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Social buffering of pesticides in bumblebees: agent-based modeling of the effects of colony size and neonicotinoid exposure on behavior within nests

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JDC, BD, and ANFV contributed equally to this work. JDC, BD, and ANFV designed the study and wrote the computational code. JDC shared relevant experimental data from a previous study and conducted the in silico experiments. JDC, BdB, BD, and ANFV analyzed results of the in silico experiments and wrote the manuscript.

Abstract

Neonicotinoids are a globally prevalent class of pesticides that can negatively impact bees and the pollination services they provide. While there is evidence suggesting that colony size may play an important role in mitigating neonicotinoid exposure in bees, mechanisms underlying these effects are not well understood. Here, a recently developed, agent-based computational model is used to investigate how the effects of sub-lethal neonicotinoid exposure on intranest behavior of bumblebees (*Bombus impatiens*) are modulated by colony size. Simulations from the model, parameterized using empirical data on bumblebee workers exposed to imidacloprid (a common neonicotinoid pesticide), suggest that colony size has significant effects on behavior neonicotinoid-sensitivity within bumblebee nests. Specifically, differences are reduced between treated and untreated workers in larger colonies for several key aspects of behavior within nests. Our results suggest that changes in both number of workers and nest architecture may contribute to making larger colonies less sensitive to pesticide exposure.

Keywords: Pollinator, Neonicotinoids, Bees, Collective behavior, Social insects, Pesticides, Agent-based modeling

1. Introduction

Agricultural use of neonicotinoid pesticides may negatively affect bees (Cresswell, 2011; Rundlöf et al., 2015; Woodcock et al., 2016; 2017) and the ecosystem services they provide (Stanley et al., 2015a) by altering worker behavior (Gill et al., 2012; Kessler et al., 2015; Whitehorn et al., 2012). Neonicotinoids disrupt learning and navigation of foraging bees (Palmer et al., 2013; Stanley et al., 2015b; Tan et al., 2015) and may impair colony growth (Laycock et al., 2012; Whitehorn et al., 2017) through reductions in resource intake (Feltham et al., 2014; Gill et al., 2012).

Workers within bee nests are likely exposed to neonicotinoids (Mitchell et al., 2017; Rundlöf et al., 2015), which can affect their locomotion and attraction to light (Tosi and Nieh, 2017), thermoregulation (Crall et al., 2018b; Potts et al., 2018; Tosi et al., 2016), and hygienic behaviors (Tsvetkov et al., 2017; Wu-Smart and Spivak, 2016). , The neonicotinoid imidacloprid reduces activity and alters spatial dynamics and social interactions within bumblebee (*Bombus impatiens*) colonies (Crall et al., 2018b).

Mechanistic models of behavior within bee colonies can consider complex impacts of pesticides and other stressors under different scenarios (Bryden et al., 2013; Cresswell, 2017; Henry et al., 2017; Laycock and Cresswell, 2013; Sponsler and Johnson, 2016). Models have been developed for populations of bumblebees (Becher et al., 2018; Betti et al., 2017; Henry et al., 2017; Sponsler and Johnson, 2016; Thorbek et al., 2016) with and without exposure to neonicotinoids.

Colony size is a factor in sensitivity to environmental stressors as it plays a role in the resilience and conservation of social insects (Fisher, 2018; Straub et al., 2015). Colony size may affect susceptibility to neonicotinoid pesticides. One study (Rundlöf et al., 2015) found no significant effects of neonicotinoid exposure on honeybees (large colony sizes, >10,000), intermediate effects on bumblebees (moderate colony size, < 500 workers), and very strong effects on solitary bees, under the same exposure conditions. Some negative effects of imidacloprid on honeybees were shown to be stronger in smaller colonies (Wu-Smart and Spivak, 2016).

The effects of colony size on worker behavior within nests in response to pesticide exposure are not well understood. Colony size may improve resilience to pesticides as a direct effect of larger population size by decreasing the chances of colony failure when a small number of workers are lost to disease or death or behavioral impairment (Straub et al., 2015). Alternatively, larger colonies could mitigate the effects of exposure on the behavior of individuals. Changes in interaction rate or nest architecture associated with larger colony size could modulate the effects of pesticide exposure on individuals and colony performance through the division of labor, behavioral development, or social interactions associated with large social insect colonies.

