Pesticide reduces bumblebee colony initiation and increases probability of population extinction

Gemma L. Baron¹, Vincent A. A. Jansen¹, Mark J. F. Brown^{1†}, Nigel E. Raine^{1,2†}

¹ School of Biological Sciences, Royal Holloway University of London, Egham, TW20 0EX, UK;

² School of Environmental Sciences, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

 \dagger These authors contributed equally to this work

Email: gemma_baron@hotmail.com

Supplementary Information

Table S1 The proportion of queens in A) the pesticide or control treatment groups, B) short or long hibernation groups, C) a combination of these, which laid eggs by 10 weeks, reared adult offspring (shown as both a proportion of the total number of queens in the experiment, and as a proportion of egg laying queens only). Data shown only includes queens which survived the whole experiment.

*Mean (S.E.) number of adult workers reared after 14 weeks of the experiment

Table S2 The average daily syrup consumption (ml) of queens during the two week pesticide treatment phase of the experiment. Data includes all queens included in the final analysis for syrup consumption (and excludes individuals for which no data were available for any of the variables in the analysis.)

Model Selection Tables

Table S3 Model selection table showing candidate generalised linear models for queen survival after hibernation. Models in bold are those within two AICc units of the best fitting model.

Table S4 Model selection table showing candidate linear models for syrup consumption by queens during the two-week pesticide exposure period. Models in bold are those within two AICc units of the best fitting model.

Table S5 Model selection table showing candidate binomial generalised linear models for presence or absence of egg laying by queens. Models in bold are those within two AICc units of the bet fitting model.

Candidate models	Loglik	AICc	Delta	Weight
Hibernation + $P1 + P2$	-422.528	851.1	0	0.489
Hibernation + $P1 + P2 +$ Infection + Thorax	-421.426	853	1.89	0.19
Hibernation + $P1$ + $P2$ + Infection	-422.474	853.1	1.94	0.186
Hibernation $*$ P1 + Hibernation $*$ P2	-421.762	853.7	2.57	0.136
Hibernation + Thorax	-431.853	867.7	16.62	0
Hibernation	-433.319	868.6	17.53	0
Hibernation * Infection	-432.172	870.4	19.29	0
Hibernation + Infection	-433.275	870.6	19.46	0
$P1 + P2$	-433.473	871	19.86	0
$P1 + P2 + Thorax$	-432.835	871.7	20.61	0
$P1 + P2 + Infection$	-433.375	872.8	21.69	0
P1 * Infection + P2 * Infection	-433.254	876.7	25.55	0
Null	-433.898	887.8	36.68	0
Thorax	-422.973	888	36.84	0
Infection	-433.8	889.6	38.49	0
Infection + Thorax	-422.832	889.7	38.58	0

Table S6 Model selection table showing candidate Cox regression models for timing of egg laying by queens. Models in bold are those within two AICc units of the best fitting model.

Table S7 Candidate binomial generalised linear models for presence or absence of adult offspring (including all queens). Models in bold are those within two AICc units of the best fitting model.

Candidate models: fixed factors	Loglik	AICc	Delta	Weight
Null	-104.63	211.3	0	0.15
Hibernation	-103.636	211.3	0.05	0.146
Hibernation + Thorax	-102.851	211.8	0.55	0.114
Thorax	-103.996	212.1	0.77	0.102
Pesticide	-104.14	212.3	1.06	0.088
Hibernation + Pesticide	-103.163	212.4	1.17	0.084
Pesticide + Thorax	-103.364	212.9	1.57	0.069
Hibernation + Infection	-103.538	213.2	1.92	0.058
Infection	-104.577	213.2	1.94	0.057
Infection + Thorax	-103.968	214.1	2.78	0.037
Pesticide + Infection	-104.092	214.3	3.03	0.033
Hibernation + Pesticide + Infection	-103.073	214.4	3.07	0.032
Hibernation + Pesticide + Infection + Thorax	-102.155	214.6	3.34	0.028

