Climatic predictors of prominent honey bee (*Apis mellifera*) disease agents: *Varroa destructor*, *Melissococcus plutonius*, and *Vairimorpha* spp.

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Abstract

Improving our understanding of how climate influences honey bee parasites and pathogens is critical as weather patterns continue to shift under climate change. While the prevalence of diseases vary according to regional and seasonal patterns, the influence of specific climatic predictors has rarely been formally assessed. To address this gap, we analyzed how occurrence and intensity of three prominent honey bee disease agents (*Varroa destructor* — hereon *Varroa* — *Melissococcus plutonius*, and

40   *Vairimorpha* spp.) varied according to regional, temporal, and climatic factors in honey bee colonies
41   across five Canadian provinces. We found strong regional effects for all disease agents, with consistently
42   high *Varroa* intensity and infestation probabilities and high *M. plutonius* infection probabilities in British
43   Columbia, and year-dependent regional patterns of *Vairimorpha* spp. spore counts. Increasing wind
44   speed and precipitation were linked to lower *Varroa* infestation probabilities, whereas warmer
45   temperatures were linked to higher infestation probabilities. Analysis of an independent dataset shows
46   that these trends for *Varroa* are consistent within a similar date range, but temperature is the strongest
47   climatic predictor of season-long patterns. *Vairimorpha* spp. intensity decreased over the course of the
48   summer, with the lowest spore counts found at later dates when temperatures were warm.
49   *Vairimorpha* spp. intensity increased with wind speed and precipitation, consistent with inclement
50   weather limiting defecation flights. Probability of *M. plutonius* infection generally increased across the
51   spring and summer, and was also positively associated with inclement weather. These data contribute
52   to building a larger dataset of honey bee disease agent occurrence that is needed in order to predict
53   how epidemiology may change in our future climate.

Introduction

55   Infectious diseases and parasites are among the dominant factors affecting honey bee (*Apis mellifera*)
56   colony health in commercial operations.[1-3] Several recent studies have investigated relationships
57   between climatic variables and overwintering colony mortality,[4-8] but similarly large-scale association
58   studies between weather patterns and specific diseases or pathogens are more rarely conducted, with
59   the notable exception of Rowland *et al.*,[9] who examined patterns of disease incidence associated with
60   climatic variables in England and Wales. Weather patterns are expected to become less stable as the
61   climate changes, with higher frequencies of extreme events along with a shifting baseline to warmer
62   temperatures and region-specific shifts in precipitation.[10-12] A better understanding of relationships
63   between honey bee disease agents and current climatic trends would be an asset for predicting how
64   these agents might change in the vicissitudes of our future climate.

65   Several etiological agents are known to occur in seasonal cycles, including but not limited to
66   *Vairimorpha* spp. (formerly *Nosema* spp.,[13] the causative agent(s) of nosemosis), *Melissococcus*
67   *plutonius* (the causative agent of European foulbrood disease, or EFB), and the mite *Varroa destructor*
68   (hereon referred to as *Varroa*, the causative agent of varroosis).[14-21] Such seasonal patterns may be
69   driven by direct effects of weather on pathogen or parasite survival and reproduction, or indirect effects
70   via associated changes within the honey bee colony. As an example, high temperatures (>35°C) reduce
71   *Varroa* survival and reproduction directly,[22,23] whereas moderately increasing temperatures during the
72   spring lead to increased honey bee brood rearing, which *Varroa* in turn requires for reproduction.[14]
73   Moreover, favorable conditions (warm temperatures with low wind and precipitation) increase dispersal
74   opportunities between colonies for disease agents, but might also affect the risk of colonies manifesting
75   diseases that are associated with poor food availability or stress (such as EFB[24]). It is thus difficult to
76   predict, *a priori*, how weather conditions may translate into changes in intensity or occurrence of
77   disease agents.

78   *Varroa* is broadly considered the most economically damaging parasite of honey bees, and its life cycle is
79   closely tied to seasonal changes in honey bee brood production.[14,25] The mites disperse to new colonies
80   through contact between bees at forage sources as well as robbing and drift (see reviews[14,25] for more
81   information about *Varroa* biology and control). *Varroa* has successfully become established in all

82   continents where honey bees are maintained, and only a handful of island territories remain *Varroa*-
83   free.[14,26] Many studies broadly highlight the impact of winter brood breaks on *Varroa* loads and
84   differences in *Varroa* population growth in temperate versus tropical climates (reviewed in Rosenkranz
85   et al.[25]). The studies that have investigated relationships with weather conditions have focused mainly
86   on temperature,[9,22,23,27] with extreme heat being associated with reduced *Varroa* reproduction[22,23] but
87   otherwise a positive relationship between *Varroa* occurrence and temperature has been observed.[9,27]
88   One study conducted by Harris *et al.,*[23] found that rainfall had no association with *Varroa* population
89   growth rates, and a recent study by Rowland *et al.*[9] found a negative correlation between *Varroa*
90   occurrence and precipitation and wind. Further studies in different regions would be helpful to clarify
91   and substantiate these findings in different geographies.

92   *Vairimorpha* spp. are microsporidian endoparasites of the honey bee midgut and, in temperate climates,
93   tend to infect bees with seasonal intensities that are normally highest in the spring and early summer,
94   then decrease in late summer and early fall.[18-21] Two species of *Vairimorpha* are known to infect honey
95   bees in Canada: *V. apis* and *V. ceranae.* with different seasonal trends. Whereas *V. apis* exhibits a regular
96   peak in average gut spore loads in the spring and often a secondary peak in in the fall,[28] *V. ceranae*
97   trends appear more variable across different countries.[29-32] Regional differences in *V. ceranae* and *V.*
98   *apis* abundance has led to speculation over whether each species' relative success is influenced by
99   climate, but it is not clear if this is the case.[17,19] *V. ceranae* is the dominant species in Canada and the
100   United States;[33,34] therefore, in these regions detection metrics that do not discriminate between
101   species (*e.g.* spore counts) likely mainly represent *V. ceranae.* Some[18,35] but not all[36] field studies have
102   found that higher temperatures correlate with lower spore loads, and some research suggests that
103   geographic region (and, by extension, climate) is an important factor influencing *Vairimorpha* spp.
104   epidemiology.[37] Indeed, in addition to warm temperatures inhibiting *V. apis* and *V. ceranae* proliferation
105   in laboratory trials,[38] inclement (cold, windy, or wet) weather limits opportunities for defecation flights
106   that help clear *Vairimorpha* spp. infections,[39,40] but interactions among these variables have, to our
107   knowledge, not yet been investigated.

108   EFB disease can cause severe damage to honey bee colonies by killing developing brood, thereby
109   weakening the colony and reducing productivity.[16] *M. plutonius* is often detected in asymptomatic and
110   apparently healthy colonies, especially those proximal to symptomatic colonies and apiaries;[16,24,41]
111   therefore, dispersal from infected units is likely an important factor governing patterns of *M. plutonius*
112   prevalence. EFB is thought to be an opportunistic disease that tends to manifest when larvae are under
113   nutritional stress, such as when colonies are producing new brood very quickly in the spring or when
114   pollen and nectar resources are low.[16,24] However, larval food supply is clearly not the only factor
115   involved, since larvae fed in excess may still develop the disease.[42] Interestingly, Milbrath[16] and Bailey[43]
116   suggest that even favorable foraging conditions could lead to temporary nutritional stress of larvae
117   during the spring if a strong nectar flow causes nurse bees to reallocate their labor to process honey.
118   Rowland *et al.*[9] found that higher precipitation (associated with poor foraging conditions) was linked to
119   higher EFB incidence in England and Wales, but no relationship was found with temperature nor wind
120   speed.

121   Given how widespread these honey bee pathogens and parasites are and their apparent associations
122   with weather conditions, we sought to investigate relationships between the disease agents and climatic
123   variables while accounting for broad differences between regions and years. To this end, we used a
124   dataset of *Varroa* infestation levels, *Vairimorpha* spp. spore intensities, and *M. plutonius* occurrence

125 across five Canadian provinces, representing a wide range of climates and geographies, to identify
126 associations with temperature, precipitation, and wind speed. While regional differences were most
127 pronounced, climatic variables were also influential and largely reveal intuitive patterns. This dataset
128 will help build the growing body of knowledge and data necessary to facilitate predictive modelling of
129 disease agent dynamics under projected climate conditions.

130

Methods

Honey bee colonies and datasets

133 This study includes analyses of a primary dataset (*Varroa*, *Vairimorpha*, and *M. plutonius* data collected
134 in 2020 and 2021) and a validation dataset (*Varroa* only, collected in 2016). Some, but not all, elements
135 of the primary and validation datasets have been previously described in several publications (see
136 details below).