Our goal is to elucidate the mechanisms underlying the effects of neonicotinoid exposure on worker behavior within bumblebee nests and how these effects are modulated by colony size. We use our spatially-explicit agent-based model called BeeNestABM (Crall et al., 2018b; Ford Versypt et al., 2018) to test the hypothesis that the effects of exposure on worker behavior within bumblebee nests are reduced in larger colonies. Bumblebee colonies range from a single individual during colony founding by a solitary queen up to several hundred workers (Michener and Laberge, 1954). Inside colonies, interactions with nestmates and nest architecture can have important effects on worker behavior. Interactions with nestmates directly affect activity and task switching in colonies (Crall et al., 2018b; Renner and Nieh, 2008) and could modulate behavioral impacts of pesticides. The physical structure of the nest (e.g., food pots and developing brood) can modulate worker behavior by carrying information on larval development and hunger (Boer and Duchateau, 2006; Dornhaus, 2005; Heinrich, 1974). Because neonicotinoids directly reduce activity, which indirectly alters spatial and social dynamics (Crall et al., 2018b), increased interactions with nestmates or nest structures in larger colonies could mitigate the impacts of neonicotinoid exposure.

2. Model description

BeeNestABM was parameterized using empirical data (Crall et al., 2018b) and simulates bumblebee activity and interactions within a nest. The code and documentation are available (Ford Versypt et al., 2018). Here, we briefly describe the model.

BeeNestABM simulates movements of bees within a nest on short time scales allowing for interactions with nestmates and nest structures. The state variables at each time step and for each bee are the x- and y-coordinates, heading angle, speed, and activity state. The simulation is initialized with a user-specified number of bees. Bees are initialized with random positions inside a nest arena (0.2 x 0.2 m for all simulations here). The model is iterated in steps of 0.5 s. Nest structures are placed in 1-cm apart in a square grid covering a centered, user-specified fraction of the arena.

The model consists of three processes that occur at each time step. First, distances between bees and the nest structures (Fig 1A) are calculated. Next, the activity state for each bee (Fig 1C-G) is updated. Finally, the coordinates for each active bee are updated using the movement rules (Fig 1B).

2.1. Activity state updates

Activity-switching probabilities depend on location on or off the nest structures (Fig 1D), proximity to nestmates (Fig 1E), and treatment group (Fig 1C-G). Workers are assigned probabilities calculated previously (Crall et al., 2018b Supplement) from the means of empirical data of automatically tracked (Crall et al., 2015) bumblebees. These probabilities represent the probabilities of switching between active (moving) and inactive (stationary) between time steps, calculated for different combinations of social interaction state and location (Fig 1). Probabilities for treated bees are set equivalent to controls for parameters that did not different significantly between control and treated workers (Fig 1F-G). Treated bees have activity-switching probabilities that make bees less likely to initiate movement either spontaneously or after physical contact with a nestmate and more likely to become immobilized, with stronger effects when treated bees are located off the nest structures (Fig 1F-G).

In the dataset (Crall et al., 2018b), workers were exposed orally to an acute dose of either 0 ng (control) or 1.0 ng imidacloprid at 87 ppb (treated) in a sucrose solution and allowed to behave freely in a nest arena. 1.0 ng was approximately equal to the cumulative imidacloprid consumed per worker in a single day of chronic feeding on nectar with environmentally realistic imidacloprid concentrations (6 ppb). While that study found qualitatively similar effects on behavior in workers exposed to imidacloprid either acutely or chronically, the behavioral effects during chronic exposure varied with time of day. To limit model complexity, we did not incorporate circadian effects but focused on acute exposure data collected at the same time of day.

2.2 Movement rules

Previously inactive bees are set at a speed sampled from the empirical speed distribution. Bees previously moving are updated to the sampled speed if it is within specified limits of the current speed; otherwise, there is a 10% probability of switching to the sampled value from the current speed. The heading angle for each bee is the weighted angular mean of a random walk angle fluctuating within 90° of the current angle and the angle toward the environmental stimuli determined by the net displacement between the bee and nest structures (Fig 1B). The environmental stimuli represent attractions to the nest structures with weights estimated using empirical data (μ = 0.067 and 0.052 for control and treated bees, respectively). After location updates, movement is truncated for each bee that would move outside the perimeter.

3. Results and Discussion

We used BeeNestABM to examine the effects of colony size and exposure treatment intensities (the proportion of workers within the colony exposed to imidacloprid) on worker behavior. We ran simulations where two aspects of colony size (numbers of nest structures and of workers) were varied simultaneously from 4 to 441, spanning the ecologically relevant colony sizes for bumblebees, to maintain a 1:1 worker:nest structure ratio across a range of treatment intensities (Fig 2). To examine the relative importance of nest architecture and number of workers, we ran simulations varying these parameters independently (Figs S1-S2). For each combination of colony size and exposure intensity, we estimated mean values across all simulations and individuals for four behavioral metrics (Figs 2A-D, S1, S2).

Exposure intensity and colony size significantly affected worker behavior within nests. Larger colonies had higher activity levels (Fig 2A), mean distance to the nest center (Fig 2B), and proportions of time spent on the nest structure (Fig 2C), with interaction rates (Fig 2D) decreasing at intermediate colony sizes. Higher treatment intensity was associated with substantial declines in activity and interaction rate (Figs

2A,D), a weak increase in D_{NC} (Fig 2B), and a weak decrease in P_{nest} (Fig 2C). Simulations isolating the effects of variation in nest size and number of workers separately suggest that the effects of colony size are driven primarily by changes in the size and number of nest structures, especially for D_{NC} and (trivially) P_{nest} (Fig S1).

