Supporting information 2 for: Simulating the ~~BeePop+ model~~ effects of pesticides on honey bee (*Apis mellifera* L.) colonies with BeePop+

Approach for simulating colony failure due to low pollen and nectar stores during winter

Conceptual approach

In this approach, colonies may fail during winter if the bees run out of food resources (*i.e.,* pollen or nectar). The colony simulation model accounts for the amount of pollen and nectar equivalents (*i.e.*, food stores) present in the hive on a daily basis. During the spring, summer and fall, food stores increase each day that pollen and nectar collected by foragers is in excess of the amount consumed by all the individuals in the hive. If less pollen and/or nectar is brought into the hive than is needed, then bees consume stored food. The food stores may be present in the hive as a result of foraging (during the spring, summer and fall) or supplemental feeding by beekeepers (sucrose and/or pollen cakes). In the winter, no new food is brought into the hive. Therefore, each day, winter bees consume stored pollen and honey (which is expressed as nectar equivalents based on sugar consumption). During winter, on any given day, if there is an insufficient amount of pollen and/or nectar to feed the winter bees, the colony dies. Colonies will survive the winter if they have sufficient stored pollen and honey on each day.

A winter bee consumes an average of 2 mg pollen per day (Appendix A of USEPA 2012) and a variable amount of nectar-equivalents (as described below). At the beginning of each simulation day (t+1), the stored nectar and pollen ($Pollen_t$ in g) is less than the day before (t) (**Equation 1**; N_{bees} is number of adult bees in hive at time t). The amount of pollen available in the hive (in g) on any given day is calculated according to **Equation 1**.

Equation 1. $Pollen_{t+1} = Pollen_t - 0.002 * N_{bees(t)}$

The amount of nectar consumed is related to temperature. During the winter, workers maintain the temperature of the hive using their body heat, specifically by vibrating their flight muscles (without moving their wings). When ambient temperatures are ≤18°C (64°F), bees form clusters that allow them to conserve heat. As the temperature outside the hive drops, the cluster contracts, with the tightest formation reached at -5°C (ambient). Clusters migrate slowly through a Langstroth hive (from the bottom up) as they consume food in a given area, moving to areas with stored honey. Clusters are composed of multiple layers of worker bees, with the queen located in the center. The inside of the cluster is typically maintained at 20°C (68°F), with a minimum of 13 °C (55°F). The outside of the cluster is cooler, typically 8 °C (46°F). Bees inside the cluster actively vibrate their flight muscles to generate heat, while bees on the outside of the cluster are motionless, essentially serving as insulators. Individual bees remain inside the cluster for approximately 12 hours, rotating out to the external portion of the cluster (Winston 1987).

Southwick (1985) observed that metabolism per mass of bee increased with temperature and with fewer bees (*i.e.*, smaller cluster). Allometric equations were published (**Equations 2 and 3**) for ambient temperatures of 2°C and 15°C that related metabolism (V_{O2} is mL oxygen per g of bees per hour) to cluster size (expressed as body weight in g; BW).

Equation 2. At 2°C: $V_{O_2} = 22.69 * BW^{-0.48}$ (R^2=0.96)

Equation 3. At 15°C: $V_{O_2} = 4.23 * BW^{-0.27}$ (R^2=0.89)

In order to relate these equations to the amount of nectar equivalents consumed, these equations need to be converted as follows. BW can be replaced with the number of bees (N) times the weight of an individual bee (0.128 g). A factor of 24 is included in the equation to convert the output from hourly to daily. The total mass of the cluster is also used to convert the calculation from volume of oxygen consumed per g of bees to the total amount of oxygen consumed. In the equations below, $V_{O2(tot)}$ is mL oxygen per day.

Equation 4. At 2°C: $V_{O_2(tot)} = [22.69 * (0.128 * N_{bees})^{-0.48}] * 24 * (N_{bees} * 0.128)$
Equation 5. At 15°C: $V_{O_2(tot)} = [4.23 * (0.128 * N_{bees})^{-0.27}] * 24 * (N_{bees} * 0.128)$

Using relevant densities of oxygen gas (*i.e.*, 0.001429 g/mL at 0oC and 0.001331 g/mL at 20oC[1]) the mass of oxygen in g, these equations can be translated to the following (with rearrangement):

Equation 6. At 2°C: $M_{O_2(tot)} = 0.09961 * N_{bees} * (0.128 * N_{bees})^{-0.48}$
Equation 7. At 15°C: $M_{O_2(tot)} = 0.01730 * N_{bees} * (0.128 * N_{bees})^{-0.27}$