137 A subset of the primary dataset's honey bee colonies and pathogen/parasite data have been previously
138 described by French *et al.*[44] (240 colonies sampled in 2020 and 2021) along with additional colonies (a
139 further 240 in both years) described here and partially reported in McAfee *et al.*[45] As described further
140 below, samples for *M. plutonius* and *Vairimorpha* spp. testing were pooled ahead of analysis (4 colonies
141 per composite sample), whereas samples taken for *Varroa* detection were not pooled. The total number
142 of samples analyzed for *M. plutonius*, *Vairimorpha* spp., and *Varroa* were therefore N = 120, 120, and
143 480, respectively, though all were derived from the same 480 colonies.

144 As previously described,[44] the colonies were located in five provinces (British Columbia (BC), Alberta
145 (AB), Manitoba (MB), Ontario (ON), and Quebec (QC)), and eight different regions. Within these regions,
146 colonies were derived from the same beekeeping operation; therefore, operational and regional
147 differences are indistinguishable. Two regions/operations were represented within AB (near Grande
148 Prairie to the north and Lethbridge to the south); three regions/operations were represented in QC
149 (near Quebec City in 2020 and near Montreal and Lac St. Jean in 2021); and BC, MB, and ON were
150 represented by one region/operation each. Colonies were placed near or far from one of eight possible
151 focal crops, which depended on region, and sampled at the start (immediately before or immediately
152 after moving into pollination), middle (peak bloom), and end (immediately before or immediately after)
153 of the pollination period, the duration of which varied depending on commercial standards for each
154 crop. In the case of corn, which is not pollinated by honey bees, colonies were sampled before sowing,
155 during sowing, and at the end of the growing season. An equal number (N = 240) of additional colonies
156 produced from the same stocks, according to the same standards, as those assigned to the focal crops
157 but located far from the pollination yards (>1.5 km, with the exception of highbush blueberries which
158 were > 1.3 km) are included in this dataset, bringing the final number of colonies to 480 across site types
159 (near or far from crops) in both years, each subject to three sampling events over time. A subset of
160 these data are also described in McAfee *et al.*[45] Colony locations in each province and at each time point
161 are illustrated in **Supplementary Figure S1**.

162 In each region, colonies were either supplied by collaborating beekeepers, owned by the relevant
163 institution, or purchased from producers, but in all cases sampling and management during the
164 experiment were conducted by the research teams. As previously described,[44] colonies were headed by

165 locally overwintered queens and colony sizes were standardized in deep Langstroth-style single- or
166 double- box pollination units according to standards for commercial pollination for each focal crop.
167 Although *Varroa* is one of the parasites of interest, which we subsequently measured, early season
168 *Varroa* control is often necessary to ensure colony vitality throughout the season; therefore, in some
169 cases colonies were treated with miticides before the experiment began if adult bee infestation levels
170 exceeded the economic threshold (>1 mite per 100 bees).[46] Specifically, in early spring, colonies in BC
171 (2020; n = 64) were treated with Formic Pro and colonies in southern Alberta (2020 and 2021; n = 48 in
172 both years) were treated with Apivar according to manufacturer instructions. Mite treatments applied
173 to colonies prior to being purchased from commercial suppliers (BC in 2021 [n = 80] and MB in 2020 and
174 2021 [n = 56 and 16, respectively]) are unknown. All other colonies in northern AB (n = 16 in both years),
175 ON (n = 40 in 2020), and QC (n = 16 in 2020 and 80 in 2021) were under university or government
176 research laboratory management for the full calendar year and miticides were not applied. These
177 differences in treatment (and other operational/regional differences) were accounted for statistically by
178 including region as an interactive term in the model and by including colony as a random intercept term
179 (accounting for differences in baseline mite levels). No colonies were treated between study
180 commencement and termination, and no colonies were treated with Fumagillin-B (a product for
181 controlling *Vairimorpha* spp.) or oxytetracycline (an antibiotic for controlling EFB disease).

182 The *Varroa* validation dataset consisted of previously published data quantifying *Varroa* infestations in
183 Canada in 2016 (described by Borba *et al.*[47]). We used these data to validate our findings relating *Varroa*
184 to regional, temporal, and climate variables and explore how length of beekeeping season relates to
185 *Varroa* occurrence. For our analysis, we only used *Varroa* infestation data (mites per 100 bees,
186 determined by the alcohol wash method[47]) from overwintered colonies belonging to, and managed by,
187 beekeeper collaborators where their mite loads were not manipulated. This includes N = 480
188 overwintered colonies located in BC, southern AB, southern ON, and southern QC. Colonies within BC
189 were located in 11 yards and were owned by 11 operators in coastal BC (<100 km from the coast; 8
190 yards) and interior BC (Thompson/Okanagan and Kootenay/Boundary regions; 3 yards), which have
191 notably different climates. Colonies in AB, ON, and QC were also distributed across multiple yards, all of
192 which are shown in Borba *et al.*[47] Each colony was sampled at up to three time points during the active
193 beekeeping season. Data were filtered to exclude colonies from which fewer than two mite wash
194 samples were obtained between May and September, as well as colonies which beekeepers moved to
195 unknown locations between mite sampling events.

196 *Pathogen testing (primary dataset)*

197 *Varroa, M. plutonius,* and *Vairimorpha* 2020 and 2021 sample analysis was performed exactly as
198 previously described.[44] Briefly, for *M. plutonius* and *Vairimorpha*, samples were analyzed as pooled
199 replicates (15 bees from 4 colonies each, yielding n = 5 replicates from each site type (near to or far from
200 focal crops) for each crop in each year. The same four colonies were pooled at each time point, and
201 pooled colonies were always located together in the same yard (and experienced the same climatic
202 conditions). *M. plutonius* analysis was performed by endpoint PCR and *Vairimorpha* spore counts were
203 determined by microscopy (hemocytometer counting) and expressed as number of spores per bee.[44]
204 Samples for *Varroa* mite counts were not pooled across colonies, and mite counts per 100 bees were
205 determined using the alcohol wash method as previously described.[47] Therefore, final sample sizes for
206 *M. plutonius* and *Vairimorpha* data were N = 60 pooled samples per time point, per year (360 samples
207 from 120 pooled four-colony units), whereas the sample size for *Varroa* was N = 240 colonies per time

208   point, per year (1,440 samples from 480 colonies). Please see the previously published methods for
209   specific details regarding the protocols used.[44]

210   *Climatic data extraction*

211   Datasets from Environment and Climate Change Canada climate stations[48] were used to extract hourly
212   and daily climate variables (mean, maximum, and minimum daily temperatures, average daytime and
213   nighttime wind speeds, and total precipitation). The climate stations that were within 30 km of the
214   colony locations were selected and the daily and hourly datasets for the corresponding time of the
215   sampling events were downloaded. The daily climate records were used to extract the daily climate
216   variables for the sampling dates and were averaged over three weeks (21 days) prior to the sampling
217   date. Averaging climatic variables across time prior to sampling dates improves model fits,[9] and three
218   weeks prior (corresponding to one honey bee brood cycle) was the longest period for which we had
219   precise knowledge of colony GPS locations. Because wind is predominantly expected to influence honey
220   bees via changing their flight behavior, and since flight only occurs during the daylight hours, we used
221   the hourly climate records to extract daytime and nighttime averages of wind speed for the sampling
222   dates, which were also averaged over three weeks prior to the sampling dates. Daytime was defined as
223   5 am to 8 pm for all locations and dates, except for September sampling in Ontario, for which daytime
224   was defined as 6 am to 7 pm to more closely match actual sunrise and sunset times late in the season.
225   For the colonies with more than one climate station within a 30 km distance, the daily, daytime, and
226   nighttime climate data for sampling dates and three weeks prior to the sampling dates were extracted
227   for each climate station independently, then spatially averaged across all climate stations within the 30
228   km distance.

229   Prior to conducting statistical modelling, correlations among climate variables in the primary dataset
230   were assessed by computing Pearson correlation coefficients (**Supplementary Figure S2**). Based on
231   these results, we included only mean temperature, average daytime wind speed, and total precipitation
232   in our statistical models (described under "Statistical analysis," as these variables had correlation
233   coefficients of $|r| < 0.4$ in 2020 and < 0.3 in 2021. We chose to use daytime wind exclusively, because
234   wind is expected to only impact honey bees during the day when they have an opportunity to fly. For
235   precipitation, we used daily totals, rather than daytime totals, because precipitation is also linked to
236   nectar production in flowers and ambient humidity regardless of when it falls, and could therefore
237   conceivably affect honey bee behavior, thermoregulation, or nutrition at any time.