Candidate models: fixed factors	Loglik	AICc	Delta	Weight
Hibernation + Pesticide	-56.119	118.5	0	0.415
Pesticide	-58.087	120.3	1.79	0.169
Hibernation + Pesticide + Infection	-56.09	120.7	2.14	0.143
Pesticide + Thorax	-57.809	121.9	3.38	0.077
Pesticide + Infection	-57.888	122.1	3.54	0.071
Hibernation + Pesticide + Infection + Thorax	-55.992	122.7	4.19	0.051
Hibernation	-59.865	123.9	5.35	0.029
Null	-61.684	125.4	6.9	0.013
Hibernation + Infection	-59.853	126	7.47	0.01
Hibernation + Thorax	-59.855	126	7.47	0.01
Infection	-61.549	127.2	8.72	0.005
Thorax	-61.593	127.3	8.81	0.005
Infection + Thorax	-61.495	129.3	10.75	0.002

Table S8 Model selection table showing candidate binomial generalised linear models for presence or absence of adult offspring (including only queens which laid eggs). Models in bold are those within two AICc units of the best fitting model.

Modelling methods: a Bayesian model for colony capacity

For a bumblebee population to persist, bumblebee colonies need to produce sufficiently many new colonies over their reproductive cycle. A minimum requirement for persistence is that the average number of colonies produced must be at least one when the bumblebee population reaches very low density. This result is underpinned by results from branching process theory: if the number of colonies produced is a random number drawn from a distribution and if the mean number of colonies is less than one, extinction is certain in the long run. In what follows we consider ultimate persistence and extinction of populations: the probability of extinction and persistence in the limit of time tending to infinity. If the mean number of daughter colonies exceeds one, the probability of extinction, starting from a single colony, is smaller than one, meaning that there is positive chance that the population persists. When new colonies are repeatedly introduced or if the initial population is of sufficient size the probability of extinction is very small and persistence virtually certain. Therefore, the mean number of colonies produced is a key parameter. If it takes value one a population is on the threshold between persistence and extinction. There is a parallel here with epidemiology and metapopulation theory, where similar threshold exists in that each infection or patch, needs to be capable, on average, of producing at least one new infection or patch over its lifetime to guarantee persistence⁴⁶, and where the key parameters are known as the basic reproductive number and metapopulation capacity, respectively. We will call the average number of colonies produced per colony the colony capacity.

If we would know the precise value of the colony capacity, it would allow us to predict the persistence of the bumblebee population. The number of new colonies produced depends on a number of factors: firstly, it depends on the average number of gynes (unmated queens) produced by a colony. These gynes must mate, survive hibernation, find a suitable nest site, and following this they will need to initiate a colony. If we can quantify these factors, we can quantify the colony capacity. Generally, the information needed to calculate the colony capacity is either unknown, or not known in sufficient detail. Because we do not have complete information about the value of these factors, there is a certain amount of uncertainty about the value of the colony capacity. What we do here is show how partial information can be used to calculate the likely value of the colony capacity, and how we can quantify the uncertainty about the components of this parameter, to arrive at a likelihood profile for the colony capacity. Using our results on the effects of pesticide exposure, we can then also find the likelihood profile of the colony capacity after pesticide use, and can use this to make predictions about persistence and extinction

In order to calculate a likelihood profile, we need a model that describes the number of daughter colonies produced. Such a model is necessarily a simplification of reality. It ignores a number of factors that exist in the real world, such as the spatial and temporal variability in the number of colonies produced, and the density dependence in the process. Density dependence we will ignore because the value of the threshold depends on the dynamics for low densities, and the value of the threshold is not affected by density dependence. Spatial patterning we assume to be incorporated in the averages in the number of colonies produced. Temporal variation and correlation can be important in environments where the colony capacity is different from one year to the next. In that case, the colony capacity is the geometric mean of the values over the years, and thus on the variance over the years. To estimate the geometric mean one would need a number of measurements over the years to quantify the colony capacity. The published information we use only covers a single year due to logistical and financial constraints on performing such large scale experiments, and we are not aware of sufficient information from across multiple years to form a precise estimate. What we do instead is use the information we have on *B*. *terrestris*, which we know is from a relatively good year. If every year were to be like this, we conclude that the population will persist and that the colony capacity in such a year is at least one. Using a Bayesian approach we calculate the likelihood of the colony capacity based on this assumption and use this to extrapolate the effect of pesticide exposure on overwintering bumblebee queens. Because we based our calculations on data from what we know to be a favourable year, our results are a conservative estimate of the likelihood that the population can become extinct through pesticide exposure, based on the uncertainty we have about parameter values.

