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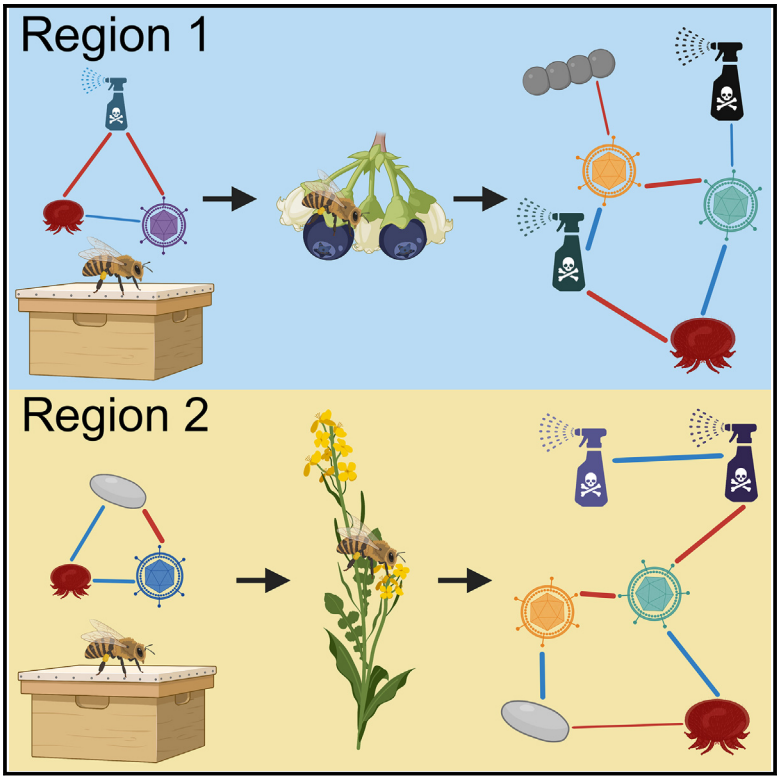
Honey bee stressor networks are complex and dependent on crop and region

Graphical abstract



Authors

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In brief

The causes of honey bee colony losses are difficult to identify and manage. French et al. find that honey bees are exposed to complex stressor networks that are crop- and region-specific. Management strategies must consider multiple stressors, as many influential stressors in these networks are currently unmanaged by beekeepers.

Highlights

- Honey bee colonies are exposed to multiple stressors all at once
- Exposure to crops during pollination increases the complexity of stressor networks
- Influential stressors that regulate network complexity are unmanaged by beekeepers
- Stressor networks differ among crops and regions

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Honey bee stressor networks are complex and dependent on crop and region

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SUMMARY

Honey bees play a major role in crop pollination but have experienced declining health throughout most of the globe. Despite decades of research on key honey bee stressors (e.g., parasitic *Varroa destructor* mites and viruses), researchers cannot fully explain or predict colony mortality, potentially because it is caused by exposure to multiple interacting stressors in the field. Understanding which honey bee stressors co-occur and have the potential to interact is therefore of profound importance. Here, we used the emerging field of systems theory to characterize the stressor networks found in honey bee colonies after they were placed in fields containing economically valuable crops across Canada. Honey bee stressor networks were often highly complex, with hundreds of potential interactions between stressors. Their placement in crops for the pollination season generally exposed colonies to more complex stressor networks, with an average of 23 stressors and 307 interactions. We discovered that the most influential stressors in a network—those that substantively impacted network architecture—are not currently addressed by beekeepers. Finally, the stressor networks showed substantial divergence among crop systems from different regions, which is consistent with the knowledge that some crops (e.g., highbush blueberry) are traditionally riskier to honey bees than others. Our approach sheds light on the stressor networks that honey bees encounter in the field and underscores the importance of considering interactions among stressors. Clearly, addressing and managing these issues will require solutions that are tailored to specific crops and regions and their associated stressor networks.

INTRODUCTION

Honey bees are key pollinators of many crops but face global threats of declining health and colony loss.[1] The valuable pollination services that western honey bees (*Apis mellifera* L.) provide come at a cost for beekeepers, who must manage parasites and pathogens that spread quickly within and among colonies.[2,3] Outside the hive, honey bees are further exposed to environmental stressors, such as toxic agrochemicals.[2] Even when armed with an ever-expanding knowledge of how individual stressors impact honey bee colonies,[4,5] beekeepers still experience substantial colony losses, particularly in North America and parts of Europe.[1] In some regions of Canada and the United States, beekeepers experience over 60% mortality in overwintering colonies, with losses often being attributed to parasites.[6–8] Colonies that are used for crop pollination also risk being exposed to pesticides and poor nutritional conditions in the landscape, as well as to greater rates of transmission of parasites and pathogens from nearby colonies.[9–11] Despite considerable efforts to study individual stressors and their lethal and sublethal impacts on bee health, including mortality, genetics, behavior, and fitness,[12] beekeepers and researchers have not

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been able to circumvent colony declines. Instead, certain parasites and pathogens are becoming increasingly resistant to treatments, thus making colony health more difficult to manage.[13,14]

Perhaps our collective failure to fully understand honey bee colony declines is caused by an undue focus on single stressors: if honey bees are naturally exposed to multiple stressors, strategies to manage single stressors may be fundamentally inadequate to address colony losses. For instance, bees may encounter a multitude of pesticides,[15] with possible additive or synergistic interactions.[16,17] Multiple viruses can also co-occur in an individual bee or colony, which may intensify the severity of infections.[18–20] Moreover, bee parasites, some of which may be relatively benign on their own, can increase in severity in the presence of other parasites, pathogens, and pesticides.[12,21] Honey bees thus have a high risk of being exposed to lethal combinations of stressors in agricultural environments. Consequently, solutions for honey bee management need to be considered within a multiple-stressor framework.[22]

The threats posed by multiple stressors are not unique to honey bees and their complex environments: they are pervasive across terrestrial, marine, and freshwater ecosystems.[23] As knowledge of the impacts of multiple stressors in biological systems continues to advance across spatial, temporal, and ecological scales,[23] analytical methodologies are needed to effectively capture and convey stressor co-occurrences and risk in the environment.[24] Network models allow for the visualization and analysis of the associations among multiple stressors[25] and comparisons across time and space.[26] Simultaneous or repeated changes to individuals (or organisms, sites, ecosystems, etc.) can be tracked, as is common in fields like psychology and medicine that use networks to model the health and behavior of human individuals.[27] Clearly, the time is opportune to apply these emerging modeling tools to honey bee management and other pressing issues in environmental management and conservation.

In this study, we applied systems theory and network methodologies to model the multiple-stressor environments of honey bee colonies that were exposed to eight economically significant crops. We conducted a 2-year field study to investigate the distribution and co-occurrence of honey bee stressors across several agricultural regions of Canada. We assayed colonies for hundreds of potential stressors at three time points throughout the pollination periods of focal crops. Networks were developed to quantify the relationships among stressor levels. Our holistic systems approach provides insight into the multiple-stressor networks that have the potential to affect honey bee colonies and we explore strategies to harness this knowledge to improve bee health.

RESULTS

Honey bee colonies experienced multiple stressors all at once

A total of 54 different stressors were detected in Canadian honey bee colonies during our study: 42 pesticides, parasitic Varroa mites, the gut microsporidian parasite *Nosema* (*Nosema ceranae* with occasional co-occurrences of *Nosema apis*), the bacterial pathogen causing European foulbrood (EFB), and nine viruses (see Table 1 for stressor abbreviations and Table S1 for a breakdown of stressors). Pesticides, in either nectar or pollen collected by the bees, and *Nosema* spp. were detected in 97% of apiaries (one apiary per site comprised four colonies). Varroa mites and EFB were each detected in 73% of apiaries. Six viruses were prevalent in $\geq$55% of apiaries: black queen cell virus (BQCV), deformed wing virus type A (DWV-A), Lake Sinai virus (LSV), sacbrood virus (SBV), and *Varroa destructor* virus-1 or deformed wing virus type B (VDV/DWV-B). The remaining three identified viruses were prevalent in $\leq$30% of apiaries: acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV), and Kashmir bee virus (KBV). Over a pollination period, an apiary always experienced multiple stressors. On average, this multi-stressor environment included 15 stressors (ranging from 6 to 24) and comprised seven pesticides (0–15), five viruses (3–8), *Nosema*, EFB, and Varroa mites. The risk that individual pesticide compounds posed to honey bees was generally low ($1.4 \times 10^{-8} < RQ < 0.64$, with a median of non-zero values of 2.9×10^{-6}; Figure S1A), where a dietary risk quotient (RQ) of 1 indicates that 50% of bees in an apiary are at risk of mortality.[28] When the RQ of all pesticides in an apiary and time point were considered (i.e., total dietary risk), risk ranged from $4.8 \times 10^{-8} < RQ < 0.76$, with the median RQ increasing to 0.0017 (excluding zero values; Figure S1B). The pesticide mefenacet was excluded from these and further analyses as its RQ could not be accurately determined.