We found evidence that the relative difference between control and treated workers varied significantly within colony size (Fig 2E-H); relative differences between control and treated workers in activity (Fig 2E), D_{NC} (Fig 2F), and P_{nest} (Fig 2G) were reduced in larger colonies, while relative differences in interaction rate were greatest at intermediate colony sizes (Fig 2H). The reduced differences between control and treated workers in larger colonies is likely driven primarily by the weaker effects of imidacloprid on activity parameters when located on the nest structures (Fig 1F-G, Fig S2). We found qualitatively similar effects for absolute differences between control and treated workers for most metrics (Fig S3), with the exception of proportion of time spent on the nest structure, which varied most with colony size (Figs 2, S3).

4. Conclusions

Our results support the hypothesis that larger colonies buffer the effects of neonicotinoid exposure on behavior within bumblebee nests: differences between control and treated workers in several key emergent behavioral parameters were reduced in larger colonies (Fig 2A-D). The dominant effects of nest structure size (Fig S1-S2) suggest that nest architecture, rather than altered rates of interaction with nestmates, is the primary driver of reduced effects of imidacloprid exposure in large colonies, although higher worker numbers did have qualitatively similar, but quantitatively weaker, effects for several metrics (Fig S2). The strong effects of nest architecture found here result directly from the empirical observation that neonicotinoids have particularly strong effects on activity when workers are located on the nest structure (Crall et al., 2018b). The specific mechanisms driving this patterns are not clear but suggest that some aspects of the nest structure (potentially including chemical signals derived from the brood (Boer and Duchateau, 2006) or from nestmates (Richardson and Gorochowski, 2015) laid on the nest structure) play a direct role in regulating worker behavior. Being on the nest architecture stimulates movement (Fig 1), potentially mitigating the negative effects of neonicotinoids on activity.

The mitigating effects of colony size likely translate into significant impacts on colony performance. Location on the nest structure is a strong proxy for direct brood care and maintenance behavior within the nest (Crall et al., 2018a). Our results lead to the testable predictions that (a) similar levels of exposure will lead to stronger effects on nest behavior in smaller colonies and that (b) this would lead to stronger reductions in per-worker nursing rates and brood growth in smaller colonies. For bumblebees, increased sensitivity at small colony sizes is especially relevant because exposure during colony initiation by a solitary queen or during early developmental stages can substantially impair colony performance (Baron et al., 2017; Leza et al., 2018).

Our model improves understanding of how colony size and pesticide exposure affect behavior within bumblebee nests. However, the scope of the model is limited to a simplified set of behaviors. In the future, the model could be expanded to more complex behaviors of bumblebees (Cameron, 1989; Crall et al., 2018a). Combining this model with colony dynamic processes at larger spatial and longer time scales, including foraging, growth, and landscape dynamics (Becher et al., 2018) could help assess how the impacts of neonicotinoids on behavior translate to long-term colony performance.