10 *A model* Several studies have quantified the success of various stages of the bumblebee life cycle. Baer and Schmid-Hempel⁷⁵ studied the number of *B. terrestris* gynes produced by a colony under field conditions (a quantity which we call m_g), and found that 18 colonies produced a total of 155 gynes. The probability to survive hibernation (which we call *ph*) and the probability to be able initiate a colony following survival (which we call p_c) were studied by Beekman and colleagues⁴². They found that 23 of 45 (51.1%) queens survived a 6-month hibernation period, and that 11 of the 23 surviving queens initiated a colony. Following over-winter survival queens need to find a nest site. The probabilities of successful mating and finding a nest site have not been studied, and we therefore have no information about values of these parameters. We have bundled these two unknown parameters together and call their product *pnm*. Table S9 provides a summary of the key parameters.

Although we do not know what the exact value of the population capacity is based on the above observations, we can quantify the certainty with which the colony capacity takes a certain value, using a Bayesian approach. The capacity of the population is the product $m_{\varphi}p_{h}p_{c}p_{nm}$ and its logarithm is $\ln m_g + \ln p_h + \ln p_c + \ln p_{nm}$. For a population to persist, the capacity of the population needs to be at least 1, or equivalently, its logarithm needs to be at least 0. Because natural *B*. *terrestris* populations persist, we also can include in our considerations that the capacity must be at least 1. We will then establish what reduction in the probability of colony initiation is produced by exposure to thiamethoxam after hibernation. By combining this with the probability profile of the colony capacity we can calculate the increase in the chances of population extinction caused by thiamethoxam exposure.

Table S9 Key model parameters and data sources.

The number of gynes produced by established colonies

Bumblebee colonies in the wild produce a variable number of daughter colonies. We assume a geometric distribution for the number of gynes, (unmated queens) with mean m_g . The probability of a colony to produce *x* gynes is:

$$
P(\underline{x} = x) = \frac{1}{1 + m_g} \left(\frac{m_g}{1 + m_g}\right)^x
$$

If we collect data from n_g colonies placed in the field, the probability to find x_1, x_2, \ldots, x_{ng} gynes is:

$$
\prod_{i=1}^{n_g} \frac{1}{1 + m_g} \left(\frac{m_g}{1 + m_g} \right)^{x_i} = (1 + m_g)^{-(n_g + k_g)} m_g^{k_g}
$$

where the total number of gynes in the n_g colonies is $k_g = \sum_{i=1}^{n_g} x_i$. Assuming an uninformed prior for m_g , the likelihood of the mean, m_g is proportional to:

$$
L(m_g|k_g, n_g) \propto (1+m_g)^{-(n_g+k_g)} m_g^{k_g}
$$

and normalized to a probability this is:

$$
P(\underline{m_g} = m_g | n_g, k_g) = \frac{L(m_g | k_g, n_g)}{\int_0^\infty L(m_g | k_g, n_g) dm_g} = \frac{(k_g + n_g - 1)!}{k_g! (n_g - 2)!} (1 + m_g)^{-(n_g + k_g)} m_g^{k_g}.
$$