Crop exposure increased the complexity of stressor networks

Using network analyses, we visualized the multi-stressor environments found in honey bee apiaries and quantified the relative role of each stressor in the network. Separate networks were developed for each focal crop, as different pesticides may be applied to each crop[29] and the prevalence of parasites and pathogens may depend on the region associated with a given crop.[6] Networks of colonies were further subdivided into three sampling time points: T1 (time point 1, before colonies were exposed to a crop), T2 (time point 2, during exposure), and T3 (time point 3, at the end of exposure). Apiaries had multiple co-occurring stressors, the levels of which tended to be either positively or negatively related to one another (Figure 1). The stressor networks of colonies became substantially more complex (i.e., increase in total stressors and interactions, indicating a potential increase in risk to colonies) after colonies were placed in focal crops. For example, the average number of stressors in a network, across all focal crops, increased 1.5-fold between T1 and T2 and 1.6-fold between T1 and T3. Across all focal crops, networks experienced a 1.1-fold increase in stressors between T2 and T3. Networks, however, were crop- and region-specific with respect to their pattern of stressor accumulation over time (Figure 2). For colonies placed in apple, canola oil, and lowbush blueberry, networks consistently gained stressors and interactions over time, whereas those in canola seed and soybean gained the most stressors at T2. The networks of colonies in cranberry and highbush blueberry had a constant number of stressors before gaining stressors at T3, while the network of colonies in corn gained stressors at T2, remaining constant up to T3. The number of stressors in a network was not related to the number of days that had elapsed since the start of the

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Table 1. List of stressor abbreviations

Stressor	Abbreviation
Acute bee paralysis virus	ABPV
Acetamiprid	ACE
Bifenazate	BIF
Boscalid	BOS
Black queen cell virus	BQCV
Carbaryl	CAL
Carbendazim	CAM
Chronic bee paralysis virus	CBPV
Chlorantraniliprole	CHL
Clothianidin	CLO
Coumaphos	COU
Cyantraniliprole	CYA
Difenoconazole	DIF
Dimethomorph	DIP
Dimethoate	DIT
Diuron	DIU
Deformed wing virus (type A unless otherwise indicated)	DWV
European foulbrood or *Melissococcus plutonius*	EFB
Fenamidone	FEA
Fenhexamid	FEH
Flupyradifurone	FLE
Fluopyram	FLM
Flonicamid	FLO
Hexythiazox	HEX
Israeli acute paralysis virus	IAPV
Imidacloprid	IMI
Kashmir bee virus	KBV
Linuron	LIN
Lake Sinai virus	LSV
Mandipropamid	MAN
Metconazole	MEC
Methoxyfenozide	MEE
Methamidophos	MEH
Methomyl	MEL
Mean number of Varroa mites per 100 bees	MIT
Napropamide	NAP
Nosema spp. spores	NOS
Novaluron	NOV
Omethoate	OME
Picoxystrobin	PIC
Prothioconazole	PRO
Pyraclostrobin	PYA
Pyrimethanil	PYI
Total dietary risk	RISK
Sacbrood virus	SBV
Spinosyn A	SPA
Spinosyn D	SPD
Spirotetramat	SPI
Spinetoram	SPM
Tebufenozide	TEB
Thiamethoxam	THA
Thiophanate-methyl	THO
Tralkoxydim	TRA
Varroa destructor virus-1 or deformed wing virus type B	VDV

Stressors found in experimental colonies are listed alongside their abbreviations (in alphabetical order) that were used in text and figures.

experiment ($\tau = 0.18$, $p = 0.25$), the length of the experiment ($\tau = -0.14$, $p = 0.72$), nor the month the sampling was conducted in ($\tau = 0.21$, $p = 0.18$).

Individual stressor networks had common and influential stressors

For each time point and focal crop, stressors were evaluated for their relative importance and influence within a network (Figure S2). Certain stressors were consistently detected across time points for some focal crops (e.g., imidacloprid in cranberry and thiamethoxam in highbush blueberry; Figure S2). Honey bees therefore experienced chronic exposure to some stressors like pesticides throughout a pollination period, as has been previously found for honey bee colonies near corn.[17] We focus here on the relative expected influence (EI) of a stressor, which indicates the strength and direction of relationships between a stressor and its co-occurring stressors. The prevalence and relative EI of a stressor differed by time point and focal crop, and perhaps on the number or presence of specific stressors in the network (Figure S2). Stressors that tend to be actively managed and highly actionable (i.e., easy to address by beekeepers), such as Varroa mites, *Nosema* spp., and EFB, had low EI relative to other stressors when averaged across focal crops. Pesticides, a less actionable group of stressors, tended to have the strongest interactions with other stressors. Viruses, another group of less actionable stressors, varied greatly across time and focal crop in both the strength and direction of their EI (see Figures 3, 4, and S3 for T1, T2, and T3, respectively).

Stressor networks differed among focal crops and regions

Crops and their respective regions differed substantially with respect to the number of stressors and interactions found within their stressor networks (Figure 5). On average, colonies exposed to lowbush blueberry had the least complex networks (i.e., fewest stressors and interactions, indicating a potentially lower risk to colonies), whereas cranberry colonies had the most complex networks (Figures 1 and 2). Across time points, the networks of colonies in cranberry differed most in terms of stressors from other networks, as well as in the combination of stressors and interactions, whereas colonies in lowbush blueberry differed most in terms of interactions. In terms of the combination of stressors and interactions, lowbush blueberry colonies differed the most from other crops at T1 and T2 (i.e., greatest network divergence),

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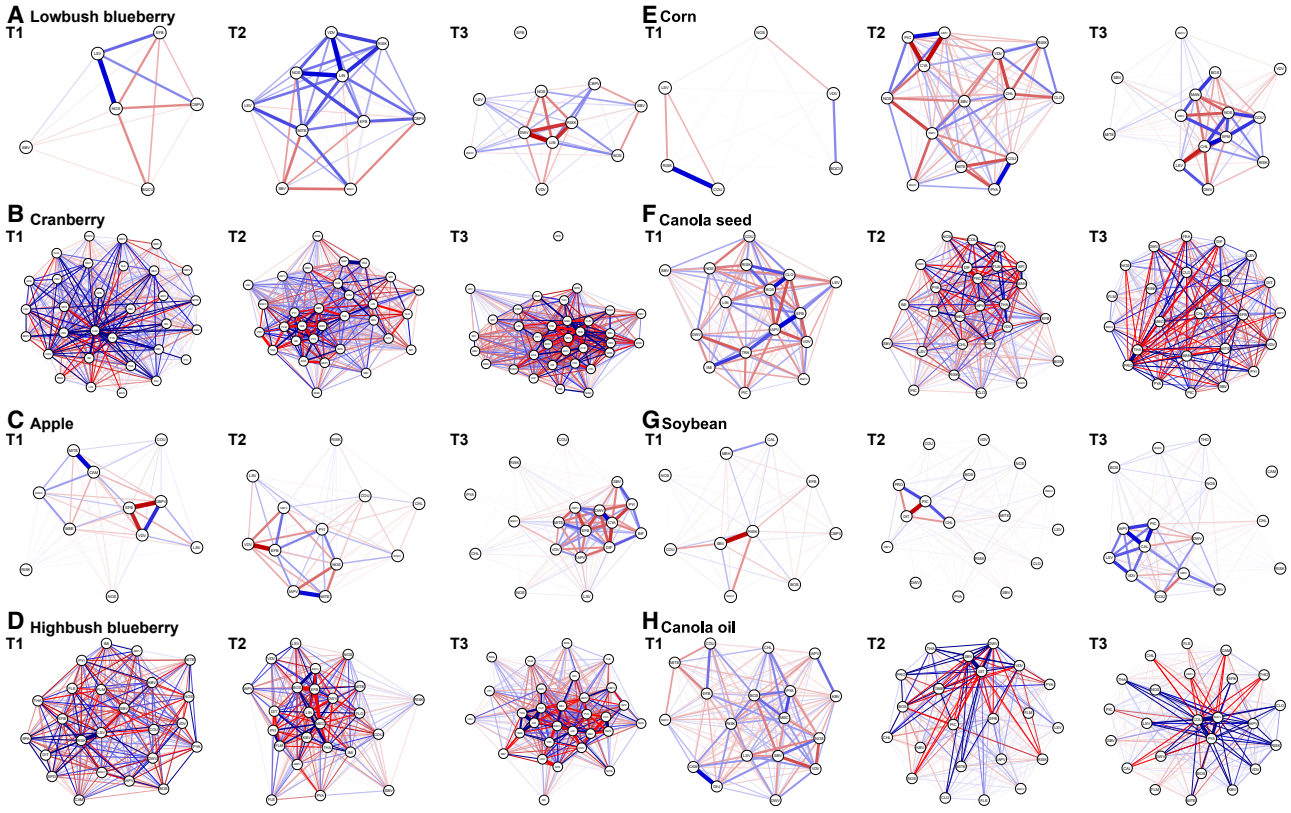

Figure 1. Honey bee colonies experienced multiple stressors all at once

Stressor networks for colonies became increasingly complex in their total number of stressors and interactions after exposure to focal crops. Stressor networks are shown for colonies placed in (A) lowbush blueberry, (B) cranberry, (C) apple, (D) highbush blueberry, (E) corn, (F) canola seed, (G) soybean, and (H) canola oil. Three time points are portrayed: before colonies were exposed to a crop (time point 1, T1), during exposure (time point 2, T2), and at the end of exposure (time point 3, T3). Each stressor (i.e., node) was found in at least one apiary in its associated crop. Interactions between stressor levels (i.e., edges) are represented by positive (blue) and negative (red) partial correlations, with a line's width and darkness indicating strength (|0–1|), and weak interactions appearing faint.
See also Figure S2, Table S2, and Data S1.

whereas colonies in cranberry differed most at T3. For the stressor networks of colonies placed in other focal crops, there was no consistent pattern in their relative (dis)similarities over time (Figure 5). When considering the temporal change of stressor networks for colonies situated in each crop (Figure S4), canola seed colonies had the greatest increase in stressors between T1 and T2, apple colonies between T1 and T3, and highbush blueberry colonies between T2 and T3. The networks of colonies placed in corn had the greatest increase in interactions between T1 and T2, and T2 and T3, and those in canola seed between T1 and T3.

DISCUSSION

Honey bees were always exposed to multiple stressors

All honey bee colonies experienced multiple-stressor environments during the pollination season, which included pesticides, Varroa mites, *Nosema* spp., EFB, and viruses. Each of these stressor groups, including five prevalent viruses, was found at more than 73% of apiaries. A single apiary at a given time point generally experienced a stressor environment containing at least one pesticide, one virus, *Nosema*, and Varroa mites/EFB. Several pesticides were moderately prevalent (across 45%–63% of apiaries): the fungicides boscalid, fluopyram, pyraclostrobin, and pyrimethanil and the insecticides clothianidin and thiamethoxam—both neonicotinoids—and chlorantraniliprole. The RQs for individual pesticides were generally low, with the median RQ = 2.9×10^{-6}. Notably, neonicotinoids and spinosyns posed greater risk, with an instance each of RQ > 0.2 (Figure S1A). When total dietary risk was considered, median risk increased by nearly three orders of magnitude to RQ = 0.0017, with seven apiaries exceeding RQ = 0.2 (Figure S1B). The European Food Safety Authority and the U.S. Environmental Protection Agency consider RQs of $\geq$0.2 and $\geq$0.4, respectively, to pose acute toxicological risks that may require mitigation.[30] The levels of total dietary risk found in our study are thus likely to threaten honey bee colonies and to require additional management.