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Figure Captions

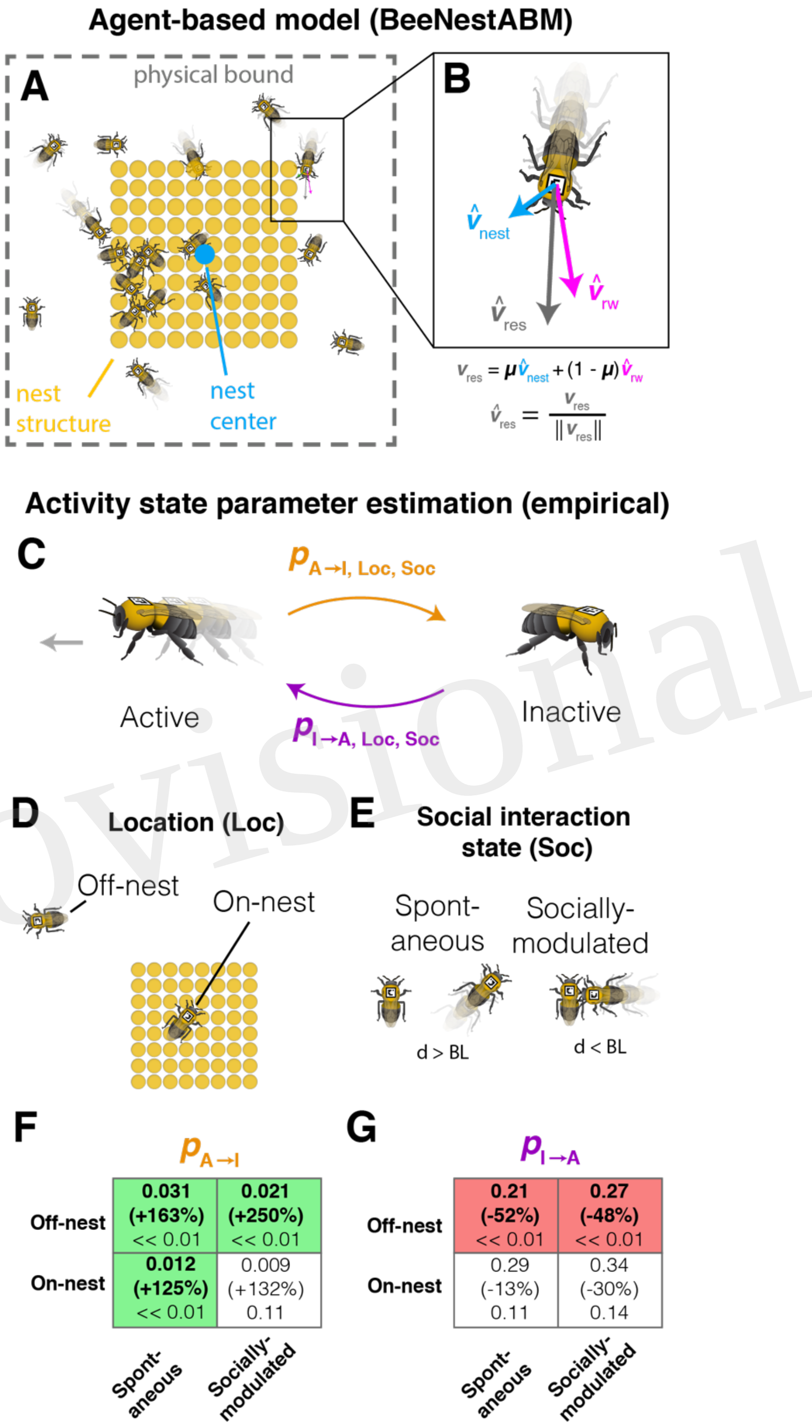


Figure 1. **Parameter estimation and model description.** (A) Schematic diagram of model simulation, including individual workers, nest structure (filled tan circles), nest center (blue circle), and physical boundaries. (B) Model for simulated worker movement showing the random walk (v_{rw}), nest attraction (v_{nest}), and resultant (v_{res}) movement vectors. (C) Activity switching probabilities (active to inactive, $p_{A\ à\ I}$, and inactive to active, $p_{I\ à\ A}$), are measured separately depending on whether bees are (D) located on or

off the nest structure or (E) in physical contact with a nestmate. (F-G) Changes in probability of switching from active to inactive ($\boldsymbol{p}_{A \rightarrow I}$, F) and switching from inactive to active state ($\boldsymbol{p}_{I \rightarrow A}$, G) as a result of imidacloprid exposure. Probability changes are shown separately for combinations of location (Loc) and social interaction state (Soc). For each box, upper values show parameter estimates for untreated bees, middle values (in parentheses) show the percent change in the estimated parameters in bees fed 1.0 ng imidacloprid (compared to controls), and lower values are p-values. Bold values indicate significant effects, with box color indicating the direction of change (red for decreasing, green for increasing). Data from Fig S5 in (Crall et al., 2018b).