238   *Statistical analysis*

239   All statistical analyses were performed in R (version 4.3.0) using R Studio (version 2023.09.1+494)[49]. The
240   primary dataset (2020 and 2021) and *Varroa* validation dataset (2016) were analyzed separately. For the
241   primary dataset, site type (whether colonies/replicates were located near or far from focal crops) was
242   first considered as a covariate but in no case was it a significant predictor, so it was dropped from the
243   models. For all models, appropriateness of fit was confirmed using tools within the DHARMa package[50]
244   Interaction terms were removed from the model if they were non-influential and if appropriateness of
245   fit was retained after doing so.

246   The *Varroa* data in the primary dataset are proportional (mites per 100 bees) and zero-heavy; therefore,
247   we analyzed the data in two ways: first, we modelled the non-zero subset of the data (153/480 samples)
248   to describe how intensity of *Varroa* infestation changed in different regions over time, then, we

249   modelled binomial *Varroa* detections (occurrence) separately. In no cases (for *Varroa* nor other
250   pathogens) were zeroes imputed. The non-zero *Varroa* data were transformed by taking the natural
251   logarithm and analyzed using a linear mixed model (lme4 package[51]) with year (two levels) as a fixed
252   effect, calendar date (continuous) and general region/operation (eight levels) as interacting effects, and
253   colony as a random intercept term. Next, *Varroa* occurrence (1 = detected, 0 = not detected) from
254   sampling dates at similar points in the beekeeping season (all in July for 2020 and all in June for 2021, no
255   repeated sampling, years analyzed separately) were extracted from the complete dataset and analyzed
256   using a generalized linear model, specifying a binomial distribution, and with region/operation (five
257   levels in each year) included as a fixed effect. The Ontario and Quebec-Montreal regions were excluded
258   from this analysis as they did not have a *Varroa* sample taken at a comparable date to the other regions.

259   To determine relationships with regional, temporal and climatic variables in the primary dataset, *Varroa*
260   occurrence from all time points and regions were analyzed using a generalized linear mixed model
261   according to the following formula: Occurrence ~ Temperature*Wind+Precipitation + Date*Region +
262   Year + (1|Colony). Here and in all cases (except for the *Varroa* validation analysis; see below), the three
263   climatic variables were initially included as a three-way interaction term, but the model was simplified if
264   this interaction was not significant. If a climate variable did not significantly predict occurrence as a main
265   or interactive effect, and if retaining the variable in the model did not significantly improve residual or
266   simulated residual distributions, the variable was dropped. *M. plutonius* occurrence was modelled using
267   the same principles, according to the formula: Occurrence ~ Wind*Precipitation + Date*Region + Year +
268   (1|Unit), where unit refers to the pooled sample group. For *Vairimorpha*, non-zero intensities (spore
269   loads; 305/360 samples) were first log10 transformed, then modelled using a linear mixed model
270   according to the formula: Intensity ~ Wind*Precipitation + Temp + Date*Region + Year + (1|Unit).
271   *Vairimorpha* data from Quebec in 2020 were removed as there were too few data points to draw
272   meaningful comparisons (< n=3 per site type and time point).

273   To validate the results of our primary analysis of *Varroa* occurrence and climate data, we used the same
274   linear model to describe *Varroa* occurrence data on the 2016 validation dataset (except that year was
275   not included, as this dataset only spans one year). This dataset comprised *Varroa* occurrence previously
276   published by Borba *et al.*[47] (see "honey bee colonies and datasets" section). The climate data were
277   downloaded and averaged for each site in the same manner as described for the 2020 and 2021 data. In
278   a second evaluation, we analyzed *Varroa* occurrence at the three different sampling events (early
279   season, mid-season, and late season) separately, as the mid-season sampling dates have the greatest
280   temporal overlap with the 2020 and 2021 data. For this analysis, we used a generalized linear model (no
281   random effects were specified as there was no repeated sampling of colonies within each sampling
282   event). Mean daily temperature data (spatially averaged across climate stations within a 30 km radius of
283   each site) for full 2016 calendar year were also obtained for the corresponding GPS locations and used
284   to estimate the beginning and end of the brood rearing season. While we do not know the actual dates
285   that substantial brood rearing commenced and ceased, we estimated it to begin on the date at which
286   the seven-day sliding average temperature was at or above 5°C (when brood rearing intensifies[52]) for at
287   least three consecutive days, and ending on the date at which the seven-day sliding average
288   temperature declined below 5°C for ten or more consecutive days. We chose these criteria on the
289   rationale that it guards against making inferences based on spurious fluctuations. While imprecise, the
290   approach is meant to illustrate broad patterns of seasonal timing in different regions.

291   *Data availability*

292   All raw data underlying these analyses are available as supplementary material. 2020 and 2021
293   pathogen, parasite, regional, temporal, climate, and GPS location data are available in **Supplementary**
294   **Data 1 & 2**, respectively. Colony locations are also depicted in **Supplementary Files 1 & 2.** The 2016
295   *Varroa* and climate data are reported in **Supplementary Data 3**.

296   **Results**

297   Analysis of the primary dataset shows that *Varroa* intensity generally increased over time, as expected,
298   but varied significantly by region and year (**Figure 1A**; see **Table 1** for statistical reporting). Notably,
299   *Varroa* intensities in BC's Lower Mainland were generally high even early in the season (April-May), with
300   levels comparable to late-season (Aug-Sept) intensities in other provinces. Indeed, selecting comparable
301   sampling dates across provinces (July sampling in 2020 and June sampling in 2021) shows that *Varroa*
302   prevalence in BC is high with respect to other provinces, with an 88% and 50% positivity rate in 2020 and
303   2021, respectively (**Figure 1B & C**).

304   We next modelled *Varroa* occurrence with regional, temporal (date, and year) and climatic (mean
305   temperature, average wind speed, and total precipitation) predictors to determine how weather
306   patterns influence occurrence probability. Date and temperature were, predictably, strongly correlated
307   with one another (Pearson's r = 0.9; **Supplementary Figure S2**). Nevertheless, temperature still
308   positively predicted *Varroa* occurrence beyond the covarying effect of date. Interestingly, although
309   *Varroa* occurrence was generally high in BC's Lower Mainland, occurrence probability did not increase
310   appreciably with time, as in most other regions (**Figure 2 A & B**). We also found a significant interactive
311   effect between temperature and wind, with higher wind speeds predicting lower *Varroa* occurrence
312   probability, but this effect was moderated by high temperatures (**Supplementary Figure S3**).
313   Precipitation was also negatively associated with *Varroa* occurrence, and the lowest occurrence
314   probabilities were observed when both wind speeds and precipitation were high (**Figure 2C**). This effect
315   was consistent across 2020 and 2021. See **Table 2** for complete statistical reporting.

316   To determine if our findings agreed with an independent dataset, we analyzed a validation dataset of
317   *Varroa* occurrence acquired in 2016 from BC, AB, ON, and QC using the same model parameters. Unlike
318   the 2020 and 2021 data, this dataset covers a longer time period, spanning dates from April to
319   November (**Figure 3A**). Climatic variables in the primary and validation datasets had comparable average
320   magnitudes, although the maximum precipitation observed in the primary dataset was notably higher
321   than the validation dataset (primary dataset: mean temperature = 15.1 °C (2.9-22.7 °C), mean
322   precipitation = 2.0 mm (0.1-19.6 mm), mean wind speed = 9.5 km/h (3.9-17.5 km/h); validation dataset:
323   mean temperature = 15.6 °C (6.7-23.0 °C), mean precipitation = 2.4 mm (0.1-9.5 mm), mean wind speed
324   = 11.6 km/h (3.4-21.3 km/h)). Early season (April-May) occurrence probabilities were again highest in
325   BC (coastal regions including the Lower Mainland) and low in QC, which might reflect differences in
326   brooding initiation in these areas, as temperatures in BC yards exceeded 5°C earlier than yards in all
327   other regions (**Supplementary Figure S4**); however, wind was not associated with an overall decrease in
328   occurrence (**Supplementary Figure S5**). Rather, temperature was the ultimate climatic predictor
329   (associated with higher occurrence probability), again above and beyond a simple covariation between
330   temperature and date (see **Table 3** for complete statistical reporting). We hypothesized that this may be
331   due to the dataset covering a wider range of temperatures across a longer season. Indeed, when we
332   inspected early, mid, and late-season occurrence separately, the mid-season occurrence – which overlap

333   with the 2020 and 2021 sampling dates to the greatest degree – do display a negative relationship with
334   wind, but no significant relationship with precipitation (**Figure 3B**).