The colonies in our study experienced a multiple-stressor environment at each point of observation and generally gained stressors after exposure to crops and across a pollination period. The potential interactions among these stressors could severely impair honey bees and colonies. Colony loss may be accelerated when hives contain multiple pesticides with large RQs (e.g., RQ $\approx$ 1).[29] The interaction between Varroa mites and sublethal concentrations of clothianidin reduces the weight of bees and

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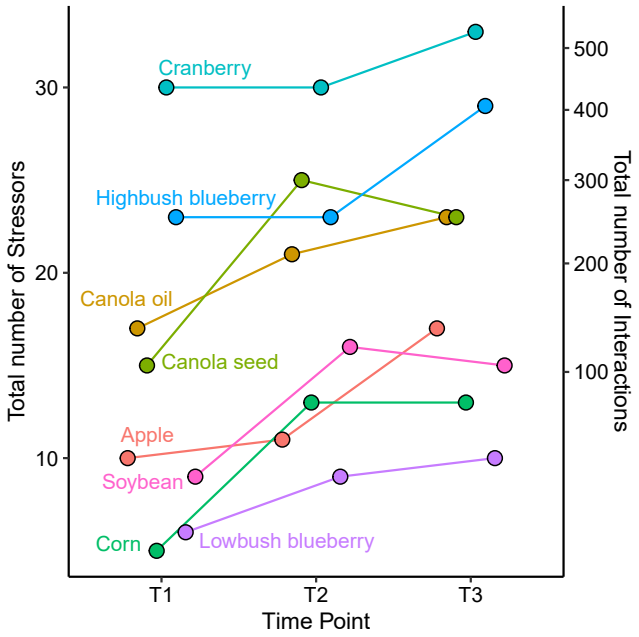

Figure 2. Crop exposure increased the complexity of stressor networks

The change in the total number of stressors and interactions per crop across three time points: before colonies were exposed to a crop (time point 1, T1), during exposure (time point 2, T2), and at the end of exposure (time point 3, T3). See also Table S1.

further impairs their grooming responses to mites.[31] Certain fungicides can make insecticides more toxic to honey bees[17,32] or cause sublethal effects—the interaction between pyraclostrobin, a fungicide found in 63% of our apiaries, and *Nosema* can degrade the intestinal lining of adult honey bees.[33] Furthermore, viruses may synergize with other stressors to become more harmful to honey bees, as has been observed between BQCV and *Nosema*[21] and between CBPV and thiamethoxam.[34] Because interactions among honey bee stressors can be so severe,[12,16,21] a shift in research and management priorities is necessary to consider how combinations of stressors interact to modulate honey bee health and colony losses. We recommend that future research efforts focus on identifying stressors that are influential, both in the context of stressor networks and as stressors relate to lethal and non-lethal effects on bee health.[12] Our study used RQs as a proxy for colony health in response to pesticides but was otherwise limited to simply quantifying biotic stressors. Research that identifies similar lethal thresholds for parasites and pathogens would be highly informative. Furthermore, studies on stressors should be informed by stressor combinations that are commonly observed in the field—at field-realistic levels and at the scale of a colony[16]—as well as by metrics, such as EI in our network models, that rank the influence of single stressors or combinations of stressors on other stressors in a given environment (see Figures 3, 4, S2, and S3).

Influential stressors are not currently managed by beekeepers

The networks of the colonies exposed to each focal crop type had certain stressors that were either directly or indirectly influential on other stressors during the pollination season (Figure S2). Stressors that are commonly managed by beekeepers, like many parasites and pathogens, tended to be less influential in a network. Parasites and pathogens likely play less of a role during the pollination season, as levels of these stressors are relatively low in worker bees.[5,20] Pesticides and viruses, which are commonly unmanaged by beekeepers, varied in their influence on other stressors (see Figures 3, 4, and S3 for T1, T2, and T3, respectively). The influence of an individual stressor, however, was highly dependent on crop and region and time point, suggesting some dependence on other stressors in the colony environment. For example, BQCV, an influential virus in some of our networks, was positively correlated in our study with LSV and SBV in canola seed colonies at T3, as has been the case in colonies that pollinate almond crops.[19] However, at other time points and in different crops and regions, this correlation becomes negative or non-significant (Table S2). We cannot discern whether these changes in association are due to the large scale of our study (using full-size colonies and broad geographic localities) versus smaller laboratory cage experiments.[16] Relationships between stressors can also change over time,[20] at varying stressor levels,[18] or depending on land cover.[35] The potential for stressor combinations to couple or decouple over spatial and temporal gradients warrants further investigation. Nevertheless, for combinations of stressors with clear associations, managing influential stressors may lessen the impacts of multiple stressors on honey bee health. If, for example, a given pesticide makes honey bees more susceptible to *Nosema*,[36] and this pesticide is influential and positively correlated with other stressors in a network, then managing the pesticide may lessen the impacts of *Nosema*. A critical component of stressor management is providing beekeepers with diagnostic services that evaluate honey bees and hive matrices for multiple stressors.[37] Viable risk management strategies or measures must also be explored with the participation of multiple stakeholders, such as beekeepers, growers, and government regulators. Traditional approaches to risk assessment[38] should be adapted to account for harmful stressor interactions, which may be any combination of parasites, pathogens, pesticides, and poor nutrition, and should reflect the dynamic nature of parasites and pathogens in colonies.[39] On a related note, stressor environments change before, during, and after the pollination season. Stressors encountered during the pollination season may persist over time or have carry-over effects that weaken colonies entering the overwintering period.[11] Our results should also be considered in tandem with studies on stressor dynamics that occur during autumn, as well as during the overwintering season, when beekeepers experience heavy colony losses.

Stressor management requires crop- and region-specific strategies

The stressor networks of colonies diverged among focal crops (Figure 5), with crops implicitly covarying with region (Figure S5). The time point at which a network gained the most stressors also depended on crop type (Figure 2), with this gain always corresponding to an increase in pesticide compounds (Table S1). These drastic differences in colonies' stressor networks indicate that crop- and region-specific solutions are needed to manage risks to honey bees and colonies. Of our eight focal crops,

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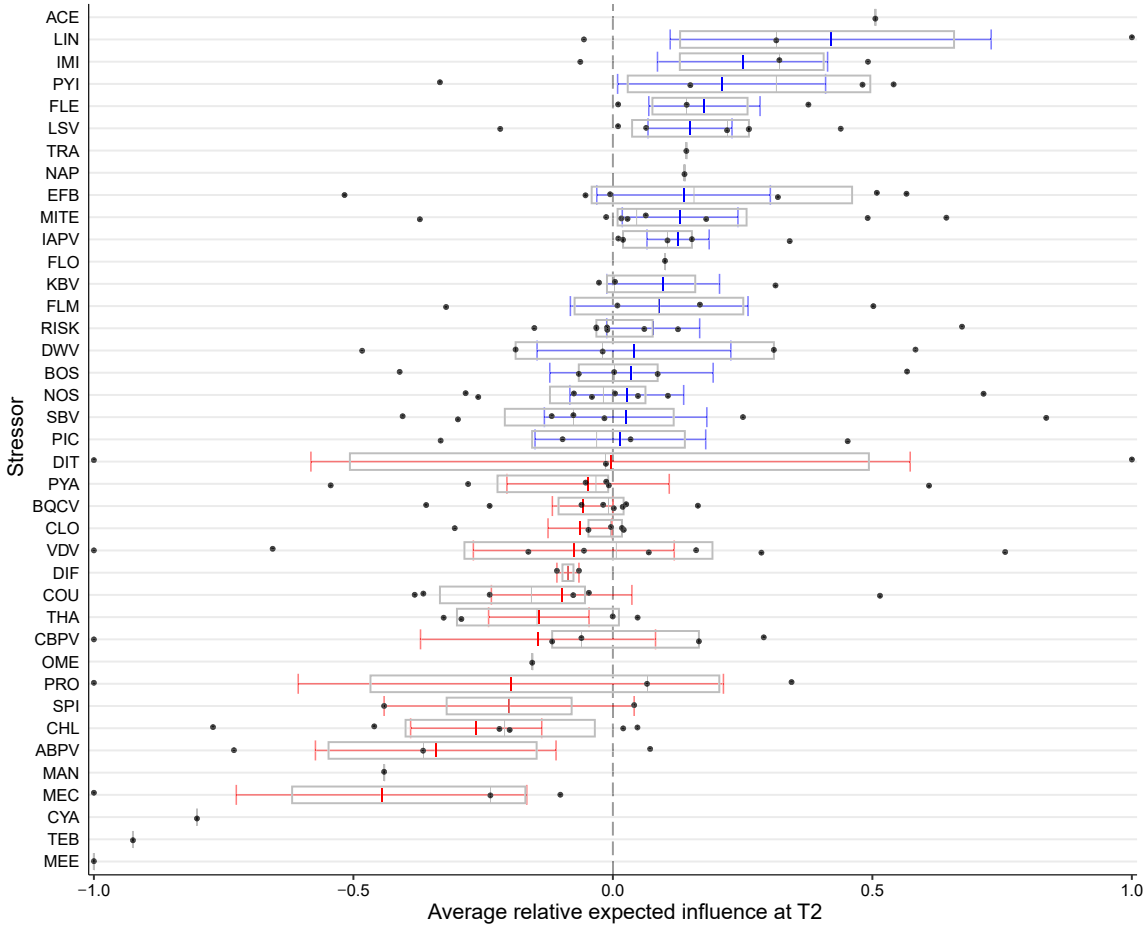

Figure 3. Networks had common and influential stressors during exposure to crops

Certain stressors were more influential in a network than others, having either stronger positive (blue) or negative (red) interactions with other stressors. Here, the relative expected influence (EI) for each stressor detected within a crop has been averaged across crops at time point 2 (T2; during exposure to a crop). The mean EI ± SEM values are shown, in descending order, as are the 25th and 75th percentiles and data points per crop.
See also Figures 4 and S3.