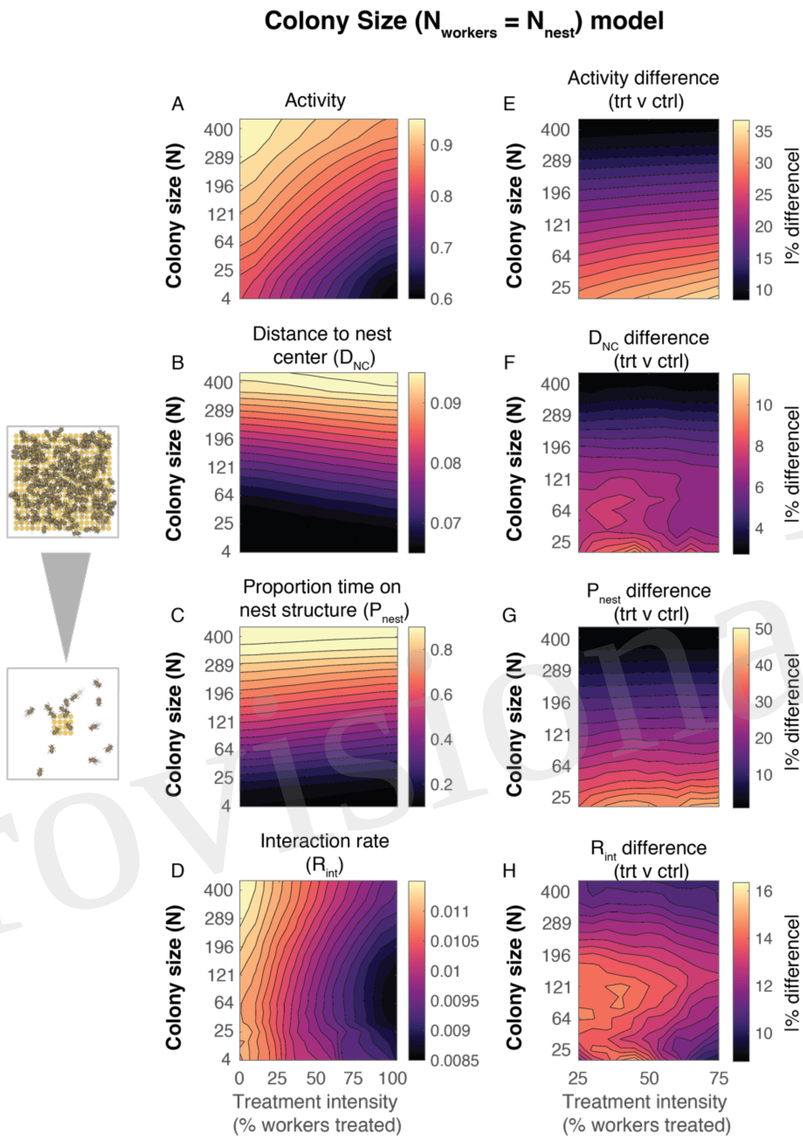


Figure 2. **Effects of colony size on emergent behavior in bumblebee nests.** Mean behavioral metrics (left column) and relative differences between treated and control workers (right column) as a function of colony size and treatment intensity (% of workers treated). Data are shown for four metrics: (A, E) activity, (B, F) distance to the nest center (D_{NC}), (C, G) proportion of time spent on the nest structure (P_{nest}), and (D, H) interaction rate (R_{int}). Values are the mean across all workers and replicate simulations at a given parameter set. The number of simulation iterations at each parameter set was varied with colony size (# of iterations = 10000/*N,* where *N* = # of workers). Phase spaces smoothed using local averaging over adjacent values (smoothing window: +/- 10% for treatment intensity, $(\text{sqrt}(N-2))^2$:$(\text{sqrt}(N+2))^2$ for $N_{nest}/N_{workers}$). For all simulations, the number of nest structure elements (N_{nest}) and the number of workers ($N_{workers}$) were varied simultaneously to maintain a 1:1 nest structure:worker ratio. Differences between control and treated workers (right column) were calculated as a percentage of mean absolute value, abs([*trt* – *ctrl*]/mean(*trt*, *ctrl*)])*100, where *trt* denotes the treated workers and *ctrl* denotes the control group.