13

Note that k_g and n_g are sufficient statistics, and there is no need to know the number of gynes produced per colony. The probability density function of $z_g = \ln m_g$ is:

$$
P(\underline{z_g}=z_g|n_g,k_g)=\frac{(k_g+n_g-1)!}{k_g!(n_g-2)!}(1+e^{z_g})^{-(n_g+k_g)}e^{z_g(k_g+1)}.
$$

with moment generation function:

$$
G_g(s) = \int_{-\infty}^{\infty} e^{sz_g} \frac{(k_g + n_g - 1)!}{k_g!(n_g - 2)!} (1 + e^{z_g})^{-(n_g + k_g)} e^{z_g(k_g + 1)} dz_g
$$

=
$$
\frac{\Gamma(k_g + 1 + s)\Gamma(n_g - s - 1)}{\Gamma(k_g + 1)\Gamma(n_g - 1)}
$$

Hibernation survival

Queens either survive hibernation, or not. A typical experiment to establish the chance, which we call *ph*, to survive consists of keeping a *nh* queens for a certain length of the hibernation period and establish the number k_h that survive. We assume that the survival of an individual is independent of the survival of others, so that the probability of having k_h out of n_h queens surviving is given by a binomial distribution:

$$
P(k_h \text{ out of } n_h \text{ queens survive} | p_h) = {n_h \choose k_h} p_h^{k_h} (1 - p_h)^{n_h - k_h}.
$$

If we do an experiment and find that there are k_h queens surviving out of n_h we can turn this around and infer the likelihood, assuming an uninformed prior for p_h , that the chance to survive is p_h as:

$$
L(\underline{p_h} = p_h | k_h, n_h) = {n_h \choose k_h} p_h^{k_h} (1 - p_h)^{n_h - k_h}
$$

and the probability of the parameter p_h is:

For ft
$$
P(\underline{p_h} = p_h | k_h, n_h) = \frac{L(\underline{p}_h = p_h | k_h, n_h)}{R(\underline{p}_h = z_h | k_h, n_h)} = (n_h + 1) {n_h \choose k} p_h^{k_h} (1 - p_h)^{n_h - k_h}
$$

$$
P(\underline{z_h} = z_h | k_h, n_h) = (n_h + 1) {n_h \choose k_h} e^{z_h(k_h + 1)} (1 - e^{z_h})^{n_h - k_h}
$$

The moment generating function of this distribution is:

$$
G_h(s) = \int_{-\infty}^0 (n_h + 1) e^{sz_h} {n_h \choose k_h} e^{z_h(k_h + 1)} (1 - e^{z_h})^{n_h - k_h} dz_h
$$

=
$$
\frac{\Gamma(n_h + 2)}{\Gamma(k_h + 1)} \frac{\Gamma(k_h + 1 + s)}{\Gamma(n_h + 2 + s)}.
$$

Initiating a colony

Following hibernation queens need to initiate a colony. Let the chance of initiating a colony be given by p_c . A typical experiment to quantify p_c consists of taking n_c queens and establishing the number k that initiate a colony. We assume that the probability of having k_c out of n_c queens initiating a colony is given by a binomial distribution:

$$
P(k_c \text{ out of } n_c \text{ queens initiate a colony} | p_c) = {n_c \choose k_c} p_c^{k_c} (1 - p_c)^{n_c - k_c}.
$$

If we do an experiment and find that there are k_c queens initiating a colony out of n_c we can also turn this around and infer, assuming an uninformed prior for p_c , the likelihood that the chance to initiate a colony is p_c as:

$$
L(\underline{p_c} = p_c | k_c, n_c) = {n_c \choose k} p_c^{k_c} (1 - p_c)^{n_c - k_c}
$$

and the probability of the parameter p_c is:

$$
P(\underline{p_c} = p_c | k_c, n_c) = (n_c + 1) {n_c \choose k_c} p_c^{k_c} (1 - p_c)^{n_c - k_c}
$$

The moment generating function of $z_c = \ln p_c$ is:

$$
G_c(s) = \frac{\Gamma(n_c+2)}{\Gamma(k_c+1)} \frac{\Gamma(k_c+s+1)}{\Gamma(n_c+2+s)}
$$