In respiration, 6 moles of oxygen molecules are required to break down 1 mol of glucose. When considering the molecular weights of oxygen (*i.e.*, 32 g/mol) and glucose (*i.e.*, 180 g/mol), for every 1 g of oxygen that is consumed, 0.94 g of glucose is consumed. Therefore, a factor of 0.94 can be applied to the equations above to generate a mass of glucose that is consumed per day by the cluster. In order to convert back to the amount of nectar consumed, the sugar content of nectar is incorporated (*i.e.*, 30%). This results in the following equations that generate a daily mass of nectar equivalents consumed by the hive (M_{nectar}; g/day):

Equation 8. At 2°C: $M_{nectar} = 0.3121 * N_{bees} * (0.128 * N_{bees})^{-0.48}$
Equation 9. At 15°C: $M_{nectar} = 0.05419 * N_{bees} * (0.128 * N_{bees})^{-0.27}$

The total amount of nectar equivalents available in the hive would be calculated in a manner similar to **Equation 1** for pollen; *i.e.*, the amount of stored nectar in the hive would be decreased the following day by the amount of nectar consumed by the hive at time t (**Equation 10**).

Equation 10. $Nectar_{t+1} = Nectar_t - M_{nectar(t)}$

The table below presents an example of how much nectar (equivalent) is consumed per day by the cluster, given different numbers and temperatures. These values translate to different food consumption value per bee that are on the same order of magnitude as the BeeREX model (*i.e.*, 29 mg/bee/day).

[1] source: http://www.engineeringtoolbox.com/gas-density-d_158.html

Table 1. Nectar consumed by clusters calculated using equations 8 and 9.

N (number of bees)	Mass of nectar consumed per day by cluster (g)		Mass of nectar consumed per day by individuals (mg)	
	2°C	15°C	2°C	15°C
500	21	9	42	18
1000	30	15	30	15
5000	70	47	14	9.5
10000	101	79	10	7.9
12500	113	92	9.0	7.4
16500	131	113	7.9	6.9
20000	144	130	7.2	6.5

The equations incorporated into Southwick 1985 were based on metabolism measures from different cluster sizes. The greatest cluster size incorporated into the dataset was approximately 1250 g, which roughly translates to 10,000 bees. There is uncertainty associated with food consumption rates for cluster sizes >10,000; however, the data presented by Southwick (1985; in Figure 1) suggest that an asymptote is reached at >5000 bees.

Another study (University of Wyoming 1932) is available to allow for comparison of metabolism rates at different temperatures. In this study, mass of CO_2 generated by hives was determined at different temperatures using winter bees. Two different hives were studied, with sizes of 12,500 and 16,500 bees. At these sizes, the modified equations above (**Equations 8 and 9**) estimate nectar consumption ranging 92-131 g/day for a colony (Table 1). Nectar consumption estimates for these hives were within a factor of 0.7-2.6 of the estimates generated using **Equations 8 and 9** (**Tables 2 and 3**). This suggests good agreement between data generated by the two studies and that **Equations 8 and 9** are predictive of nectar consumed by winter bees. The Wyoming study included a third hive, which involved reduced temperature exposures to summer bees. Their metabolism rates were almost a factor of 3 higher than those of the winter bees.

Table 2. Comparison of estimated mass of nectar consumed by Hive 1 (N_{bees} = 12,483) from Wyoming study (based on CO_2 generated) to nectar consumed using Equations 8 and 9.

Temp (°C)	Reported amount of CO_2 generated (mg CO_2/bee-h)	Estimated mass of nectar consumed (g nectar/colony-d)		Factor difference
		Converted from reported amount of CO_2 generated	Based on Equation 8 or 9	
13.8	0.29	196	92*	2.1
13.3	0.21	141	92*	1.5
2.9	0.15	103	113**	0.9
2.8	0.13	87	113**	0.8

*Calculated using Equation 9.
**Calculated using Equation 8.

Table 3. Comparison of estimated mass of nectar consumed by Hive 2 (N_{bees} = 16,500) from Wyoming study (based on CO_2 generated) to nectar consumed using Equations 8 and 9.

Temp (°C)	Reported amount of CO_2 generated (mg CO_2/bee-h)	Estimated mass of nectar consumed (g nectar/colony-d)		Factor difference
		Converted from reported amount of CO_2 generated	Based on Equation 8	
7.42	0.378	340	131	2.6
6.22	0.182	164	131	1.3
6.2	0.174	157	131	1.2
6.25	0.148	133	131	1.0
6	0.135	122	131	0.9
5.8	0.139	125	131	1.0
5.74	0.163	147	131	1.1
5.69	0.15	135	131	1.0
5.48	0.115	104	131	0.8
5.83	0.111	100	131	0.8
5.81	0.107	96	131	0.7
5.68	0.111	100	131	0.8
5.82	0.116	104	131	0.8
5.7	0.126	113	131	0.9

Implementation in BeePop+ model

Stored pollen: When average ambient temperature on day t is ≤18°C, amount of pollen available in the hive on any given day is calculated according to **Equation 1**.