colonies placed in cranberry bogs experienced the most stressors, particularly pesticides, followed by colonies in highbush blueberry fields (Figure 2; Table S1). The complexity of these two networks warrants further investigation into the impacts of multiple stressors, particularly pesticides, on colonies located in these crop systems and in this region of British Columbia, where most of these sites were located. Colonies that pollinate cranberries experience low productivity and survival, possibly in relation to a combination of parasites, pathogens, and poor nutrition.[11] Colonies that pollinate highbush blueberries also have poor quality diets, which may compound the effects of other stressors.[40] In this instance, networks can help to identify which stressors are co-occurring at a single time point and that may interact synergistically. Networks can also account for temporal dynamics over discrete or continuous time periods. Stressor environments may change seasonally or annually, may depend on the length of time colonies spent in certain crops (e.g., Table S3), or may spread or compound over time if colonies are used for multiple pollination jobs in a beekeeping season.[41] Our colonies in corn and soybean experienced the second and third fewest number of stressors, yet these crops have been associated with greater honey bee mortality than other crops.[25] Networks are equally useful in identifying influential stressors in these less complex stressor environments.[25] Furthermore, although we associate the pesticides detected in our colonies with a focal crop, the land use and land cover that surround colonies, including non-focal crops, may increase the risk of pesticide exposure to foraging honey bees.[30] Identifying risk across heterogeneous landscapes would provide beekeepers with a more comprehensive assessment of risk when managing their colonies. Minimizing risk in the landscape must be a joint effort between beekeepers and growers, by adopting crop- and region-specific best management practices.[42]

The complexity of the stressor environments detected in our experimental colonies underscores the need to strategize the management of multiple stressors with more nuance. Multidisciplinary projects, spanning multiple countries and stakeholders, have developed frameworks to evaluate and manage the scale and impact of stressors.[43] In marine systems, frameworks have been proposed that consider stressor regulation and/or

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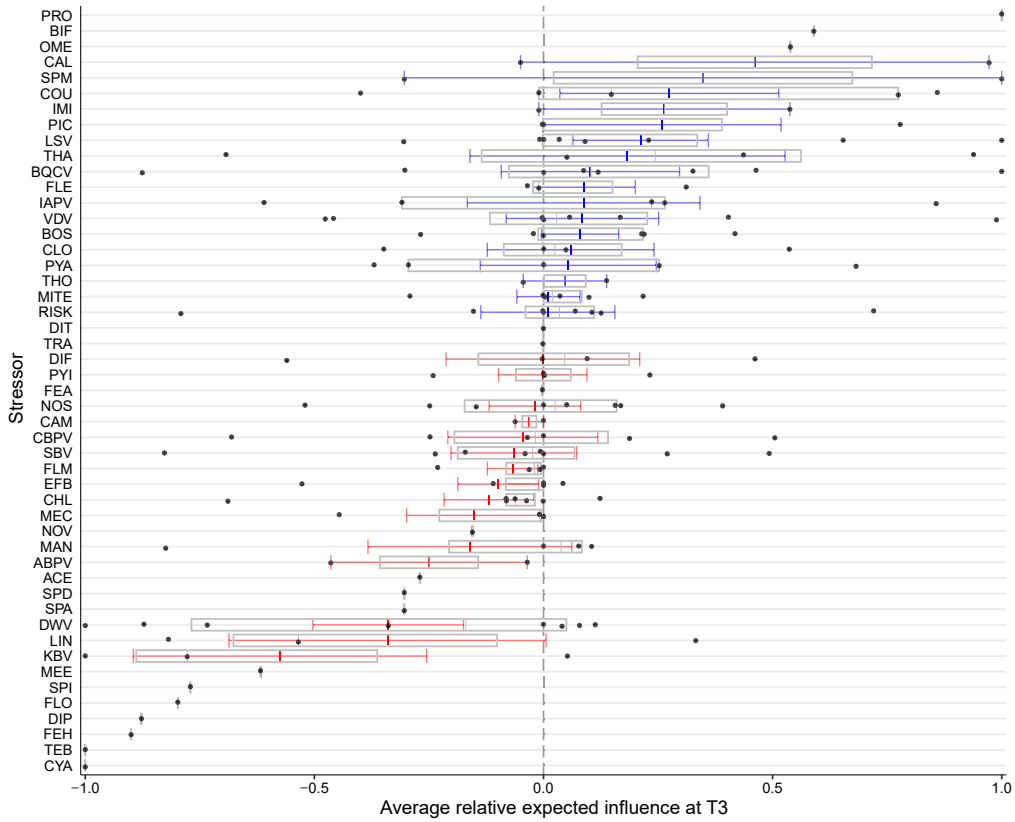

Figure 4. Networks had common and influential stressors at the end of exposure to crops

Certain stressors were more influential in a network than others, having either stronger positive (blue) or negative (red) interactions with other stressors. Here, the relative expected influence (EI) for each stressor detected within a crop has been averaged across crops at time point 3 (T3; at the end of exposure to a crop). The mean EI ± SEM values are shown, in descending order, as are the 25th and 75th percentiles and data points per crop.
See also Figures 3 and S3.

management, and species- and region-specific strategies, while allowing for developments in research, knowledge translation, and policy.[26] Networks have been applied to a diverse set of problems in conservation, such as maintaining genetic connectivity among target populations[44] or spatial connectivity among habitats.[45] Networks that capture multiple-stressor environments have great potential in conservation to quantify and predict environmental change and to develop management strategies.[26] Our study exemplifies the need to further explore the complex stressor environments of honey bees to build a more comprehensive framework for managing honey bee and colony health.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Field site selection and study design
- METHOD DETAILS
  - Sampling of colonies and laboratory analyses
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2024.03.039.

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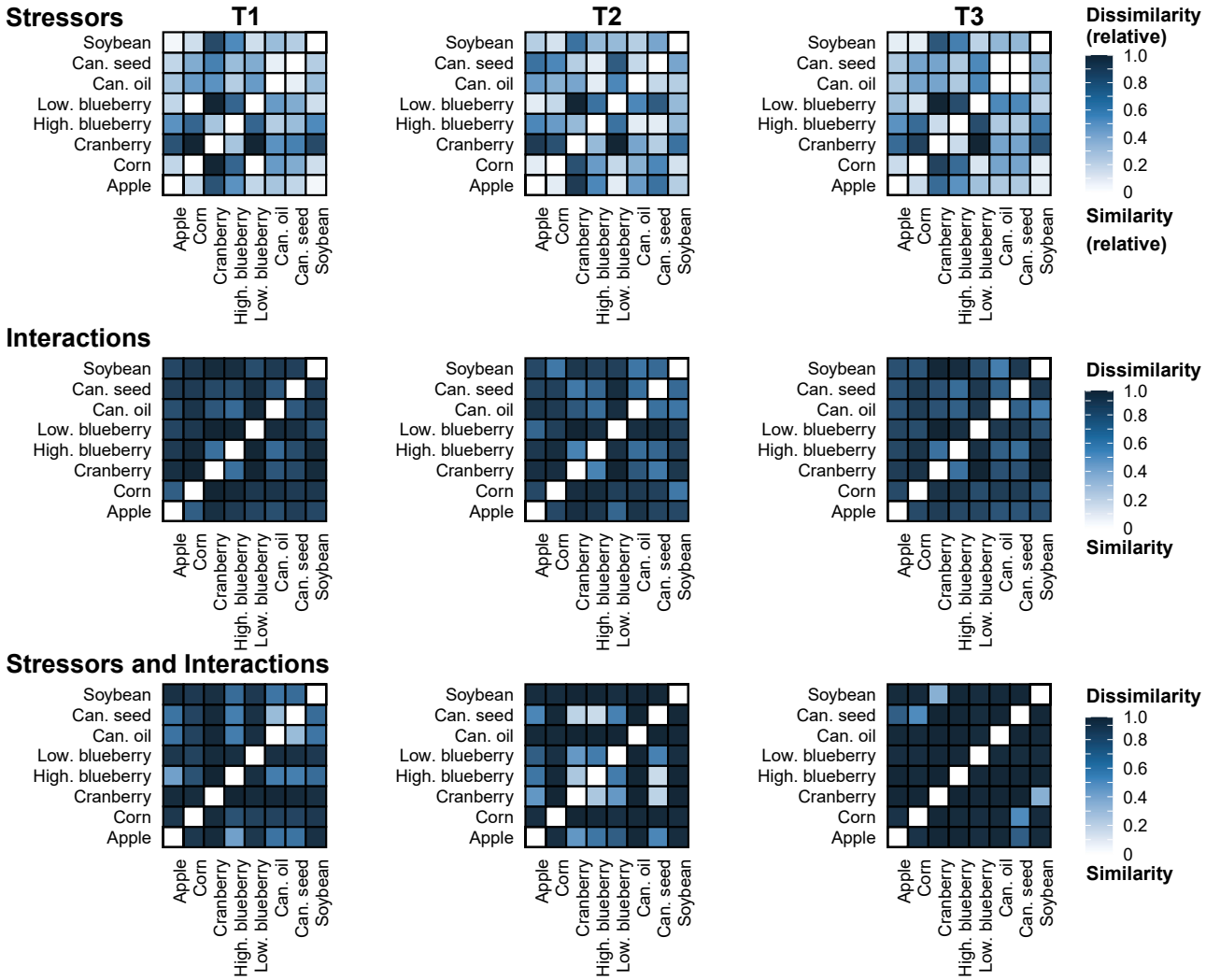

Figure 5. Stressor networks differed among focal crops and regions

Networks of colonies were compared among eight focal crop types at three time points (i.e., columns): before colonies were exposed to a crop (time point 1, T1), during exposure (time point 2, T2), and at the end of exposure (time point 3, T3). Each row of heatmaps uses a specific network dissimilarity measure: stressors were compared using NetLSD, with values scaled to 0–1; interactions were compared using Jaccard distance; and the combination of stressors and interactions was compared using portrait divergence. A value of 0 corresponds to two crops being identical in stressors, interactions, or the combination of the two; a value of 1 indicates that two crops greatly differ in these properties. Canola is abbreviated to "can," lowbush to "low," and highbush to "high."
See also Figure S4.