335   Next, we used the primary dataset to analyze relationships between *Vairimorpha* and the same regional,
336   temporal and climatic variables as for *Varroa*. Spores were detected in 95% of replicates (114/120) for at
337   least one time point; therefore, we used a linear mixed model with regional, temporal, and climatic
338   variables as predictors of intensity and did not model *Vairimorpha* presence/absence. We again
339   observed strong regional effects, but the patterns varied greatly from year to year (**Figure 4A & B**; see
340   **Table 5** for statistical reporting). For example, while patterns were similar in BC and southern AB across
341   years, samples from northern AB and MB tended to have higher intensities in 2020 than 2021. While
342   patterns over time within regions varied, overall spore intensities decreased as temperatures warmed
343   and the season progressed (**Figure 4C**), but the effect of temperature was marginally not significant
344   (Type II Wald χ^2 test; χ^2 = 3.09, df = 1, N = 480, p = 0.079) after accounting for the effect of date (**Table**
345   **5**). Wind speed and precipitation significantly interacted, with spore intensities increasing with higher
346   precipitation, but only under windier conditions (**Figure 4D**).

347   *M. plutonius* occurrence varied by region as well, but there was no significant effect of year (**Table 6**).
348   Overall detection probability was high in BC and markedly increased over time between May and June,
349   whereas zero detections were recorded in ON throughout the season (**Figure 5A**). Temperature was not
350   a significant predictor after accounting for date, and was removed from the model; however, wind and
351   precipitation significantly interacted, with increased *M. plutonius* occurrence probability under wetter,
352   windier conditions as well as drier, less windy conditions, and lower occurrence probability under
353   intermediate conditions (**Figure 5B**).

354   **Discussion**

355   We confirm several trends reported or proposed elsewhere,[9,16,40,43] and add clarity to relationships that
356   have been more inconsistent,[18,35,36] which are discussed further below. By analyzing data from colonies
357   across Canada, we are also able to comment on prevalence of disease agents in some of the major
358   beekeeping regions in this country; most notably, that *Varroa* and *M. plutonius* are more prevalent in BC
359   than other provinces, at least within our sampling breadth, which was concentrated on two operations
360   in the Lower Mainland area near Vancouver.

361   *Climatic effects*

362   We found that temperature, wind, and precipitation were all influential factors for predicting *Varroa*
363   occurrence, even after accounting for regional/operation and temporal effects, with temperature having
364   a positive effect while wind and precipitation had a negative effect. That temperature has a positive
365   impact above and beyond the effect of date aligns with expectations, as warmer temperatures promote
366   brood nest expansion and foraging, giving the mites more opportunities to reproduce and possibly
367   disperse. The negative effect of precipitation and wind are also intuitive, as such conditions limit bee
368   flight (reducing dispersal opportunities), as well as possibly brood production via reduced foraging
369   (reducing mite population growth rates).

370   Interestingly, Rowland *et al.*[9] also found a positive effect of temperature and negative effects of wind
371   and precipitation in an analysis of *Varroa* prevalence in the UK, which is consistent with our primary
372   findings. However, our primary findings were only partially validated by our analysis of an independent

373   *Varroa* dataset derived from similar regions in Canada.[47] There, we found that temperature was the
374   dominant climatic driver and the negative effect of wind was only observed among samples taken mid-
375   season (June to early August), which is the sample point overlapping the most with the data in our initial
376   analysis. Temperature and wind metrics had similar average magnitudes and spanned similar ranges in
377   both the primary and validation datasets, so we expected to observe similar effects. We suspect that the
378   different results are largely due to the two datasets being acquired over different time scales: while the
379   2020 and 2021 data were acquired from samples typically taken 2-4 weeks apart during the late spring
380   and summer (with the exception of Ontario in 2020), the 2016 validation dataset spanned a
381   considerably longer portion of the beekeeping season. It is sensible that the climatic variable promoting
382   reproduction (an exponential process), rather than dispersal, would be most influential in this temporal
383   scale.

384   Some research suggests that *Varroa* reproduction is inhibited when ambient temperatures surpass
385   35°C,[22,23] and such conditions will likely become more common as the climate changes and the
386   frequency of extreme heat events increases.[53-55] Hot temperatures can also be harmful to honey
387   bees,[7,56,57] but not always.[58] British Columbia and, to some extent, Alberta experienced a severe heat
388   wave between June 25 and July 2, 2021,[59] which falls within our study period. Temperatures reached
389   >40°C in some parts of the Lower Mainland during this time, but the event was not strongly reflected in
390   our data due to a combination of there being lower temperatures at our specific apiary locations and
391   our process of computing three-week averages of daily means. Unfortunately, our sampling did not
392   continue for long enough after the heat dome to measure any potential effect on *Varroa* reproduction.

393   Reported relationships between *Vairimorpha* spore intensities and temperature have been somewhat
394   inconsistent, and our data add some clarity to the matter. A number of studies highlight broad seasonal
395   patterns (with spore intensities decreasing between spring and fall)[18,19,21,32], and some describe a
396   negative relationship with temperature which is likely due to increased flight days,[18,35] or possibly
397   reduced ability to proliferate,[38] though this is contradicted by Rangel *et al.*[36] In our analysis, we found
398   that temperature did not significantly predict a decline in spore intensities beyond the covarying effect
399   of date, which supports the idea that flight days, and not temperature *per se,* is the limiting factor.
400   Further support of this reasoning is the interaction we observed between precipitation and wind **(Figure
401   4D)**. These variables limit defecation flight opportunities independent of temperature, and while neither
402   was sufficient to predict spore intensities on their own, precipitation did promote higher spore
403   intensities under windier conditions. This makes sense given that the average total precipitation
404   observed was not extreme (typically not more than 5 mm) and was therefore unlikely to sufficiently limit
405   flights on its own. The likely crux of defecation flights as the operational factor could also be why Rangel
406   *et al.* found no effect of temperature in their study of *Vairimorpha* in feral colonies in Texas. Those
407   samples were collected in July over the course of 20 years, when one would expect the temperatures to
408   be more than sufficient to enable flight days regardless of year-to-year deviations.

409   *M. plutonius* is often described as an opportunistic pathogen that is triggered to cause EFB disease
410   based on a combination of nutritional deficiencies,[16,24,60] pathogen genotype,[61,62] and host genotype,[63]
411   among other factors. Given the possible connection with nutrition, it is conceivable that inopportune
412   foraging conditions could promote disease and increase *M. plutonius* occurrence, but it has been
413   suggested that strong nectar flows might actually also promote *M. plutonius* proliferation due to
414   diversion of nursing care.[16,43] While Wardell *et al.*[60] states that no obvious climatic conditions account
415   for disease incidence,[60] this is not surprising for such a multifactorial issue, and others have postulated

416   that climate may indeed play a role in part due to the strong regional differences that have been
417   observed.[24,61]

418   We found both strong regional differences and a relationship with wind and precipitation, but no
419   relationship with temperature after accounting for sampling date (**Figure 5**; **Table 6**). The highest
420   occurrence frequencies were observed under both favourable (low wind, low precipitation) and
421   unfavourable (high wind, high precipitation) conditions, with lower occurrence in intermediate zones.
422   This pattern, while at first puzzling, may actually reflect both predictions: Increased foraging leads to a
423   temporarily poorer brood care due to a shift from nursing to nectar-processing activities, while
424   suboptimal foraging conditions also lead to poorer brood care via insufficient nutrition. It is also possible
425   that favourable conditions lead to higher *M. plutonius* occurrence probability because this increases
426   opportunities for dispersal, which is particularly relevant for this highly transmissible pathogen.[16]
427   Rowland *et al.* previously reported that high precipitation, but not wind, was positively linked to higher
428   EFB incidence,[9] and our results may differ in part because we analyzed *M. plutonius* occurrence, which is
429   distinct from clinical diagnosis of EFB disease. The two metrics are different but nevertheless connected,
430   especially as only 100-200 *M. plutonius* spores are required to cause disease.[42,43] We expect that our
431   method of PCR detection would be more sensitive to dispersal events in which the pathogen is
432   transmitted but does not necessarily cause disease, which is consistent with our observation of higher
433   occurrence under favourable climatic conditions.