In Feb-March, the queen begins laying eggs. When larvae are present, a subset of adult bees become nurse bees. The number of nurse bees ($N_{nursesbees(t)}$) is calculated by dividing the number of larvae by 2. The model assigns the youngest adult bees to be nurse bees. Individual nurse bees consume pollen at the intake rate entered by the user in the GUI (I_{pollen}; in g). This intake value is used during summer as well. The amount of pollen consumed by the hive is then calculated using **Equation 11.**

Equation 11: $\mathrm{Pollen}_{t+1} = \mathrm{Pollen}_t - 0.002 * (\mathrm{N}_{\mathrm{bees(t)}} - \mathrm{N}_{\mathrm{nursebees(t)}}) - I_{pollen} * \mathrm{N}_{\mathrm{nursebees(t)}}$

Note that if day t is a foraging day, bees will consume fresh pollen before stored pollen. Excess pollen brought into the hive will be added to the stored pollen.

Stored Nectar: When average ambient temperature on day t is ≤18°C and ≥8.5°C, amount of nectar available in the hive on any given day is calculated according to **Equation 12** (based on **Equations 9 and 10**). When the average ambient temperature is <8.5°C, **Equation 13** is used (this is based on **Equations 8 and 10**). In these equations, Nectar_t = mass of nectar (in g) stored in the hive on day t.

Equation 12: $Nectar_{t+1} = Nectar_t - 0.05419 * N_{bees} * (0.128 * N_{bees})^{-0.27}$

Equation 13: $Nectar_{t+1} = Nectar_t - 0.3121 * N_{bees} * (0.128 * N_{bees})^{-0.48}$

Note that if day t is a foraging day, bees will consume fresh nectar before stored nectar. Excess nectar brought into the hive will be added to the stored nectar.

<u>Supplemental feeding:</u>
Supplemental feeding by beekeepers includes pollen paddies or sucrose solution. The user can choose whether or not supplemental feeding is simulated. The dates (month and day) of the simulation where pollen paddies or sucrose are provided each year are indicated.

For pollen paddies:

- If pollen paddies are present at day t (based on user-defined date range for supplemental pollen feeding), the pollen needs of the bees are met and no stored pollen is consumed (*i.e.*, **Equation 1** is not implemented).
- If the bees forage for pollen while paddies are present, they store all incoming pollen. Bees do not eat incoming pollen.
- If it is not a foraging day and there is no paddy, bees eat stored pollen.
- Bees do not receive pesticide dose through pollen paddies.

For sucrose:

- The amount of sucrose fed to the bees is converted internally to nectar equivalents using **Equation 14**.
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Equation 14: $Nectar_{supplement} = Soln * p * {}^{0.3}/_{S}$

  - Soln = the user input volume of sucrose solution, with units of L.
  - If the user selects "Light" sucrose, it is assumed that the solution is 25% sugar (i.e., S = 0.25). The corresponding density (p) is: 1106 g/L[2].
  - If the user selects "heavy" sucrose, it is assumed that the solution is 50% sugar (i.e., S = 0.5). The corresponding density (p) is: 1232 g/L[2].
- Each day, bees automatically move the supplemental sucrose (expressed as nectar-equivalents) into storage. The amount moved on a given day is the total amount of nectar-equivalents fed divided by the number of days where the feeding occurs over each user specified interval.
- If day t is a foraging day, bees will consume fresh nectar first to meet their daily needs. If their needs are not met by the incoming nectar, they consume stored nectar.
- If it is not a foraging day, bees eat stored nectar (which included the supplemental sucrose added to the total stored nectar).
- Bees consume nectar as follows:
  - When the average ambient temperature is >18.5°C, non-winter feeding routines will be implemented.

[2] http://onlinelibrary.wiley.com/doi/10.1002/9780471790990.oth1/pdf

- When the average ambient temperature is ≤18°C and ≥8.5°C, **Equation 9** is used: $M_{nectar} = 0.05419 * N_{bees} * (0.128 * N_{bees})^{-0.27}$
- When the average ambient temperature is <8.5°C, **Equation 8** is used: $M_{nectar} = \quad 0.3121 * N_{bees} * (0.128 * N_{bees})^{-0.48}$

- Bees do not receive pesticide dose through supplemental sucrose solution.

Colony Failure: If the colony runs out of either pollen or nectar, and no supplemental feeding is available, the colony fails (i.e., if $Pollen_t$ ≤0 or $Nectar_t$ ≤ 0, colony fails).

Sources:

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