Calculating the effect of pesticide exposure

In an experiment the effect of pesticide was assessed on the probability of initiating a colony. In a control experiment it was established that *kpc* out of n_{pc} queens established a colony. Among the treated queens, k_{pt} out of n_{pt} queens establish a colony. We assume that the probability to establish a colony under pesticide is reduced to $p_{cp} = p_c r_c$. What is the likelihood of r_c ? Note that the control data k_{pc} and n_{pc} need to be obtained with the same protocol (apart from pesticide treatment) as k_{pt} and n_{pt} , (for instance because the same hibernation period needs to be used) so that it might not be possible to combine the data k_{pc} , n_{pc} with k_c and n_c . The probability of having k_{pc} out of n_{pc} queens initiating a colony is given by a binomial distribution:

$$
P(k_{pc} \text{ out of } n_{pc} \text{ queens initiate a colony} | p_c) = {n_{pc} \choose k_{pc}} p_c^{k_{pc}} (1 - p_c)^{n_{cp} - k_{cp}}.
$$

Using an uninformed prior the likelihood that the chance to initiate a colony is p_c as:

$$
L(\underline{p_c} = p_c | k_{pc}, n_{pc}) = {n_{pc} \choose k_{pc}} p_c^{k_{pc}} (1 - p_c)^{n_{pc} - k_{pc}}
$$

and the probability of the parameter p_c is:

$$
P(\underline{p_c} = p_c | k_{pc}, n_{pc}) = (n_{pc} + 1) {n_{pc} \choose k_{pc}} p_c^{k_{pc}} (1 - p_c)^{n_{pc} - k_{pc}}
$$

and the pdf of the logarithm of $z_c = \ln p_c$ is:

$$
P(\underline{z_c} = z_c | k_{pc}, n_{pc}) = (n_{pc} + 1) {n_{pc} \choose k_{pc}} e^{z_c(1 + k_{pc})} (1 - e^{z_c})^{n_{pc} - k_{pc}}.
$$

When treated with pesticide, k_{pt} out of n_{pt} queens establish a colony. We assume that the probability to establish a colony under pesticide is reduced to $p_{cp} = p_c r_c$, where r_c is the reduction in the chance of initiating a colony caused by the pesticide. What is the probability of r_c ? The probability of having k_{pt} out of n_{pt} queens initiating a colony is given by a:

$$
P(k_{pt} \text{ out of } n_{pt} \text{ queens initiate a colony} | p_{cp}) = {n_{pt} \choose k_{pt}} p_{cp}^{k_{pt}} (1 - p_{cp})^{n_{pt} - k_{pt}}
$$

=
$$
{n_{pt} \choose k_{pt}} (p_{c}r_{c})^{k_{pt}} (1 - p_{c}r_{c})^{n_{pt} - k_{pt}}
$$

Assuming an uninformed prior for $p_c r_c$, and assuming that the probability of p_c is inferred from the control, the likelihood of r_c is:

$$
L(\underline{r_c} = r_c, \underline{p_c} = p_c | k_{pt}, n_{pt}, k_{pc}, n_{pc}) = {n_{pt} \choose k_{pt}} (p_c r_c)^{k_{pt}} (1 - p_c r_c)^{n_{pt} - k_{pt}} (n_{pc} + 1) {n_{pc} \choose k_{pc}} p_c^{k_{pc}} (1 - p_c)^{n_{pc} - k_{pc}}
$$

The probability of these parameters, given the data, is:

$$
P(p_c, r_c | k_{cp}, n_{cp}, k_{ct}, n_{ct})
$$
\n
$$
= \frac{(n_{pc} + 1) {n_{pc} \choose k_{pc}} {n_{pt} \choose k_{pt}} (p_c r_c)^{k_{pt}} (1 - p_c r_c)^{n_{pt} - k_{pt}} p_c^{k_{pc}} (1 - p_c)^{n_{pc} - k_{pc}}
$$
\n
$$
= k_{pc} (n_{pt} + 1) {n_{pc} \choose k_{pc}} {n_{pt} \choose k_{pt}} (p_c r_c)^{k_{pt}} (1 - p_c r_c)^{n_{pt} - k_{pt}} (n_{pc} + 1) {n_{pc} \choose k_{pc}} p_c^{k_{pc}} (1 - p_c)^{n_{pc} - k_{pc}} dr_c dp_c
$$