434   *Regional effects*

435   We observed strong regional effects for all three disease agents, with distinctly high incidence of *M.*
436   *plutonius* and *Varroa* in BC, and variable levels of *Vairimorpha* spp. from year to year. However, we note
437   that in our experiment we are unable to distinguish between effects of region and effects of beekeeping
438   operation. We and others have previously observed an association between EFB and highbush blueberry
439   pollination,[16,60,64] and there is a high density of highbush blueberry fields in British Columbia's Lower
440   Mainland; however, two controlled field trials (including one derived from these data) do not support
441   the notion that engaging in blueberry pollination causes *M. plutonius* infection.[45,65] Therefore, perceived
442   associations may actually be due to the seasonal timing of blueberry pollination. EFB outbreaks have
443   also been observed in parts of Europe, where authorities responded with strict control measures
444   including hive destruction and exclusion zones around affected apiaries.[66] Especially given the high
445   transmissibility of *M. plutonius* to neighboring colonies,[16,24,41] strict control measures are essential for
446   containment. BC has no provincial mandate for destroying or otherwise controlling affected colonies
447   and apiaries, whereas in Ontario, where we observed zero *M. plutonius* detections, EFB is a reportable
448   disease with enforceable movement restrictions and sanitation procedures.

449   British Columbia, at least in our sampling region of the Lower Mainland, also appears to suffer from high
450   incidence of *Varroa* mites, particularly early in the season. This is despite colonies being treated for
451   *Varroa* (in early April) before the beginning of the study. Although we did not formally test this, we
452   speculate that this may be in part due to the region having notably mild winters, which lead to shorter
453   brood breaks and earlier recommencement of brood rearing. These conditions mean that beekeepers
454   have a shorter broodless window during which to apply a miticide treatment, and remaining mites are
455   able to begin reproducing earlier. However, we note that the 2016 dataset contained yards in BC's
456   interior (the Thompson/Okanagan and Kootenay/Boundary regions), as well as the Lower Mainland and
457   coastal regions, but the patterns observed there do not entirely agree with this idea. Based on average

458 daily temperatures in the interior and Lower Mainland/coastal apiaries for 2016, we estimated that the
459 spring brooding date in the interior was later, similar to the regions in other provinces. However, we still
460 observed relatively high early-season *Varroa* incidence in the interior, with fitted probabilities of ~0.5
461 (higher than all other regions). Therefore, factors yet unaccounted for are clearly at play, such as
462 possibly beekeepers' winter and early spring mite management procedures, and the timing of these in
463 relation to when the experiment began.

464 **Conclusion**

465 We present an analysis of common honey bee disease agents and how they vary according to regional,
466 temporal, and climatic factors. Our results largely support patterns expected based on existing data and
467 knowledge of how these disease agents reproduce and disperse. Nevertheless, confirmation of these
468 trends is important on a nation-wide scale and across many climatic and ecotypes. Our analysis also
469 provides additional insight into more ambiguous associations, particularly for how temperature affects
470 *Vairimorpha* spore intensities and relationships between weather and *M. plutonius* occurrence. In most
471 of our models, we observed a large degree of unexplained variation, suggesting that other factors, such
472 as bee genetics, coinfections, or landscape factors may also influence pathogen and parasite dynamics,
473 which were not investigated here. We hope that this and future studies will inform meaningful global
474 predictive models of disease agents under future climate projections.