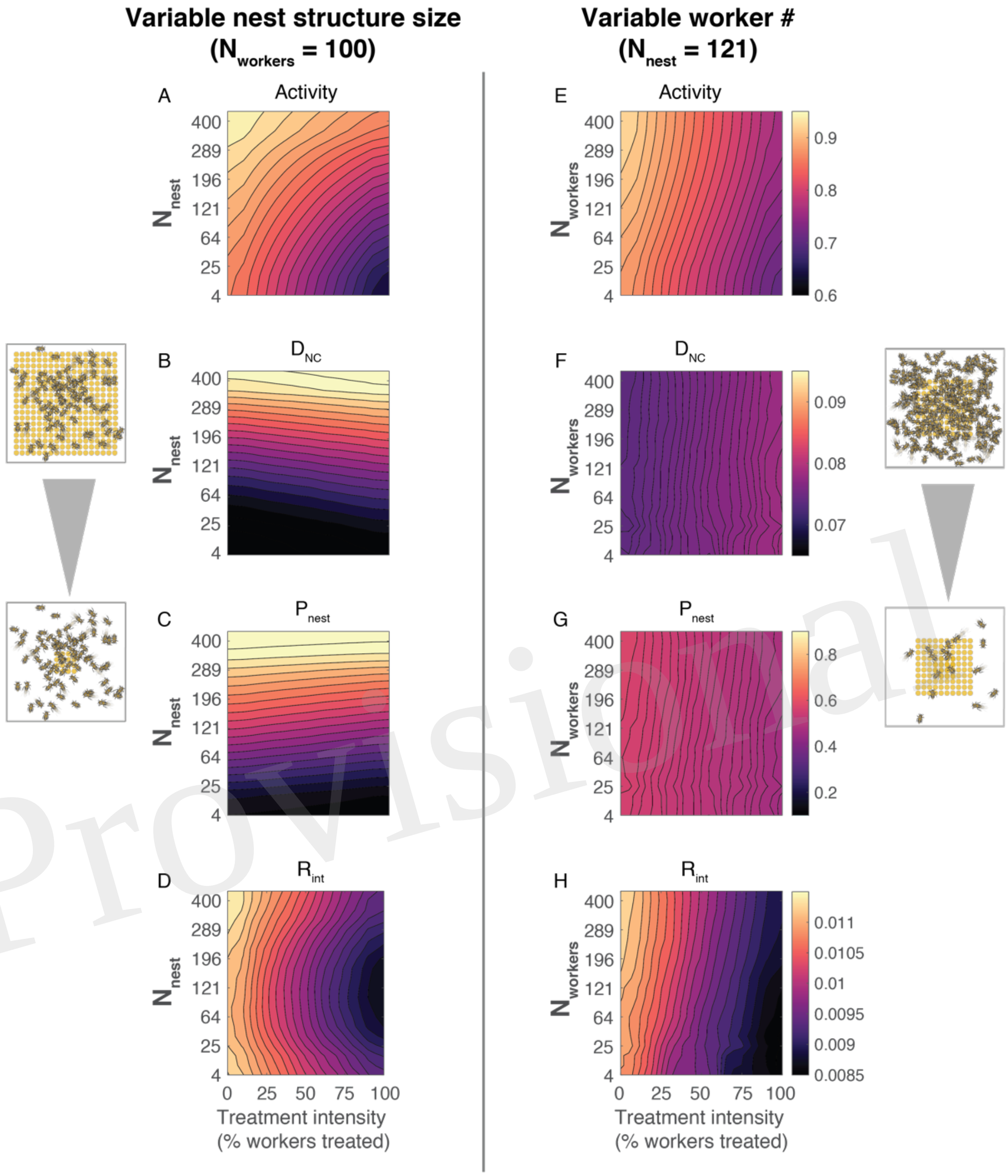


Figure S1. **Independent effects of nest structure size and worker number on emergent behavior in bumblebee nests.** Mean behavioral metrics when the number of nest structural elements (N_{nest}, left column) and number of workers ($N_{workers}$, right column) are independently varied. Data are shown for four metrics: (A, E) activity, (B, F) distance to the nest center (D_{NC}), (C, G) proportion of time spent on the nest structure (P_{nest}), and (D, H) interaction rate (R_{int}). Value are the mean across all workers and replicate simulations at a given parameter set. The number of simulation iterations at each parameter set was varied with colony size (# of iterations = 10000/*N*, where *N* = # of workers). Phase spaces smoothed using local averaging over adjacent values (smoothing window: +/- 10% for treatment intensity, (sqrt(N-2))^2:(sqrt(N+2))^2 for N_{nest}/$N_{workers}$)For each metric, identical color scales are used for the left and right columns.

