)]
49. Martin, S.J.; Highfield, A.C.; Brettell, L.; Villalobos, E.M.; Budge, G.E.; Powell, M.; Nikaido, S.; Schroeder, D.C. Global Honey Bee Viral Landscape Altered by a Parasitic Mite. *Science* **2012**, *336*, 1304–1306. [[CrossRef](#)] [[PubMed](#)]
50. Alaux, C.; Ducloz, F.; Crauser, D.; Le Conte, Y. Diet Effects on Honeybee Immunocompetence. *Biol. Lett.* **2010**, *6*, 562–565. [[CrossRef](#)] [[PubMed](#)]
51. Corona, M.; Branchiccela, B.; Alburaki, M.; Palmer-Young, E.C.; Madella, S.; Chen, Y.; Evans, J.D. Decoupling the Effects of Nutrition, Age, and Behavioral Caste on Honey Bee Physiology, Immunity, and Colony Health. *Front. Physiol.* **2023**, *14*, 1149840. [[CrossRef](#)] [[PubMed](#)]
52. Frizzera, D.; Strobl, V.; Yañez, O.; Seffin, E.; Zanni, V.; Annoscia, D.; Neumann, P.; Nazzi, F. Interactions between Agrochemicals and Parasites Endangering Insect Populations. *Environ. Int.* **2025**, *202*, 109664. [[CrossRef](#)] [[PubMed](#)]
53. Bottero, I.; Dominik, C.; Schweiger, O.; Albrecht, M.; Attridge, E.; Brown, M.J.F.; Cini, E.; Costa, C.; De la Rúa, P.; de Miranda, J.R.; et al. Impact of Landscape Configuration and Composition on Pollinator Communities across Different European Biogeographic Regions. *Front. Ecol. Evol.* **2023**, *11*, 1128228. [[CrossRef](#)]
54. Laterza, I.; Ragone, G.; Grossi, G.; Cappellari, A.; Agostinacchio, M.F.; Seclì, G.A.; Bari, G.; Porro, R.; Addante, R.; Cornara, D.; et al. Agricultural Intensification at Local and Landscape Scales Impacts Sweet Cherry Production through Altered Pollination Services. *Agric. Ecosyst. Environ.* **2025**, *386*, 109586. [[CrossRef](#)]
55. Huang, W.-F.; Solter, L.F.; Yau, P.M.; Imai, B.S. *Nosema ceranae* Escapes Fumagillin Control in Honey Bees. *PLoS Pathog.* **2013**, *9*, e1003185. [[CrossRef](#)] [[PubMed](#)]
56. Schmid-Hempel, P.; Aeby, M.; Barribeau, S.; Kitajima, T.; du Plessis, L.; Schmid-Hempel, R.; Zoller, S. The Genomes of *Crithidia Bombi* and *C. Expoeki*, Common Parasites of Bumblebees. *PLoS ONE* **2018**, *13*, e0189738. [[CrossRef](#)] [[PubMed](#)]
57. Graystock, P.; Yates, K.; Evison, S.E.F.; Darvill, B.; Goulson, D.; Hughes, W.O.H. The Trojan Hives: Pollinator Pathogens, Imported and Distributed in Bumblebee Colonies. *J. Appl. Ecol.* **2013**, *50*, 1207–1215. [[CrossRef](#)]
58. Sanzani, S.M.; Kubaa, R.A.; Jabri, B.-E.; Zaidat, S.A.E.; Addante, R.; Admane, N.; Djelouah, K. Rapid and Accurate Detection of the Most Common Bee Pathogens; *Nosema ceranae*, *Aspergillus flavus*, *Paenibacillus larvae* and Black Queen Cell Virus. *Insects* **2025**, *16*, 810. [[CrossRef](#)] [[PubMed](#)]
59. Kukiela, D.; Esperón, F.; Higes, M.; Sánchez-Vizcaíno, J.M. A Sensitive One-Step Real-Time RT-PCR Method for Detection of Deformed Wing Virus and Black Queen Cell Virus in Honeybee *Apis mellifera*. *J. Virol. Methods* **2008**, *147*, 275–281. [[CrossRef](#)] [[PubMed](#)]
60. de Miranda, J.R.; Dainat, B.; Locke, B.; Cordoni, G.; Berthoud, H.; Gauthier, L.; Neumann, P.; Budge, G.E.; Ball, B.V.; Stoltz, D.B. Genetic Characterization of Slow Bee Paralysis Virus of the Honeybee (*Apis mellifera* L.). *J. Gen. Virol.* **2010**, *91*, 2524–2530. [[CrossRef](#)] [[PubMed](#)]
61. De Miranda, J.R.; Cornman, R.S.; Evans, J.D.; Semberg, E.; Haddad, N.; Neumann, P.; Gauthier, L. Genome Characterization, Prevalence and Distribution of a Macula-Like Virus from *Apis mellifera* and *Varroa destructor*. *Viruses* **2015**, *7*, 3586–3602. [[CrossRef](#)] [[PubMed](#)]
62. Chantawannakul, P.; Ward, L.; Boonham, N.; Brown, M. A Scientific Note on the Detection of Honeybee Viruses Using Real-Time PCR (TaqMan) in *Varroa* Mites Collected from a Thai Honeybee (*Apis mellifera*) Apiary. *J. Invertebr. Pathol.* **2006**, *91*, 69–73. [[CrossRef](#)] [[PubMed](#)]
63. Arismendi, N.; Bruna, A.; Zapata, N.; Vargas, M. PCR-Specific Detection of Recently Described *Lotmaria passim* (Trypanosomatidae) in Chilean Apiaries. *J. Invertebr. Pathol.* **2016**, *134*, 1–5. [[CrossRef](#)] [[PubMed](#)]
64. Reim, T.; Thamm, M.; Rolke, D.; Blenau, W.; Scheiner, R. Suitability of Three Common Reference Genes for Quantitative Real-Time PCR in Honey Bees. *Apidologie* **2013**, *44*, 342–350. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.