475 **Acknowledgements**

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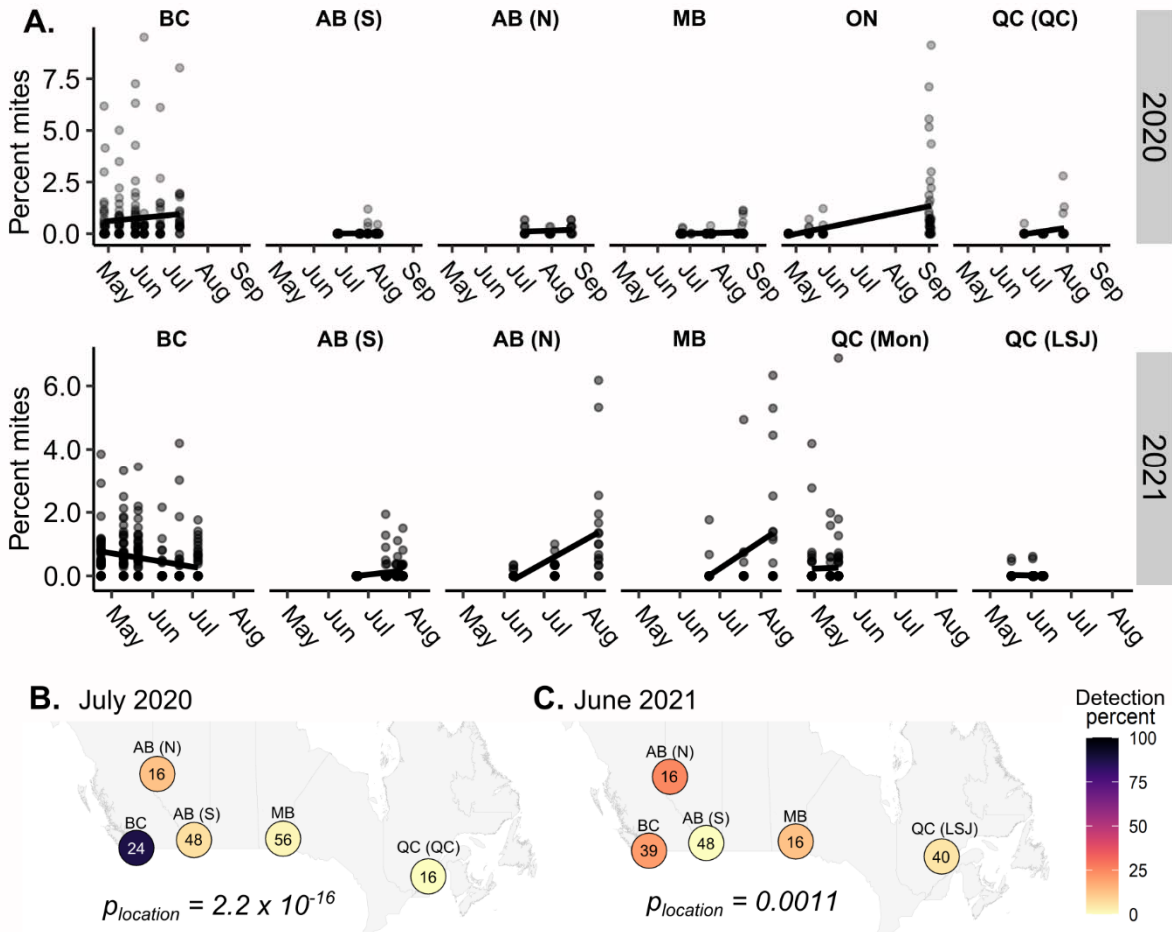
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648 **Figure 1. Regional patterns of *Varroa destructor* intensity and occurrence.** A) *Varroa* intensities (non-
649 zero mites per 100 bees) varied significantly by year and a date-by-region interaction. Statistical
650 parameters are described in **Table 1**. BC = British Columbia, AB (S) = Southern Alberta, AB (N) = Northern
651 Alberta, MB = Manitoba, ON = Ontario, QC (QC) = Quebec, near Quebec City, QC (Mon) = Quebec, near
652 Montreal, QC (LSJ) = Quebec, near Lac St. Jean. B & C) Region effects illustrated as occurrence rates at
653 each site, restricted to comparable sampling dates (July dates in 2020 and June dates in 2021, no
654 repeated sampling). Logistic regression analysis shows significant regional differences in occurrence
655 frequencies in 2020 (C; χ^2 = 82.6, df = 4, p < 0.001) and less pronounced, but still significant differences
656 in 2021 (D; χ^2 = 18.3, df = 4, p = 0.0011). Numbers inside circles indicate sample sizes.
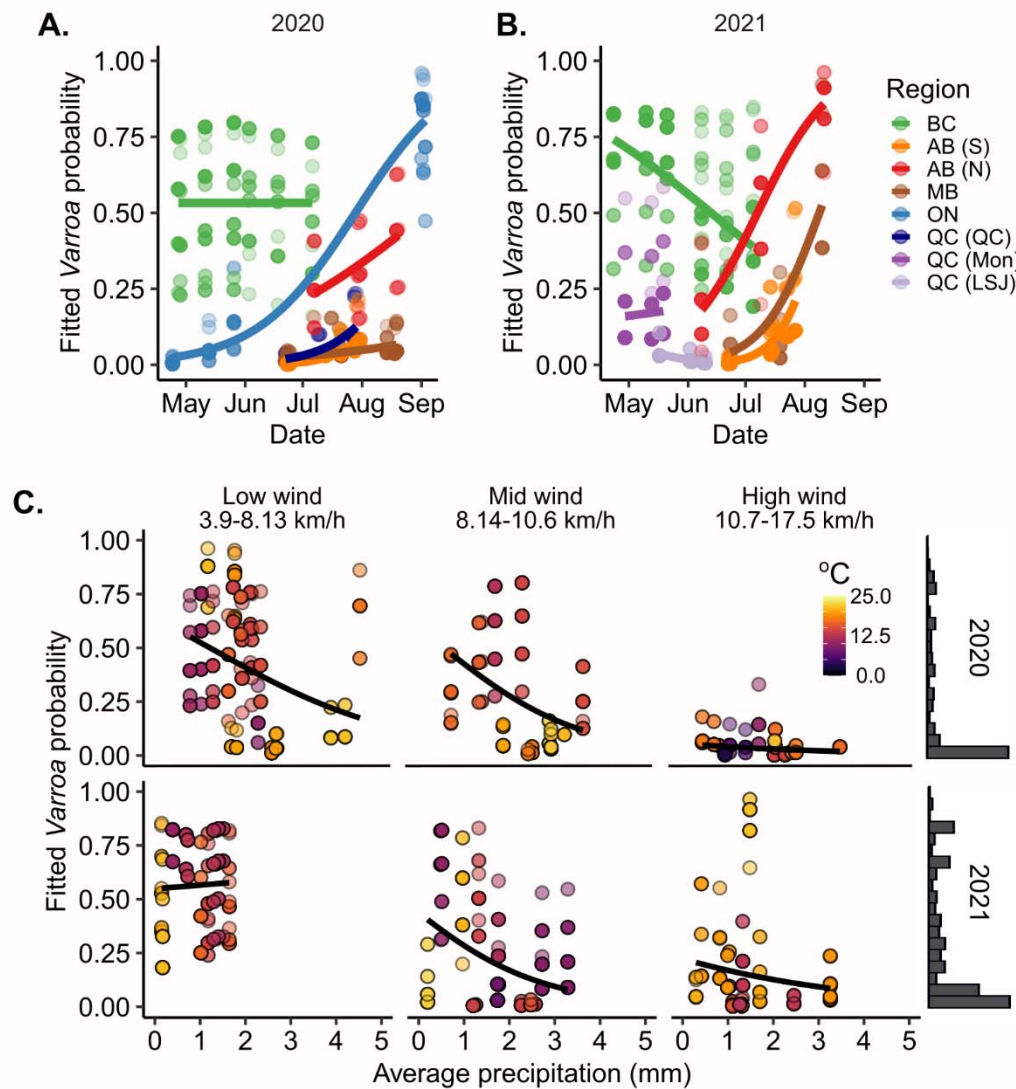
Figure 2. *Varroa destructor* occurrence is linked to regional, temporal and climatic variables. A & B) Fitted *Varroa* occurrence probabilities (1 = present, 0 = absent) vary by year and a date-by-region interaction, among other influential factors. Statistical parameters fully reported in **Table 2.** C) Occurrence probabilities significantly decrease with higher wind speeds, but the effect is reduced when temperatures are warm (see also **Supplementary Figure S2**). Occurrence also significantly decreases with precipitation. Histograms show densities of fitted probabilities.
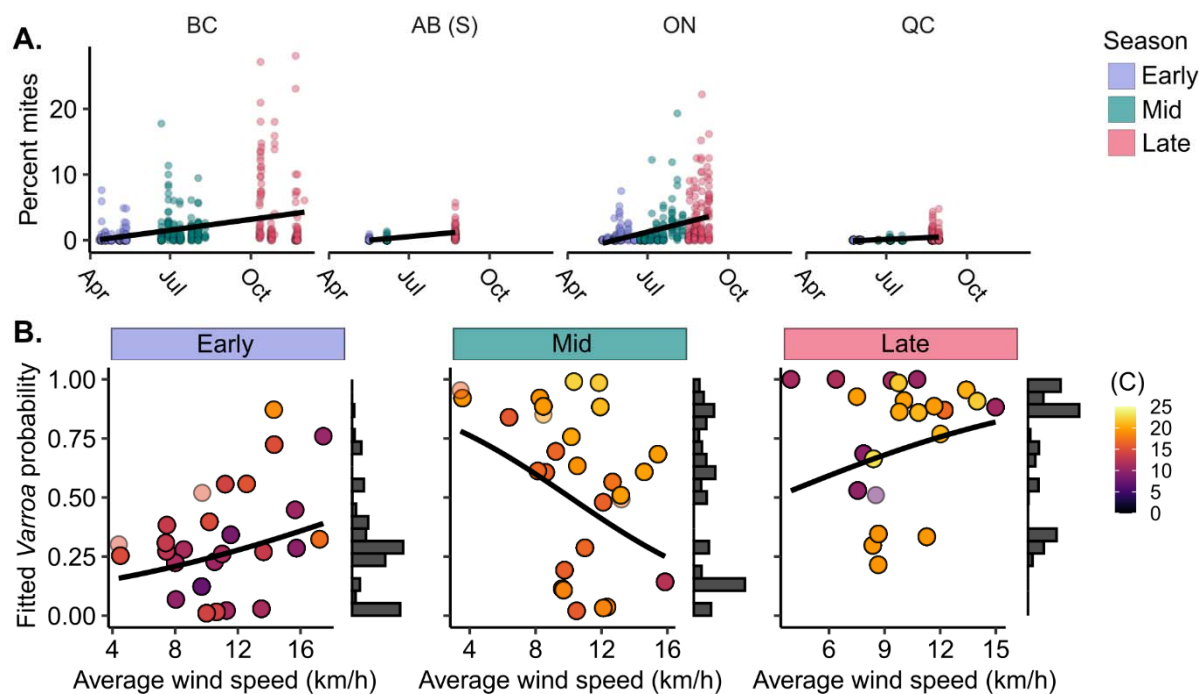
Figure 3. Validation analysis: *Varroa* **occurrence probability decreases with wind mid-season, but temperature drives overall trends.** A) Validation dataset of *Varroa* intensity and occurrence in BC, AB, ON, and QC during the 2016 beekeeping season (divided by early, mid-season, and late sampling events). Data were modelled according to the same formula as the 2020/2021 dataset, with results described in **Table 3**. The positive effect of temperature on *Varroa* occurrence probability was exacerbated, not mitigated, under windier conditions (**Supplementary Figure S4**). B) Examining each sampling time point separately, only mid-season *Varroa* occurrence (n = 480) is negatively associated with wind. Mid-season sampling (June to mid-August) dates have the greatest overlap with 2020 and 2021 sampling dates. Model parameters and outcomes for mid-season data are reported in **Table 4**.

674
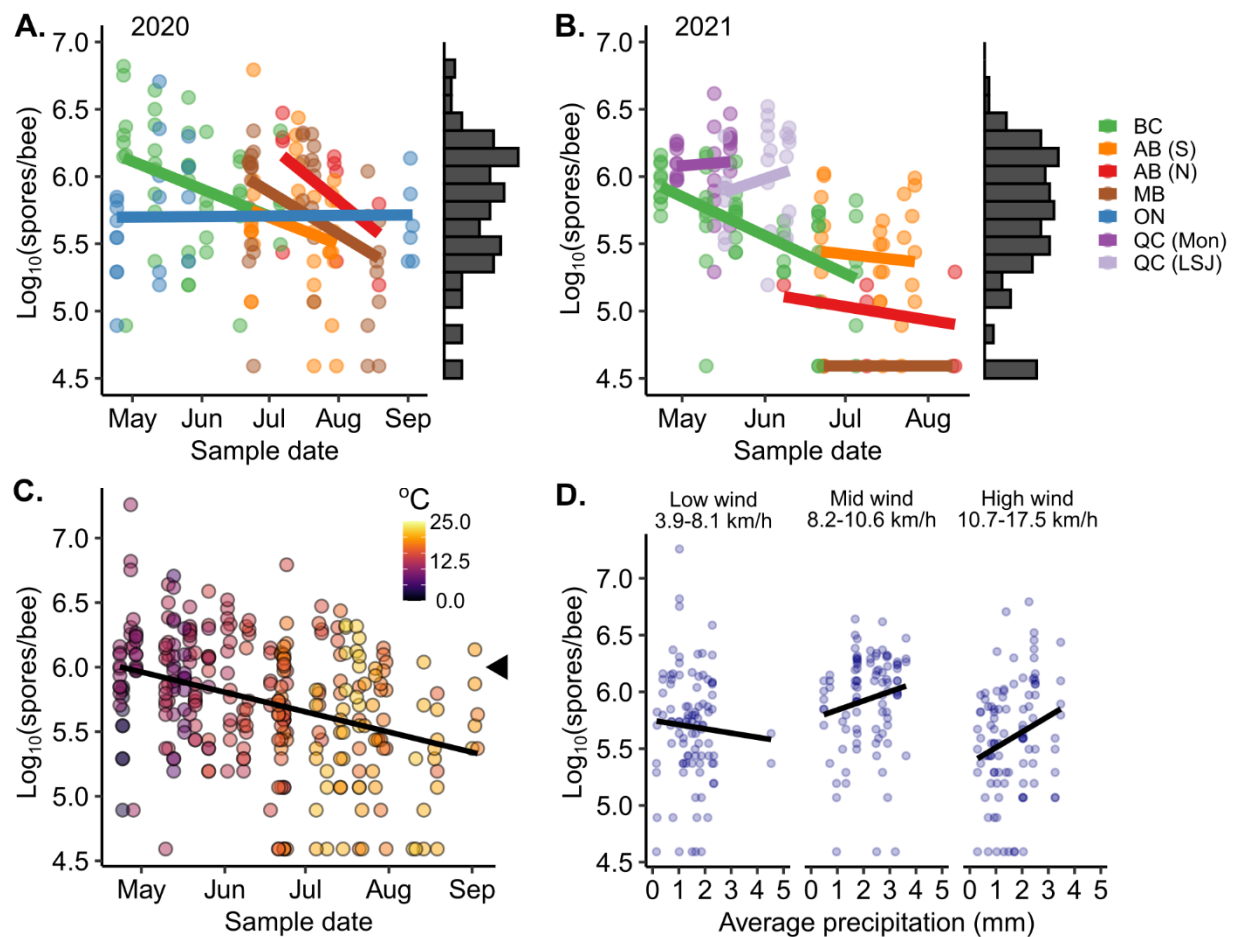
Figure 4. *Vairimorpha* spore intensities are linked to regional, temporal and climatic variables. Statistical parameters fully reported in **Table 3**. Spore intensities vary significantly by region in 2020 (A) and 2021 (B). Spore intensities generally decrease over time during the spring and summer, but this trend varies by region. Histograms show spore intensity bins. C) The general decrease over time coincides with increased temperatures. The black triangle indicates the economic threshold of 1 million spores/bee. D) Spore intensities significantly increase with precipitation, but only under windy conditions, illustrated in three equidensity wind speed bins. BC = British Columbia, AB (S) = Southern Alberta, AB (N) = Northern Alberta, MB = Manitoba, ON = Ontario, QC (Mon) = Quebec, near Montreal, QC (LSJ) = Quebec, near Lac St. Jean.