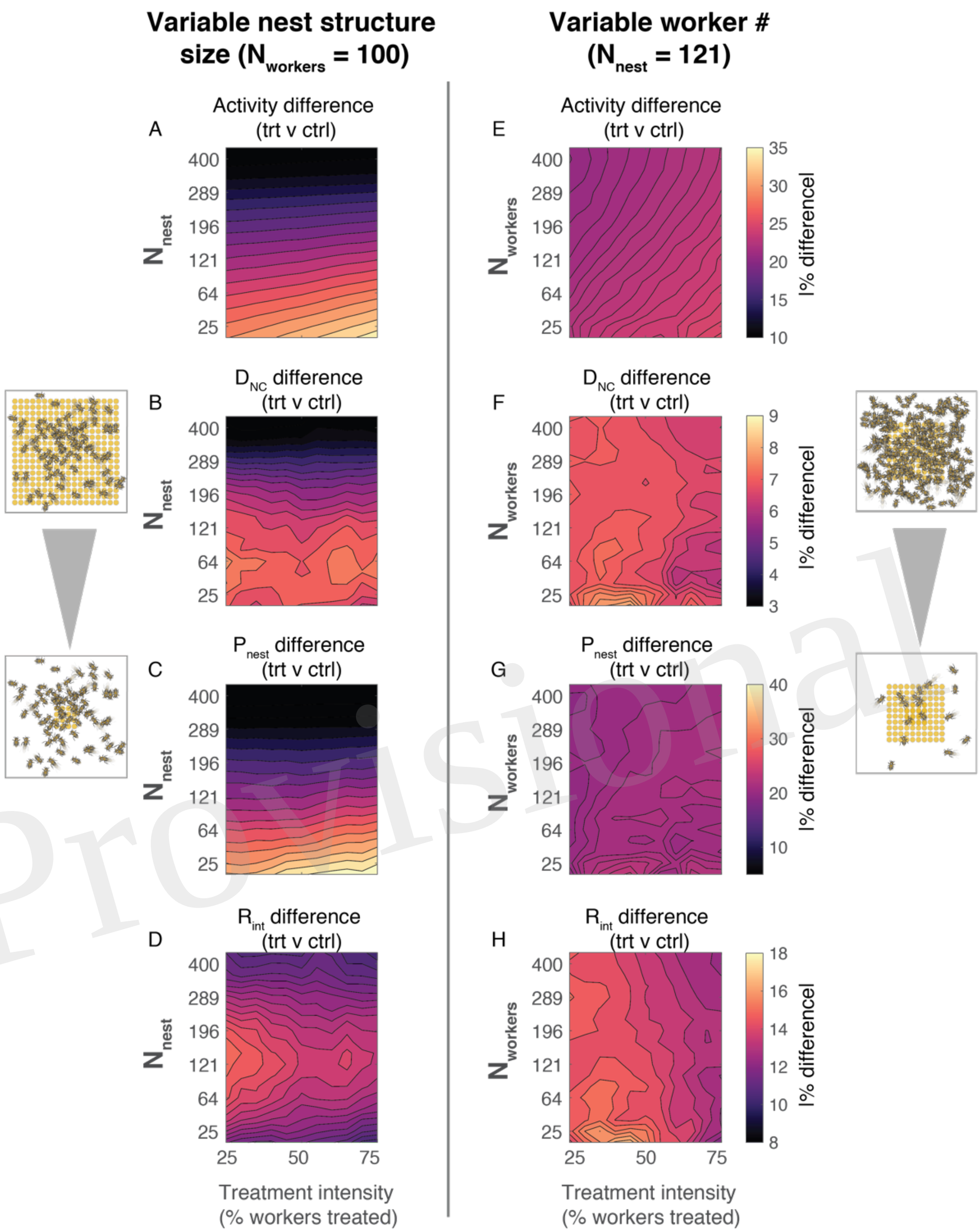


Figure S2. **Independent effects of nest structure size and worker number on relative effect size of imidacloprid.** Relative difference between treated and untreated workers when the number of nest structures (N_{nest}, left column) and number of workers ($N_{workers}$, right column) are independently varied. Data are shown for four metrics: (A, E) activity, (B, F) distance to the nest center (D_{NC}), (C, G) proportion of time spent on the nest structure (P_{nest}), and (D, H) interaction rate (R_{int}). Differences calculated as in Fig 2. The number of simulation iterations at each parameter set was varied with colony size (# of iterations = 10000/*N,* where *N* = # of workers). Phase spaces smoothed using local averaging over adjacent values (smoothing window: +/- 10% for treatment intensity, (sqrt(N-2))^2:(sqrt(N+2))^2 for N_{nest}/$N_{workers}$). For each metric, identical color scales are used for the left and right columns.