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AUTHOR CONTRIBUTIONS

Conceptualization, methodology, supervision, and funding acquisition, M.M.G., S.F.P., S.E.H., R.W.C., P.G., E.G.-N., D.B., L.J.F., and A.Z.; investigation and writing – review & editing, all authors; formal analysis and visualization, S.K.F.; writing – original draft, S.K.F. and A.Z.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
AccuStart II PCR Supermix	Quanta Biosciences	Cat # 95317
SsoAdvanced Universal Probes Supermix	Bio-Rad Laboratories	Cat # 172-52(80/81/82/84/85)
Acetic acid in acetonitrile in the presence of anhydrous sodium acetate and magnesium sulfate	Agriculture & Food Laboratory, ISO/IEC 17025 accredited Laboratory Services Division, University of Guelph	https://afl.uoguelph.ca/
Methanol/ammonium acetate and acetonitrile	Agriculture & Food Laboratory, ISO/IEC 17025 accredited Laboratory Services Division, University of Guelph	https://afl.uoguelph.ca/
Deuterium labelled internal standards, matrix matched blanks, and spikes	Agriculture & Food Laboratory, ISO/IEC 17025 accredited Laboratory Services Division, University of Guelph	https://afl.uoguelph.ca/
Critical commercial assays		
NucleoSpin Tissue kit	Macherey-Nagel GmbH & Company KG	Cat # 740952
NucleoSpin RNA kit	Macherey-Nagel GmbH & Company KG	Cat # 740955
iScript cDNA Synthesis kit	Bio-Rad Laboratories	Cat # 170889(0/1)
SSoAdvanced Universal SYBR Green Supermix	Bio-Rad Laboratories	Cat # 172-52(70/71/72/74/75)
Deposited data		
Raw and analyzed stressor data	This paper	Table S2
Oligonucleotides		
See Table S4 for primers and probes used in PCR and qPCR assays	This paper	Supplemental information
Software and algorithms		
R version 4.2.1	R Core Team	https://www.r-project.org/; RRID:SCR_001905
Python version 3.8	Python Software Foundation	https://www.python.org; RRID:SCR_008394
Applied Biosystems 7500 Fast Real-Time PCR Software version 2.3	Thermo Fisher Scientific	https://www.thermofisher.com
CFX Manager Software	Bio-Rad Laboratories	https://www.bio-rad.com; RRID:SCR_017251
Other		
Counting Chamber [Helber]	Hawksley Medical and Laboratory Equipment	https://hawksley.co.uk/
Upright Microscope [Eclipse Ci-L]	Nikon	https://www.microscope.healthcare.nikon.com/products/upright-microscopes/eclipse-ci-series
Applied Biosystems Veriti 96-Well Thermal Cycler	Thermo Fisher Scientific	https://www.thermofisher.com; RRID:SCR_021097
Applied Biosystems 7500 Fast System	Thermo Fisher Scientific	https://www.thermofisher.com; RRID:SCR_018051
CFX384 Touch Real-Time Detection System	Bio-Rad Laboratories	https://www.bio-rad.com; RRID:SCR_018057
Agilent 1260 InfinityLab High-Performance Liquid Chromatograph System	Agilent	https://www.agilent.com
SCIEX 5550 ESI-MS/MS system	SCIEX	https://sciex.com
Agilent 7890 Gas Chromatograph	Agilent	https://www.agilent.com

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RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Amro Zayed (zayed@yorku.ca).

Materials availability

This study did not generate new unique materials.

Data and code availability

The unique dataset generated from this study is provided as a separate supplemental file (Data S1). This study did not generate unique code, software, or algorithms. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Field site selection and study design

We conducted a two-year field study in regions that represented Canada's diverse climates and dominant crops[46] to identify the multiple-stressor environments that managed honey bee colonies are exposed to and to assess how they vary across Canada. Experimental sites were situated in high-density agricultural regions that had at least one of eight focal crops, spanning five Canadian provinces, with crop type implicitly covarying with region (see Figure S5). Each year, hives containing honey bee colonies were placed at a site located in (or if not possible, directly adjacent to) a focal crop for the pollination period of that crop. Four colonies (one apiary) were situated at each site, resulting in five sites per crop, and a total of 60 apiaries throughout the study (see Table S3). In 2020, colonies were placed in commodity canola (i.e., canola oil, total of five sites distributed across Alberta and Manitoba), hybrid seed-production canola (i.e., canola seed, Alberta), corn (Ontario), cranberry (five sites distributed across British Columbia and Québec), highbush blueberry (British Columbia), and soybean (Manitoba). In 2021, colonies were placed in apple and lowbush blueberry crops (Québec), canola oil (five sites distributed across Alberta and Manitoba), canola seed (Alberta), and cranberry and highbush blueberry (British Columbia). Although corn is wind-pollinated and soybean does not rely heavily on honey bee pollination,[47] both can overlap with nearby beekeeping operations.

The colonies were standardized by the number of brood chambers (either singles or doubles) per focal crop and started with a one-year-old laying queen. Colonies were inspected prior to inclusion in our experiments to ensure that they were healthy and free from obvious signs of disease. Following established methods,[48] sites within a certain crop system were located at least 3 km apart, to ensure that workers from different apiaries would not forage in the same area. The likelihood of honey bees foraging away from their colony tends to decrease with distance.[49]

METHOD DETAILS

Sampling of colonies and laboratory analyses

Colonies were sampled at three time points across the pollination period for a focal crop (see Table S3), to account for temporal differences in exposure to pesticides[5] or in pathogen/parasite dynamics.[20] Time Point 1 (T1) occurred at the start of the pollination period, prior to crop bloom and before colonies were exposed to a focal crop. For all crops except corn, Time Point 2 (T2) occurred during the pollination period, when >50% of a crop was in bloom; Time Point 3 (T3) occurred immediately at the end of the pollination period, either before or after colonies were removed from sites, and at the end of blooming and exposure to a focal crop. For colonies placed in corn, T2 occurred during the seeding of neonicotinoid insecticide-treated seed and T3 occurred at the end of the corn season.

At each time point, we assessed apiary levels of parasites, pathogens, and pesticides (see Table 1 for detected stressors). We collected nurse bees, as they can spread parasites and pathogens throughout the colony while feeding and cleaning,[5] by scooping bees from frames with open brood. We also collected hive matrices (i.e., bee bread/pollen and nectar) to quantify 239 agrochemicals using standard methods.[50,51] All samples were immediately placed on dry ice in the field and relocated to a -80°C freezer for storage. Samples from the four colonies of an apiary were pooled in the laboratory, yielding approximately 8 g of bee bread, 12 mL of nectar, and 200 nurse bees per site and time point. Concurrently, approximately 1000 nurse bees were collected from each apiary (i.e., pooled from subsampling each of the four colonies) to quantify *Varroa destructor* mite parasitism. These nurse bees were preserved in 70% ethanol and stored at room temperature until mites were counted using an alcohol wash method.[52] A mean mite abundance was calculated (average mites per 100 bees per apiary), with the number of bees being approximated by comparing the weight of 50 bees to that of the entire sample. Samples of bee bread and nectar were sent to the Agriculture and Food Laboratory (University of Guelph, Guelph, Ontario) for multiresidue pesticide analysis. Bees were also sent to the National Bee Diagnostic Centre (Beaverlodge, Alberta) to test for pathogens and viruses (see Table S4 for primers). Samples were prepared following standard methods.[20,53] The infection levels of nine viruses (ABPV, BQCV, CBPV, DWV-A, IAPV, KBV, LSV, SBV, and VDV-1/DWV-B) were quantified using qPCR with RP49 as a reference gene.[20] *Vairimorpha* (formerly *Nosema*) spores were quantified using standard microscopy

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methods,[20,53] and *N. ceranae* and *N. apis* were identified by end-point PCR.[54] *Melissococcus plutonius*, the causative agent of EFB, was detected by performing qPCR[20] with a 7500 Fast Real-Time PCR System and associated software (Applied Biosystems, Foster City, USA), and using β-actin as a reference gene.[54]

QUANTIFICATION AND STATISTICAL ANALYSIS

To compare the risk that different pesticides pose to honey bees, we assigned a dietary risk quotient (RQ) to each agrochemical[28,55] (Figure S1A) based on its field concentration in nectar and pollen, its median lethal dose (LD_{50} or dose that kills 50% of bees in test cages), and a honey bee's approximate daily consumption of nectar and pollen, where:

$$RQ = \frac{\left(\text{residue in nectar } \left(\mu g\ kg^{-1}\right) \times 140 \times 10^{-6}\ kg\ bee^{-1}\right) + \left(\text{residue in pollen } \left(\mu g\ kg^{-1}\right) \times 9.6 \times 10^{-6}\ kg\ bee^{-1}\right)}{\text{acute oral } LD_{50}\ \left(\mu g\ bee^{-1}\right)}$$

The LD_{50}s that were used to calculate our risk quotients were compiled from: the ECOTOXicology Knowledgebase,[56] the Pesticide Properties DataBase,[57] the European Food Safety Authority,[58] and reports from the Pest Management Regulatory Agency[59] and the World Health Organization.[60] An LD_{50} was used if it was derived using workers from any subspecies of *Apis mellifera*, followed the Organisation for Economic Co-operation and Development guidelines for acute oral [or diet] toxicity tests on honey bees,[61] and provided per bee or per body weight dosages. If tests using a high purity of the active ingredient were unavailable, studies on formulations or a combination of agrochemicals were used. Agrochemical concentrations that fell below the limits of quantification or detection were assigned a concentration equal to that limit. The average of acceptable LD_{50}s for an agrochemical was calculated, using lower or upper limits if necessary. At each site and time point, we summed across the dietary risk of all pesticides to obtain the 'total dietary risk' for an apiary (Figure S1B). For simplicity, we assumed the risk of multiple co-occurring pesticides to be additive,[29] because we wanted to facilitate comparisons among apiaries with varying stressor environments, and generally, we lack sufficient information on non-additive effects to effectively model this process in our risk estimate.