684
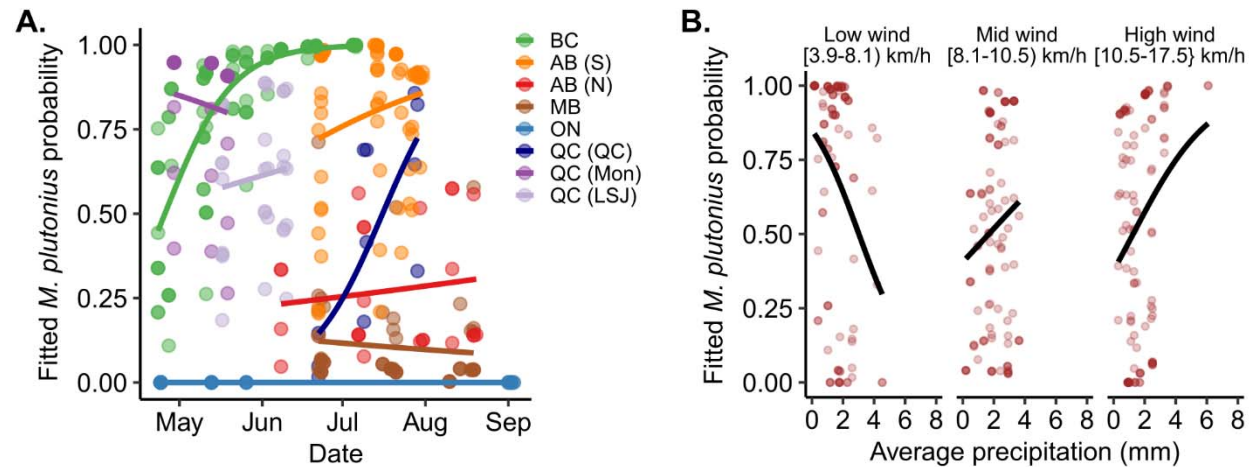
Figure 5. *Melissococcus plutonius* occurrence is linked to region, precipitation, and temperature.
Occurrence was fitted with generalized linear mixed model with statistical parameters reported in **Table 4**. A) Occurrence varies significantly by region. B) Wind and precipitation significantly interact, as illustrated by plotting fitted probabilities against precipitation in three equidensity wind bins. *M. plutonius* occurrence decreases with precipitation when wind speed is low, but increase with precipitation when wind is medium-high. BC = British Columbia, AB (S) = Southern Alberta, AB (N) = Northern Alberta, MB = Manitoba, ON = Ontario, QC (QC) = Quebec, near Quebec City, QC (Mon) = Quebec, near Montreal, QC (LSJ) = Quebec, near Lac St. Jean.

693 **Tables**

694 **Table 1. Linear mixed model of non-zero mite intensities[a] by date, region, and year**

Analysis of deviance table (Type II Wald χ^2)	χ^2	df	p	Code[b]
Date[c]	15.8	1	< 0.001	***
Region	25.7	7	< 0.001	***
Year	5.0	1	0.026	*
Date x Region	22.7	7	0.0019	**
Parameter	**Coef**	**$t_{(366)}$**	**p**	**Code**
(Intercept) [Date=0, Region=BC, Year=2020]	-0.39	-4.04	< 0.001	***
Date	0.06	0.75	0.45	
Region [AB S]	0.66	0.78	0.43	
Region [AB N]	-0.99	-4.33	< 0.001	***
Region [MB]	-0.86	-2.35	0.019	*
Region [ON]	-0.06	-0.26	0.80	
Region [QC QC]	-0.39	-0.46	0.65	
Region [QC Mon]	-0.1	-0.16	0.87	
Region [QC LSJ]	-0.26	-0.21	0.84	
Year [2021]	0.24	2.23	0.026	*
Date x Region [AB S]	-1.27	-1.44	0.15	
Date x Region [AB N]	0.57	3.47	< 0.001	***
Date x Region [MB]	0.81	3.05	0.0020	**
Date x Region [ON]	0.16	1.20	0.23	
Date x Region [QC QC]	1.02	1.18	0.24	
Date x Region [QC Mon]	-0.09	-0.17	0.86	
Date x Region [QC LSJ]	0.23	0.13	0.89	
Random effect	**Variance**	**sd**	**Levels**	**Obs**
Random intercept [Colony]	0.31	0.55	232	385

Full model R^2 = 0.57

Formula = ln(mite percent) ~ Year + Date*Region + (1|Colony)

Family = Gaussian

695 [a]Mite intensities (non-zero mites per 100 bees) were transformed by taking the natural logarithm

696 [b]Significance codes: p < 0.001 = ***, p < 0.01 = **, p < 0.05 = *

697 [c]Date was scaled prior to modelling

698

699 **Table 2. Generalized linear mixed model of mite occurrence**

Analysis of deviance table (Type II Wald χ^2)	χ^2	df	p	Code[a]
Temperature[b]	4.3	1	0.037	*
Wind[b]	3.0	1	0.084	
Precipitation[b]	9.2	1	0.0024	**
Date[b]	6.1	1	0.013	*
Region	85.7	7	< 0.001	***
Year	6.5	1	0.011	*
Temperature x Wind	4.8	1	0.029	*
Date x Region	29.6	7	< 0.001	***
Parameter	**Coef**	**z**	**p**	**Code**
(Intercept) [Temperature=0, Wind=0, Precipitation=0, Date=0, Region=BC, Year=2020]	0.37	1.0	0.31	
Temperature	0.73	2.3	0.023	*
Wind	0.61	2.3	0.022	*
Precipitation	0.27	3.0	0.0024	**
Date	-0.44	-1.2	0.23	
Region [AB S]	-8.00	-5.9	< 0.001	***
Region [AB N]	-2.23	-3.5	< 0.001	***
Region [MB]	-5.40	-6.0	< 0.001	***
Region [ON]	-0.81	-1.3	0.18	
Region [QC QC]	-3.86	-2.6	0.0095	**
Region [QC Mon]	-2.12	-1.6	0.11	
Region [QC LSJ]	-6.95	-4.5	< 0.001	***
Year [2021]	0.67	2.5	0.011	*
Temperature x Wind	0.47	2.2	0.029	*
Date x Region [AB S]	4.10	3.7	< 0.001	***
Date x Region [AB N]	1.39	2.7	0.0077	*
Date x Region [MB]	1.53	2.6	0.011	*
Date x Region [ON]	1.40	3.9	< 0.001	***
Date x Region [QC QC]	2.01	1.3	0.20	
Date x Region [QC Mon]	0.48	0.4	0.67	
Date x Region [QC LSJ]	-3.52	-1.8	0.079	
Random effect	**Variance**	**sd**	**Levels**	**Obs**
Random intercept [Colony]	1.56	1.25	479	1426