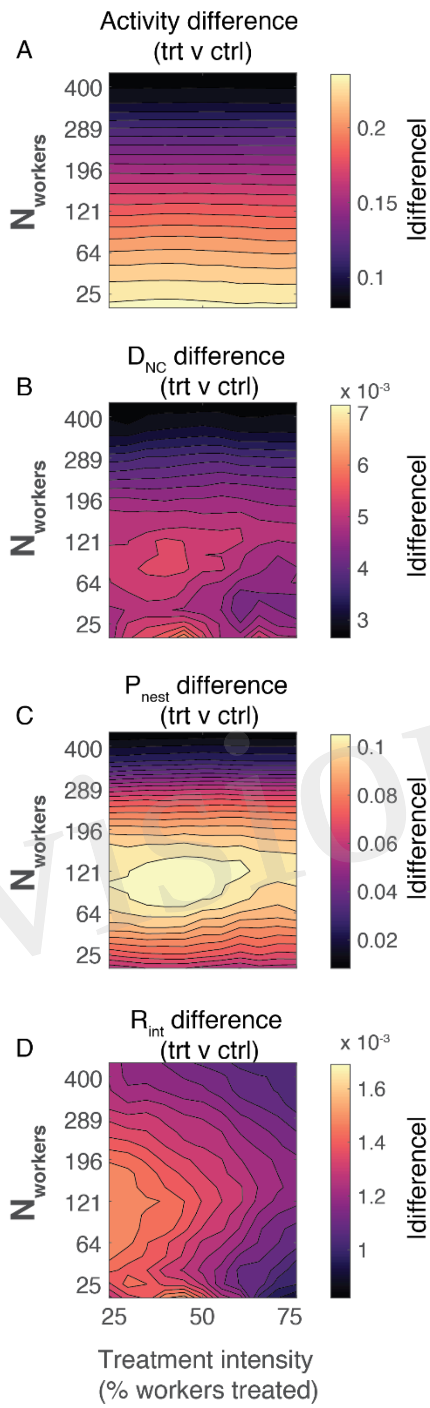
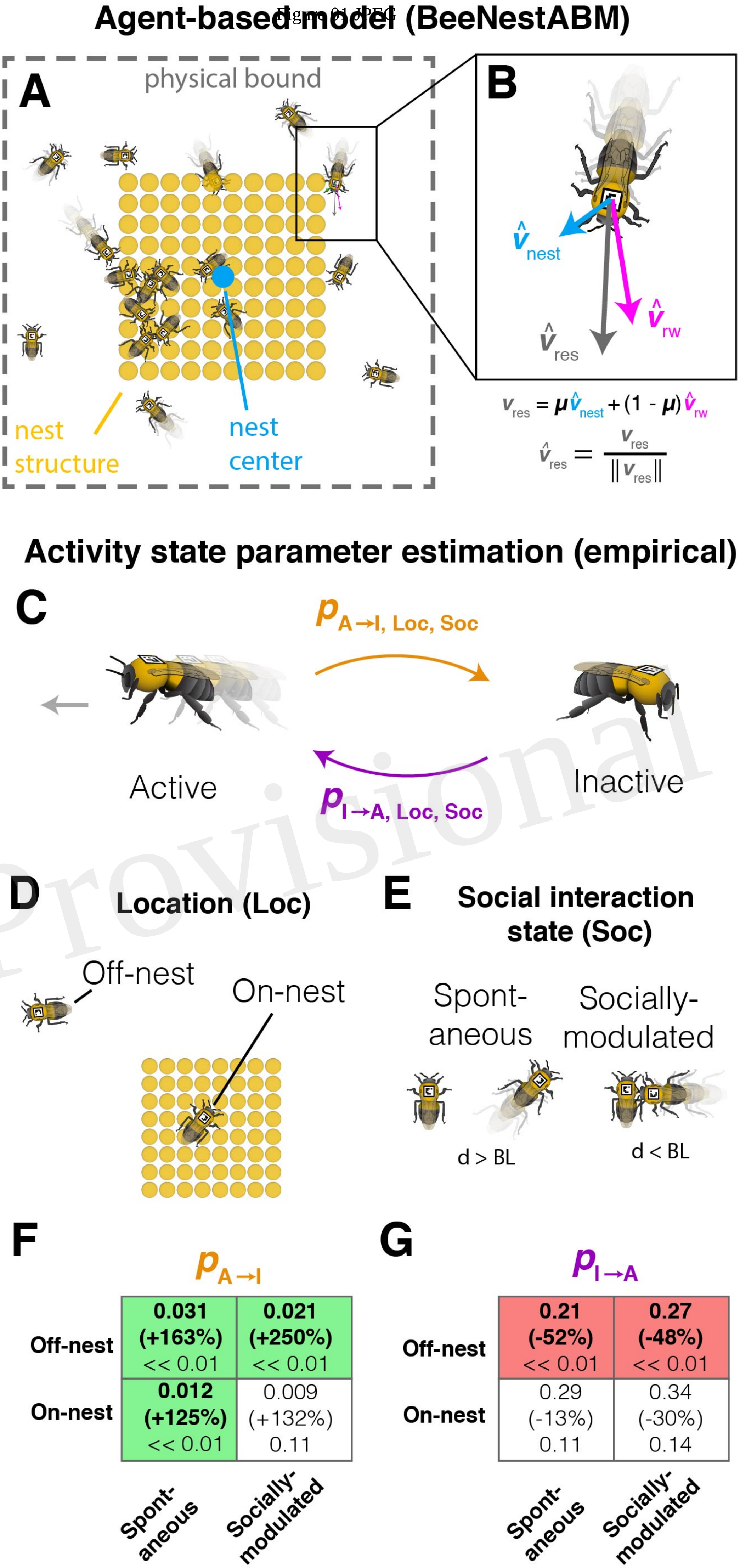


Figure S3. **Absolute differences between treated and control workers across treatment intensity and colony size**. Simulations are the same as in Fig 2**.** Relative differences for (A) activity, (B) distance to the nest center (D_{NC}), (C) proportion of time spent on the nest structure (P_{nest}), and (D) interaction rate (R_{int}) are shown as the absolute difference (rather than % difference in the right column of Fig S2).



Agent-based model (BeeNestABM)
A
physical bound
nest structure
nest center
B
$\hat{v}_{nest}$
$\hat{v}_{rw}$
$\hat{v}_{res}$
$v_{res} = \mu \hat{v}_{nest} + (1 - \mu)\hat{v}_{rw}$
$\hat{v}_{res} = \frac{v_{res}}{\|v_{res}\|}$
Activity state parameter estimation (empirical)
C
$p_{A \to I, Loc, Soc}$
Active
Inactive
$p_{I \to A, Loc, Soc}$
D
Location (Loc)
Off-nest
On-nest
E
Social interaction state (Soc)
Spont-aneous
Socially-modulated
d > BL
d < BL
F
$p_{A \to I}$
Off-nest
0.031 (+163%) << 0.01
0.021 (+250%) << 0.01
On-nest
0.012 (+125%) << 0.01
0.009 (+132%) 0.11
Spont-aneous
Socially-modulated
G
$p_{I \to A}$
Off-nest
0.21 (-52%) << 0.01
0.27 (-48%) << 0.01
On-nest
0.29 (-13%) 0.11
0.34 (-30%) 0.14
Spont-aneous
Socially-modulated

Figure 02.JPEG