All statistical analyses for this study were conducted in R (version 4.2.1).[62] First, we derived summary statistics of the stressors found in honey bee colonies across Canada, to illustrate their multiple-stressor environments. We quantified the number of stressors and stressor groups found in an apiary across the pollination period for a focal crop. We also tested for correlations between the number of stressors detected and: the number of days since the start of the experiments; the total number of days of the experiments; and the numerical month the stressors were detected in; using Kendall's τ correlation coefficients with $\alpha = 0.05$. Next, we used networks to model stressor combinations and partial correlations for each focal crop and time point. We used the 'lvm' function from *psychonetrics* (version 0.10),[63] which preserved the identity of co-occurring stressors at each site and time point. We converted our data to wide format using code from 'ml_tsdlvm1' and standardized each stressor across time using a non-parametric quantile transformation.[63] Network models used an identity matrix for the factor loadings matrix, full-information maximum likelihood estimation, and a conjugate gradient, C++ based optimizer. The models were run in a C++11 environment facilitated by *RcppArmadillo* (version 0.11.4.3.1),[64] on high-speed computing networks provided by the Digital Research Alliance of Canada. Differences in network structure among crops and across time were evaluated using *netrd* (version 0.3.0).[65] To prepare data for use by *netrd*, network matrices were converted to graphs using *NetworkX* (version 3.1)[66] and the Python (version 3.8) environment was facilitated by *reticulate* (version 1.29).[67] Graphs were compared for dissimilarity in nodes (i.e., stressors) using NetLSD, edges (i.e., interactions) using Jaccard distance, and the combination of nodes and edges using Network Portrait Divergence.[65] Characteristics of the networks were determined and visualized using *qgraph*,[68] including the number of stressors and interactions. Centrality metrics ranked stressors based on their connectedness to other stressors (strength), their indirect connectedness and influence (closeness), their ability to bridge stressors (betweenness), and their weighted and signed influence on neighbors (expected influence).[27] Additional statistical details of experiments can be found in the results and figure legends.

Current Biology, Volume *34*

Supplemental Information

Honey bee stressor networks are complex and dependent on crop and region

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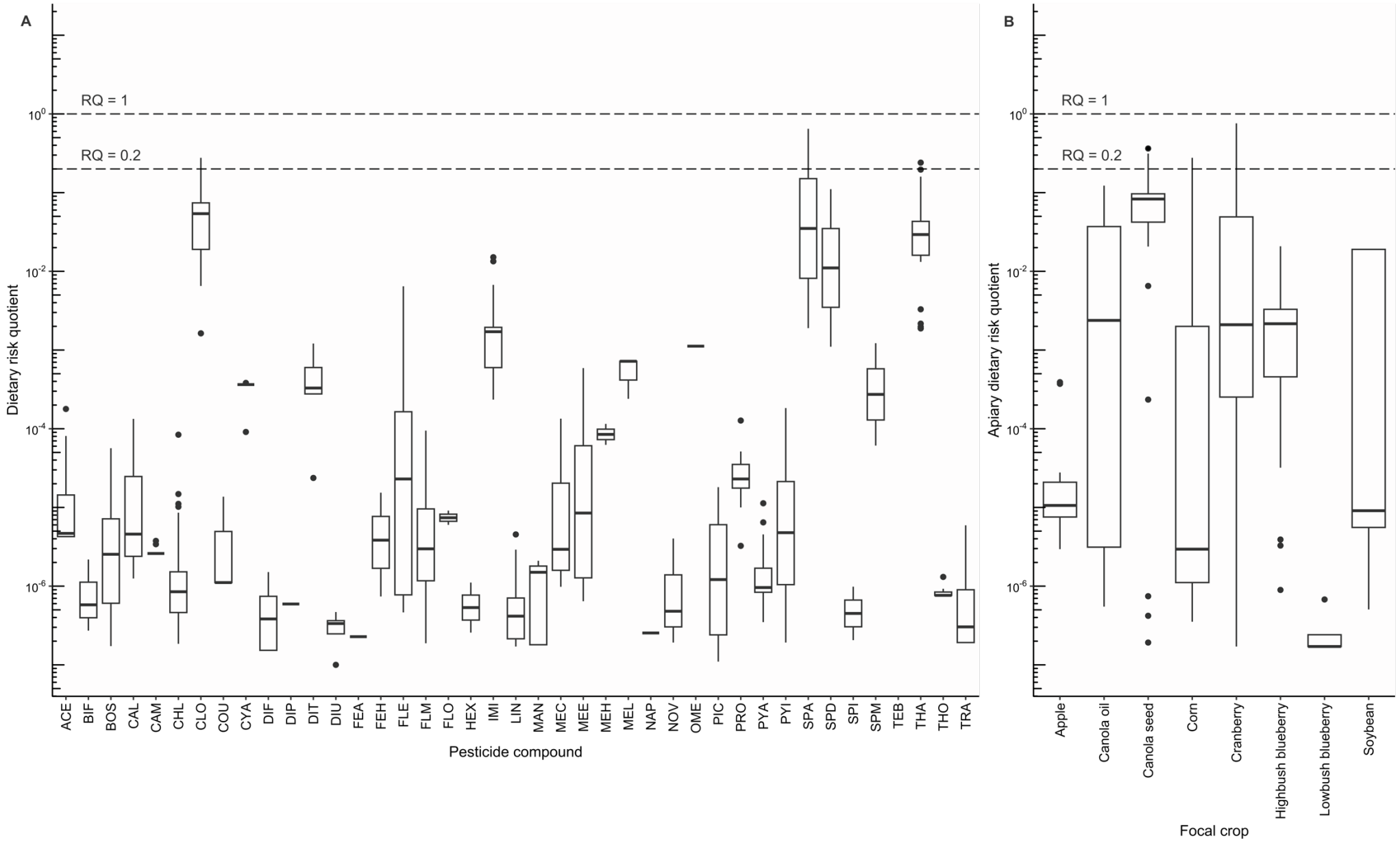

Figure S1. Apiaries were exposed to levels of pesticides that exceeded a risk threshold, Related to the STAR Methods.
Dietary risk quotients (RQs) for (A) 41 pesticide compounds found in the experimental colonies, with data points spanning eight focal crops, three time points, and two years; and (B) for apiaries placed in each of eight focal crops, with data points spanning three time points, and two years. The box plots show the median, the 25th and 75th quantiles, non-outlier minima/maxima as whiskers, and outliers as points. The dashed lines represent RQ = 1 (red), which would kill 50% of honey bees, and RQ = 0.2 (blue) the European Food Safety Authority's level of concern or honey bees' acute oral exposure to pesticides.

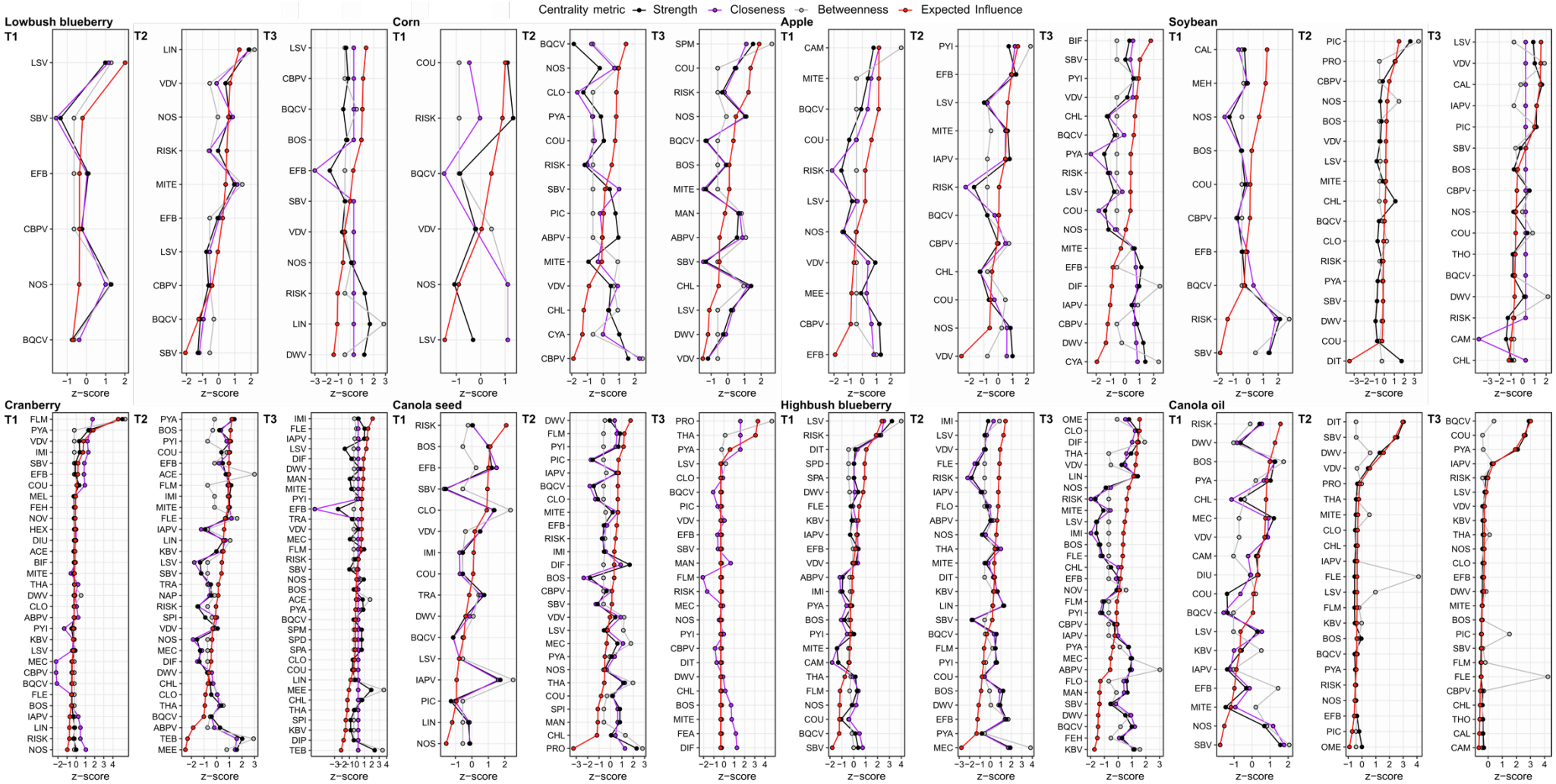

Figure S2. Stressors varied in their relative importance and influence across crops/regions and time, Related to Figure 1.
Measures of network centrality for each focal crop, represented by a *z*-score in relation to all other stressors for that crop. Stressors are found along the y-axes. 'Strength' indicates the un-signed (absolute) weight of interactions from a single stressor to all other stressors, 'Closeness' indicates how indirectly connected and influential a stressor is in a network, 'Betweenness' indicates the importance of a stressor as a bridge between stressors, and 'Expected Influence' indicates a stressor's weighted and signed influence on neighbours (Borsboom et al. 2021). Stressors are organized by descending Expected Influence.