Full model R^2 = 0.64

Formula = Occurrence ~ Temperature*Wind+Precipitation + Date*Region + Year + (1|Colony)

Family = Binomial

700 [a]Significance codes: p < 0.001 = ***, p < 0.01 = **, p < 0.05 = *

701 [b]Variables were scaled (z scored) prior to fitting

702

703   **Table 3. *Varroa* model validation: Full-season data**

Analysis of deviance table (Type II Wald χ^2)	X^2	df	p	Code[a]
Temperature[b]	7.5	1	< 0.001	***
Precipitation[b]	0.24	1	0.62	
Wind[b]	2.9	1	0.089	
Date[b]	44.9	1	< 0.001	***
Region	113	4	< 0.001	***
Date x Region	28.9	4	< 0.001	**
Temperature x Wind	16.3	1	< 0.001	***
Parameter	**Coefficient**	**z**	**p**	**Code**
(Intercept) [Temperature=0, Wind = 0, Precipitation=0, Date=0, Region=BC.coast]	0.52	2.6	0.0086	**
Region [AB.S]	-0.80	-1.8	0.067	
Region [ON]	-0.13	-0.45	0.66	
Region [QC]	-3.63	-8.9	< 0.001	***
Date	0.74	5.2	< 0.001	***
Precipitation	-0.066	-0.49	0.62	
Temperature	0.41	2.3	0.024	*
Wind	0.24	1.9	0.059	
Region [BC.int] x Date	1.4	3.5	< 0.001	***
Region [AB.S] × Date	1.9	4.2	< 0.001	***
Region [ON] × Date	0.59	1.6	0.11	
Region [QC] × Date	1.1	2.6	0.0099	**
Temperature × Wind	-0.45	-4.0	< 0.001	***
Random effects	**Variance**	**sd**	**Levels**	**Obs**
Random intercept [Colony]	1.33	1.15	480	1344

Full model R^2 = 0.65

Formula = Occurrence ~ Temperature*Precipitation + Date + Region + Year + (1|Colony)

Family = Binomial

704   [a]Significance codes: p < 0.001 = ***, p < 0.01 = **, p < 0.05 = *

705   [b]Variables were scaled prior to fitting

706 **Table 4. *Varroa* model validation: Mid-season data**

Analysis of deviance table (Type II Wald χ^2)	X^2	df	p	Code[a]
Temperature[b]	1.7	1	0.20	
Precipitation[b]	0.007	1	0.93	
Wind[b]	7.0	1	0.0081	**
Date[b]	2.6	1	0.11	
Region	115	4	< 0.001	***
Date x Region	22.5	3	< 0.001	***
Temp x Wind	4.4	1	0.036	*
Parameter	**Coefficient**	**z**	**p**	**Code**
(Intercept) [Temperature = 0, Region = BC.coast, Wind = 0, Precipitation = 0, Date = 0]	-0.41	-0.91	0.36	
Region [BC.int]	2.1	2.6	0.0094	**
Region [AB.S]	-4.9	-1.6	0.10	
Region [ON]	-0.31	-0.57	0.57	
Region [QC]	-3.7	-5.1	< 0.001	***
Date	1.4	-0.93	0.35	
Precipitation	-0.061	-0.084	0.93	
Temperature	2.3	2.1	0.039	*
Wind	0.89	1.2	0.23	
Region [BC.int] x Date	-12.0	-3.4	< 0.001	***
Region [AB.S] x Date	NA	NA	NA	
Region [ON] × Date	4.87	1.67	0.097	
Region [QC] × Date	-0.91	-0.23	0.82	
Temperature × Wind	-2.0	-2.0	0.042	*

Full model R^2 = 0.43

Formula = Occurrence ~ Temperature*Precipitation + Date + Region

Family = Binomial

707 [a]Significance codes: p < 0.001 = ***, p < 0.01 = **, p < 0.05 = *
708 [b]Variables were scaled prior to fitting

709 **Table 5. Linear mixed model of *Vairimorpha* spp. spore intensities[a]**

Analysis of deviance table (Type II Wald χ^2)	X^2	df	p	Code[b]
Wind[c]	0.00	1	0.99	
Precipitation[c]	0.53	1	0.47	
Temperature[c]	3.09	1	0.079	
Date[c]	21.4	1	< 0.001	***
Region	38.5	6	< 0.001	***
Year	32.3	1	< 0.001	***
Wind x Precipitation	9.41	1	0.0020	**
Date x Region	14.2	6	0.027	*

Parameter	Coefficient	t	p	Code
(Intercept) [Wind= 0, Precipitation=0, Temperature=0, Date=0, Region=BC, Year=2020]	5.7	54.7	< 0.001	***
Wind	-0.02	-0.31	0.76	
Precipitation	0.02	0.79	0.43	
Temperature	0.17	1.76	0.080	
Date	-0.46	-4.37	< 0.001	***
Region [MB]	0.06	0.35	0.73	
Region [AB N]	0.16	0.70	0.49	
Region [ON]	0.26	1.49	0.14	
Region [QC LSJ]	0.74	3.41	< 0.001	**
Region [QC Mon]	0.92	2.62	0.009	*
Region [AB S]	0.18	0.66	0.51	
Year [2021]	-0.45	-5.68	< 0.001	***
Wind x Precipitation	0.10	3.07	0.002	**
Scaled × Region [MB]	0.06	0.49	0.62	
Scaled × Region [AB N]	0.19	0.96	0.34	
Scaled × Region [ON]	0.31	3.25	0.001	**
Scaled × Region [QC LSJ]	0.29	0.99	0.32	
Scaled × Region [QC Mon]	0.37	1.25	0.21	
Scaled × Region [AB S]	0.11	0.65	0.52	

Random effect	Variance	sd	Levels	Obs
Random intercept [Unit]	0.043	0.21	114	296

Full model R^2 = 0.50

Formula = Spores ~ Wind*Precipitation + Temperature + Date*Region + Year + (1|Unit)

Family = Gaussian

710 [a]Spore intensities were log_{10} transformed
711 [b]Significance codes: p < 0.001 = ***, p < 0.01 = **, p < 0.05 = *
712 [c]Variables were scaled prior to fitting

713   **Table 6. Generalized linear mixed model of *M. plutonius* occurrence**

Analysis of deviance table (Type II Wald χ^2)	X^2	df	p	Code[a]
Wind[b]	0.0001	1	0.99	
Precipitation[b]	0.41	1	0.52	
Date[b]	5.4	1	0.021	*
Region	25.0	7	< 0.001	***
Year	0.75	1	0.39	
Wind x Precipitation	6.7	1	0.0099	**
Date x Region	12.1	7	0.096	

Parameter	Coefficient	z	p	Code
(Intercept) [Wind = 0, Precipitation=0, Date=0, Region=BC]	5.8	4.4	< 0.001	***
Wind	0.53	0.72	0.47	
Precipitation	0.21	0.41	0.68	
Date	3.8	3.8	< 0.001	***
Region [MB]	-8.6	-4.8	< 0.001	***
Region [AB.N]	-7.3	-4.0	< 0.001	***
Region [ON]	-24.7	-0.03	0.97	
Region [QC.QC]	-7.1	-3.1	0.002	**
Region [QC.LSJ]	-4.7	-2.5	0.012	*
Region [QC.Mon]	-4.6	-1.5	0.14	
Region [AB.S]	-4.5	-1.7	0.084	
Year [2021]	-0.56	-0.86	0.39	
Wind × Precipitation	2.2	2.6	0.01	*
Date × Region [MB]	-3.7	-2.9	0.004	**
Date × Region [AB.N]	-3.3	-2.6	0.011	*
Date × Region [ON]	-3.8	-0.0051	1.0	
Date × Region [QC.QC]	1.2	0.53	0.60	
Date × Region [QC.LSJ]	-3.7	-1.9	0.056	
Date × Region [QC.Mon]	-5.2	-1.9	0.06	
Date × Region [AB.S]	-2.5	-1.5	0.15	

Random effects	Variance	sd	Levels	Obs
Random intercept [Unit]	2.7	1.6	120	360

Full model R^2 = 0.93

*Formula = Occurrence ~ Temperature*Precipitation + Date + Region + Year + (1|Unit)*

Family = Binomial

714   [a]Significance codes: p < 0.001 = ***, p < 0.01 = **, p < 0.05 = *
715   [b]Variables were scaled prior to fitting