From this we can calculate the marginal probability of r_c as:

$$
P(r_c|k_{pt}, n_{pt}, k_{pc}, n_{pc}) =
$$

$$
\int_0^{\min(1,1/r_c)} k_{pc}(n_{pt}+1) {n_{pc} \choose k_{pc}} {n_{pt} \choose k_{pt}} (p_c r_c)^{k_{pt}} (1-p_c r_c)^{n_{pt}-k_{pt}} p_c^{k_{pc}} (1-p_c)^{n_{pc}-k_{pc}} dp_c.
$$

The logarithm r_c , denoted $z_r = \ln r_c$, is distributed as:

$$
\int_0^{\min(1,e^{-zr})} k_{pc}(n_{pt}+1) \binom{n_{pc}}{k_{pc}} \binom{n_{pt}}{k_{pt}} p_c^{k_{pt}} e^{z_r(k_{pt}+1)} (1-p_c e^{z_r})^{n_{pt}-k_{pt}} p_c^{k_{pc}} (1-p_c)^{n_{pc}-k_{pc}} dp_c.
$$

In our calculation we integrated this numerically.

Calculating the probability of the colony capacity

To calculate the probability of the capacity, *C*, we calculated the probability of $z = \ln C = \ln m_g + \ln p_h + \ln p_c + \ln p_{nm}$. We have no information on the value of *pnm*, and therefore assume that it is uniformly distributed on the interval [0;1]: The probability density of *z* is then given by:

$$
P(z) = P(\underline{z} = z | p_{nm}) = \int \int P(\underline{z_c} = z_c) P(\underline{z_h} = z_h) P(\underline{z_g} = z - z_h - z_c - \ln p_{nm}) \mathrm{d}z_h \mathrm{d}z_c
$$

The procedure above was used to calculate the probability of the colony capacity in the untreated situation. Note that, although there is no need to do so here in detail, should we want to calculate the probability distribution of z we could do this using the generating function of the logarithms of the other variable.

After treatment, the logarithm of the colony capacity is given by $z_t = \ln m_g + \ln p_h + \ln p_{cp} + \ln p_{nm} = z + z_r$. To calculate this probability distribution (the heat map) of the logarithm of the capacity affected by pesticide exposure we calculate the marginal probability of z_t .

$$
\int_{\forall z+z_r=z_t} P(z)P(\underline{z_r}=z_t-z)\mathrm{d}z.
$$

Next, we take into account that we know that the population is extant. Given this fact, we know that the colony capacity, measured as the geometric mean over the years needs to exceed one. Our measurement only covers a single year. However, because we know the year from which we have data was a good year for bumblebees the fact that the population is extant implies that the colony capacity in this year has to be at least one (if the colony capacity were to be less than one even in a good year the population would certainly be extinct). We can now use the probability for $z = \ln C$ that we have calculated as $P(z=z)$ as a prior and then add the information that the colony capacity for this year must be at least one, and that the value of *z* for this particular year must be at least zero. This gives the posterior:

$$
P'(z) = \begin{cases} 0 & \text{if } z < 0\\ \frac{P(z)}{\int_0^\infty P(z)dz} & \text{if } z > 0. \end{cases}
$$

The procedure above was used to calculate probability of the colony capacity in the untreated situation. To calculate this probability distribution (the heat map) of the logarithm of the capacity affected by pesticide exposure we calculate, using $z_t = z + z_r$.

$$
\int_{\forall z+z_r=z_t} P'(z)P(\underline{z_r}=z_t-z)\mathrm{d}z.
$$