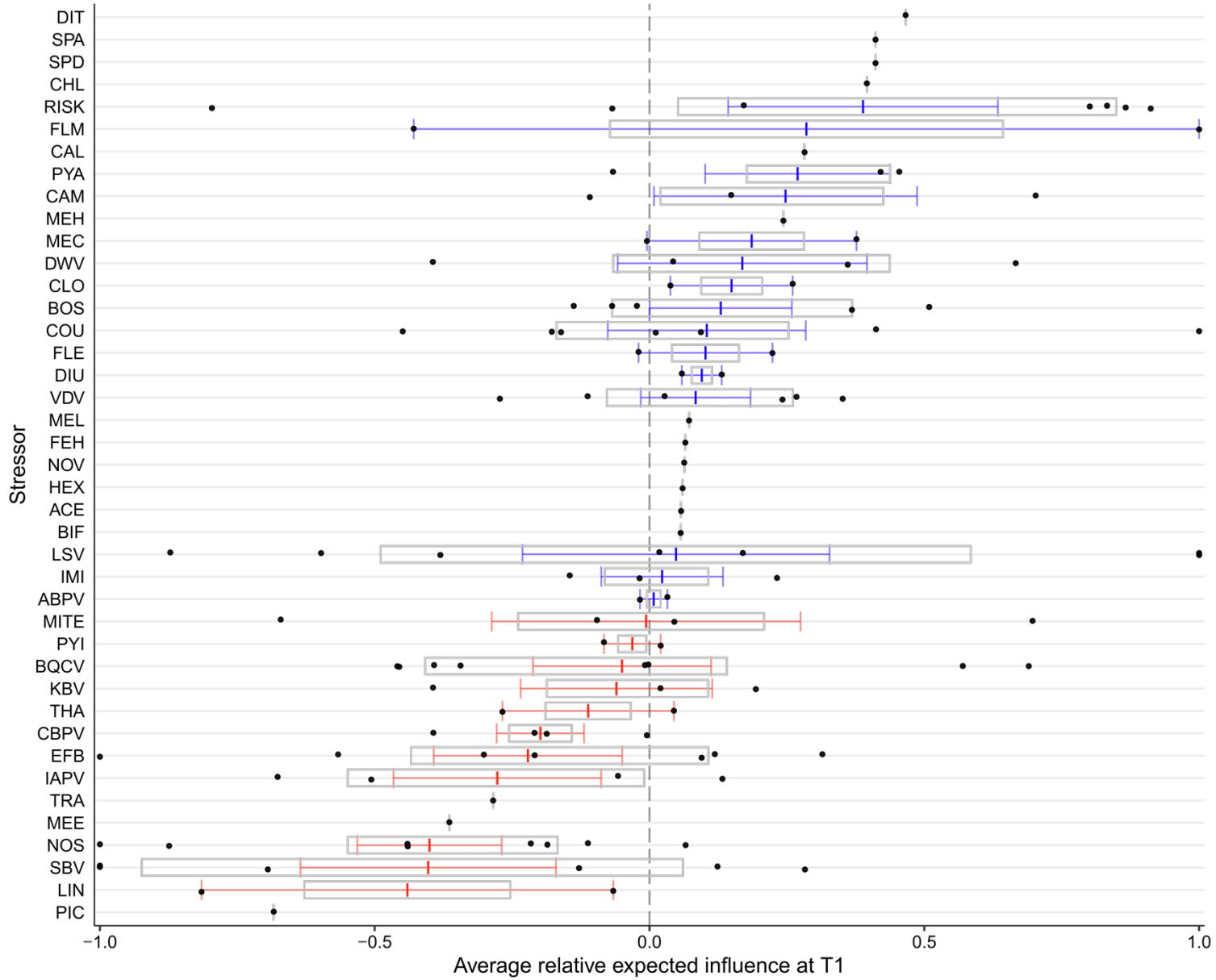

Figure S3. Networks had common and influential stressors before exposure to crops, Related to Figures 3 and 4.
Certain stressors were more influential in a network than others, having either stronger positive (blue) or negative (red) interactions with other stressors. Here, the relative Expected Influence (EI) for each stressor detected within a crop has been averaged across crops at Time Point 1 (T1; before exposure to a crop). The mean EI ± SEM values are shown, in descending order, as are the 25th and 75th percentiles, and data points per crop. See also Figures 3 and 4.

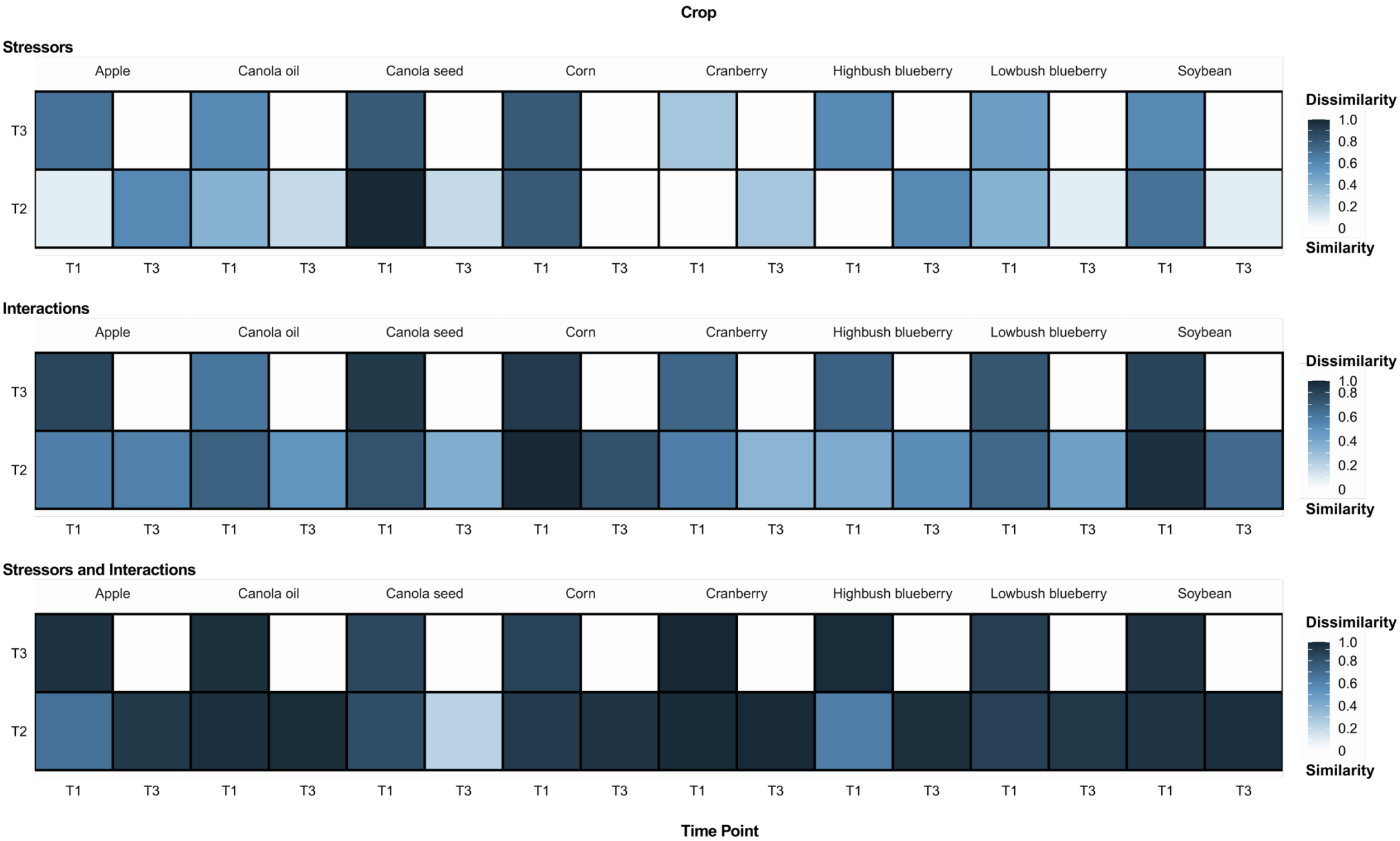

Figure S4. Stressor networks varied over time, Related to Figure 5.
For each focal crop, networks were compared among three time points: before colonies were exposed to a crop (Time Point 1; T1), when colonies were expected to experience considerable agrochemical exposure from a focal crop (Time Point 2; T2), and at the end of exposure to a focal crop (Time Point 3; T3). Each heat map uses a different network dissimilarity measure: stressors were compared using NetLSD (scaled to 0–1), interactions using Jaccard Distance, and the combination of stressors and interactions using Portrait Divergence. A value of 0 corresponds to two time points being identical in stressors, interactions, or the combination of the two for a given crop; a value of 1 indicates that two time points greatly differ in these properties. See also Figure 5.

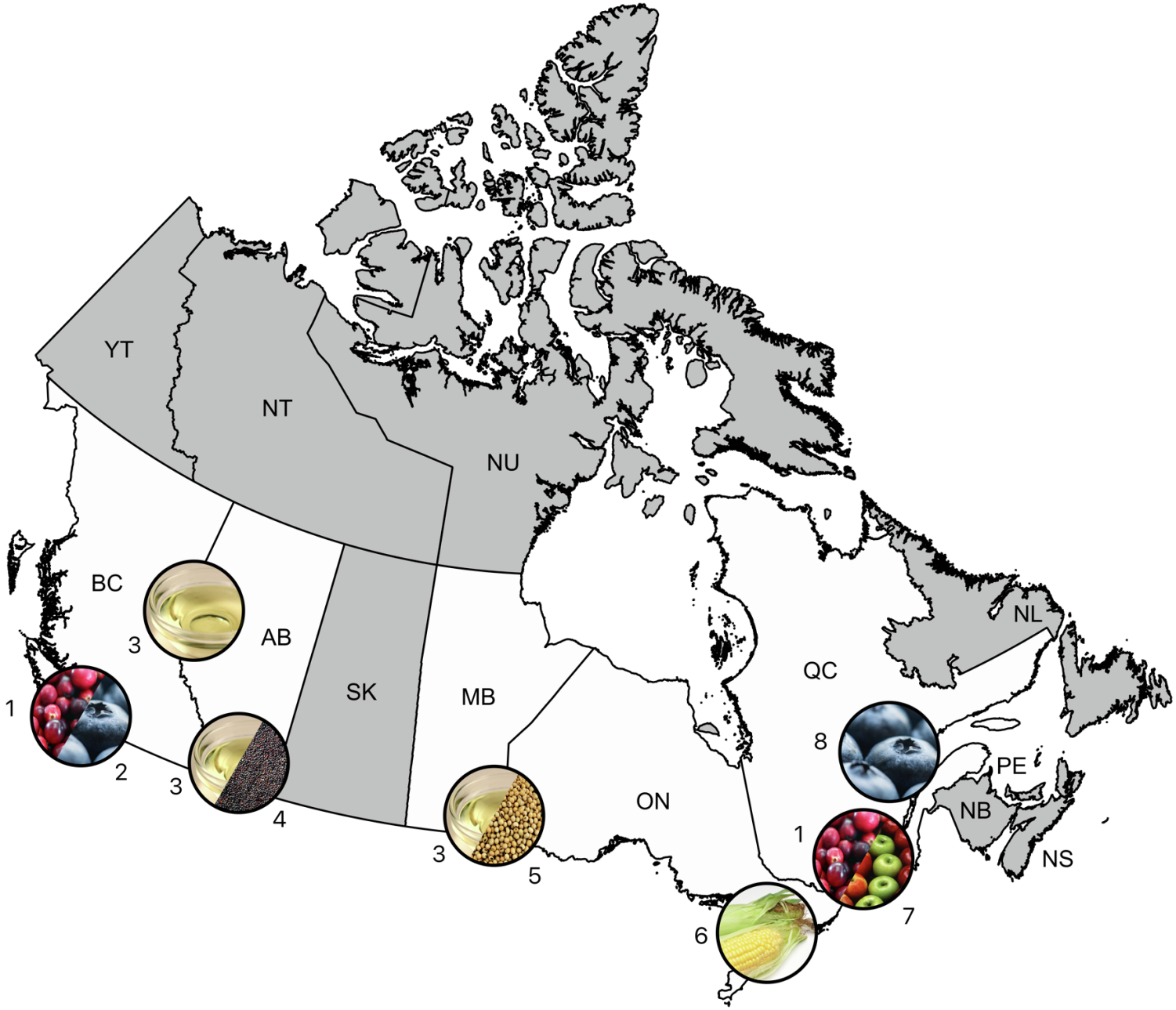

Figure S5. Study regions and focal crop types of study sites across Canada, Related to the STAR Methods.
Apiaries were placed in the following focal crops: 1 = cranberry[S1], 2 = highbush blueberry[S2], 3 = canola oil[S3], 4 = canola seed[S4], 5 = soybean[S5], 6 = corn[S6], 7 = apple[S7], 8 = lowbush blueberry[S2]. Provinces that contained our study regions and sites are white. BC = British Columbia, AB = Alberta, MB = Manitoba, ON = Ontario, QC = Québec. The map was created with QGIS (version 3.32.1) using Canadian provincial and territorial boundary data (Statistics Canada 2016). Stock images for crops are licensed under the Creative Commons.

Crop	Stressors			Interactions			Viruses			EFB			Mites			*Nosema*			Pesticides		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Apple	10	11	17	45	55	136	4	5	7	1	1	1	1	1	1	1	1	1	3	3	7
Corn	5	13	13	10	78	78	3	5	6	0	0	0	0	1	1	1	1	1	1	6	5
Cranberry	30	30	33	435	435	528	9	8	7	1	1	1	1	1	1	1	1	1	18	19	23
Canola oil	17	21	23	136	210	253	7	7	8	1	1	1	1	1	1	1	1	1	7	11	12
Canola seed	15	25	23	105	300	253	6	7	6	1	1	1	0	1	1	1	1	1	7	15	14
Highbush blueberry	23	23	29	253	253	406	8	8	9	1	1	1	1	1	1	1	1	1	12	12	17
Lowbush blueberry	6	9	10	15	36	45	4	5	6	1	1	1	0	1	0	1	1	1	0	1	2
Soybean	9	16	15	36	120	105	3	6	7	1	0	0	0	1	0	1	1	1	4	8	7

Table S1. The number of stressors and interactions in honey bee networks, Related to Figure 2.
The total number of stressors found in Canadian honey bee colonies per crop (excluding Dietary Risk) and their associated number of network interactions. Three time points are portrayed for each of eight focal crops: before colonies were exposed to a crop (Time Point 1; T1), during exposure (Time Point; T2), and at the end of exposure (Time Point 3; T3). Stressors are further broken down into: number of virus types; the presence of *Melissococcus plutonius* (European foulbrood or EFB), *Varroa destructor* mites, and *Nosema* spp.; and the number of different pesticide compounds. See also Figure 2.

Crop	Year (Number of Sites in Province)		Study length (days)	Sampling time points		
				T1	T2	T3
Apple	2021 (5 in QC)		20	April 29	May 13	May 19
Corn	2020 (5 in ON)		131	April 24	May 13 – 26	September 2
Canola oil	2020	(1 in sAB)	34	June 24	July 13	July 28
		(2 in MB)	56	June 24	July 16	August 19
		(2 in nAB)	44	July 7	July 20–31	August 19–20
	2021	(1 in sAB)	30	June 22	July 14	July 22
		(2 in MB)	48	June 23	July 19	August 10
		(2 in nAB)	64	June 8	July 9	August 11
Canola seed	2020 (5 in sAB)		38	June 23–24	July 14–21	July 29–31
	2021 (5 in sAB)		35	June 22–23	July 14–15	July 23–27
Cranberry	2020	(3 in BC)	33	June 3	June 18	July 6
		(2 in QC)	36	June 22	July 10	July 28
	2021 (5 in BC)		27	June 8	June 21	July 5
Highbush blueberry	2020 (5 in BC)		29	April 27–28	May 11	May 26
	2021 (5 in BC)		28	April 23	May 10	May 21
Lowbush blueberry	2021 (5 in QC)		24	May 17	June 2–3	June 9–10
Soybean	2020 (5 in MB)		58	June 22–23	July 20–21	August 14–19

Table S3. The sampling periods for each crop and region, Related to the STAR Methods.
The timing and length of sampling for each crop type, year sampled, and the number of sites (i.e., apiaries) per province and year. Three time points are portrayed: before colonies were exposed to a crop (Time Point 1; T1), during exposure (Time Point; T2), and at the end of exposure (Time Point 3; T3). Province codes are: BC = British Columbia, sAB/nA = southern/northern Alberta, MB = Manitoba, ON = Ontario, QC = Québec.

Target	Literature reference	Amplicon size (base pairs)	Primer/probe	Sequence (5'-3')
Nosema apis	S17	297	NosaRNAPol -F	AGC AAG AGA CGT TTC TGG TAC CTC A
			NosaRNAPol -R	CCT TCA CGA CCA CCC ATG GCA
Nosema ceranae	S17	662	NoscRNAPol -F	TGG GTT CCC TAA ACC TGG TGG TTT
			NoscRNAPol -R	TCA CAT GAC CTG GTG CTC CTT CT
EFB	S18	79	Melisso-F	CAG CTA GTC GGT TTG GTT CC
			Melisso-R	TTG GCT GTA GAT AGA ATT GAC AAT
			Melisso-Probe MGB	6'FAM-CTTGGTTGGTCGTTGACMBGNFQ
β-Actin	S18	121	β-Actin-F	CCT GGA ATC GCA GAT AGA ATG C
			β-Actin-R	CAA GAA TTG ACC CAC CAA TCC ATA
			β-Actin-probe	HEX-TCA CTG CCC TAG CAC CAT CCA CA-TAMRA
ABPV	S19	197	ABPV-F6548	TCATACCTGCCGATCAAG
			KIABPV-B6707	CTGAATAATACTGTGCGTATC
KBV	S20	106	KBV-F	TGAACGTCGACCTATTGAAAAA
			KBV-R	TCGATTTTCCATCAAATGAGC
CBPV	S19	296	CBPV1-qF1818	CAACCTGCCTCAACACAG
			CBPV1-qB2077	AATCTGGCAAGGTTGACTGG
LSV 1-4	S21	152	LSV1-4-F-2157	CGTGCGGACCTCATTTCTTCATGT
			LSV1-4-R-2309	CTGCGAAGCACTAAAGCGTT
DWV	S19	136	DWV-F8668	TTCATTAAAGCCACCTGGAACATC
			DWV-B8757	TTTCCTCATTAACTGTGTCGTTGA
VDV	S22	140	VDV-F2	TATCTTCATTAAAACCGCCAGGCT
			VDV-R2a	CTTCCTCATTAACTGAGTTGTTGTC
BQCV	S19	294	BQCV-qF7893	AGTGGCGGAGATGTATGC
			BQCV-qB8150	GGAGGTGAAGTGGCTATATC
IAPV	S20	587	IAPV-F1aF	GCGGAGAATATAAGGCTCAG
			IAPV-F1a R	CTTGCAAGATAAGAAAGGGGG
SBV	S19	335	SBV-qF3164	TTGGAACTACGCATTCTCTG
			SBV-qB3461	GCTCTAACCTCGCATCAAC
RP49 (ribosomal protein 49)	S19	205	RP49-qF	AAGTTCATTCGTCACCAGAG
			RP49-qB	CTTCCAGTTCCTTGACATTATG

Table S4. The names and gene sequences of the primers and probes assayed, Related to the STAR Methods.
Assays were performed via PCR and qPCR to identify target organisms, with sequences compiled from the associated reference.

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