



Sensitivity analyses for simulating pesticide impacts on honey bee colonies

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ABSTRACT

We employ Monte Carlo simulation and sensitivity analysis techniques to describe the population dynamics of pesticide exposure to a honey bee colony using the VarroaPop + Pesticide model. Simulations are performed of hive population trajectories with and without pesticide exposure to determine the effects of weather, queen strength, foraging activity, colony resources, and Varroa populations on colony growth and survival. The daily resolution of the model allows us to conditionally identify sensitivity metrics. Simulations indicate queen strength and forager lifespan are consistent, critical inputs for colony dynamics in both the control and exposed conditions. Adult contact toxicity, application rate and nectar load become critical parameters for colony dynamics within exposed simulations. Daily sensitivity analysis also reveals that the relative importance of these parameters fluctuates throughout the simulation period according to the status of other inputs.

1. Introduction

Insect pollinator species richness and diversity has been in decline for a half century (Vanbergen, 2013). Honey bee colonies have increased globally but have shown significant decline in Europe and North America (Spleen et al., 2013; Steinhauer et al., 2014; Lee et al., 2015). Honey bees and wild pollinators are important for increased crop yields through higher quality harvests (Garibaldi et al., 2013) and have been estimated worldwide to support nearly 10% of agricultural production (Gallai et al., 2009). Declines in honey bee populations could potentially lead to unstable yields over time of pollinator-dependent crops (Sinnathamby et al., 2013). Pollination services have been valued at over \$29 billion/year for U.S. agriculture with over half the contribution by honey bees (Calderone, 2012). Multiple stressors have been identified that threaten honey bee health, these include parasites and pests, pathogens, poor nutrition, and pesticide exposure (Goulson et al., 2015; Pettis and Delaplane, 2010). Insecticide effects on honey bee colonies can be through direct mortality, but sublethal exposures leading to adverse outcomes at the hive level also occur (Johnson, 2015). Generally, from a pesticide regulatory perspective,

short-term experiments are used to derive ecological exposure levels that are protective of direct mortality (Steege et al., 2015). However, sublethal effects can have a lower threshold for significant colony level effects and regulatory processes are adapting to address these effects for pollinators (EPA, 2012). Regulatory agencies assess potential risks to honey bees from pesticides through a tiered process that includes individual-based effects data, colony-based assessments under controlled test conditions and less controlled, but more environmentally-relevant conditions where bees forage freely. Model implementation is also tiered. Since extrapolating short-term exposures to colony-level effects over multiple seasons is problematic (Becher et al., 2013), simulation models that estimate stage-based exposure and subsequent effects on colony population dynamics have been identified as an important component of pollinator risk management (Becher et al., 2013). There is a need for a more detailed colony model to inform the design and interpretation of higher-tier studies, interpret the relevance of sublethal and lethal effects and estimate the effect of pesticides in conjunction with other known honey bee stressors (e.g., Varroa mites).

Detailed honey bee simulations for pesticides must consider the structure of the colony, which includes different life stages and castes

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(i.e., eggs, larvae, pupae, drones, workers, foragers, queen), as well as environmentally relevant magnitudes of exposure through direct or indirect routes (stage-specific pollen, nectar, honey, bee bread, and royal jelly consumption). Pesticide exposures include non-target exposure from agricultural use (Johnson et al., 2010) but also intentional use to extirpate hive pests (e.g. miticides). Such a simulation model therefore makes predictions for exposure concentrations and effects for all these combinations and must be compared to available data for these same combinations for verification and validation purposes. There have been a number of studies of managed bee colonies that have demonstrated a range of pesticide residues detected in bees and hive matrices (e.g., honey, pollen, wax). Chauzat et al. (2011) found that within honey bee colony matrices, pollen loads and beeswax had the highest frequency of occurrence of multiple pesticides used either directly within a hive or for agricultural uses. Unintentional exposure to agriculturally applied pesticides was high with a detection rate of > 40%. Honey bees can be lethally impacted by exposure to pesticides but are most likely exposed to concentrations lower than lethal limits. At sublethal levels, pesticide exposure has been associated with changes in individual bee behavior such as reduced foraging efficiency and decreases in colony queen production (Henry et al., 2012; Schneider et al., 2012; Whitehorn et al., 2012; DeGrandi-Hoffman et al., 2013). Overall, the most frequently detected pesticides and the two that occur in the highest quantity are those used by beekeepers to control *Varroa* mites (coumaphos and fluvalinate) (Mullin et al., 2010).

When submitted pesticides fail screening assessment, more realistic and taxa-specific lines of evidence can be requested and evaluated before making a final registration decision, for instance additional empirical exposure and effects data. Requirements for a higher-tier honey bee colony model were identified in Fischer and Moriarty (2014) with a goal of addressing questions that cannot be answered with individual-level tests, semi-field and field studies. In addition, Sponsler and Johnson (2016) identify key components of exposure modeling that are often lacking in population and colony-level models: environmental heterogeneity and in-hive pesticide distribution. The ability to model all possible exposure pathways, from foraging dynamics to intra-colony interactions, is another important requirement. Fischer and Moriarty (2014) included a formal evaluation of existing candidate models that assessed the risks to honey bees from pesticides. None of the existing honey bee models were determined to be currently suitable for regulatory usage because of a variety of issues that included lack of linkage between foragers and surrounding landscape, insufficient testing with empirical data, lack of sensitivity analysis to understand controlling factors, non-incorporation of multiple stressors and insufficient documentation for some of the models. For the USEPA, an existing USDA model is being evaluated (USEPA, 2012, 2014) to simulate honey bee colony dynamics and provide an additional line of evidence for the pesticide evaluation process. VarroaPop is a population model that predicts the population growth and behavior of a honey bee (*Apis mellifera*) colony infested by *Varroa* mites (*Varroa destructor*). The model was developed as an extension of a honey bee population model, BEEPOP, that was created by DeGrandi-Hoffman et al. (1989) to simulate colony dynamics. The modified version of BEEPOP can then be used to translate mite effects on individuals and predict outcomes at the colony level and parameterized for specific environments (Purucker et al., 2007). VarroaPop uses weather conditions, mite population dynamics, and age-structured honey bee colony input parameters to calculate honey bee and mite population growth.

We updated the existing VarroaPop model to predict population growth and behavior by leveraging existing cohort development dynamics. Existing features included daily tracking of colony population size and demographics in which weather conditions, mite population dynamics, and age-structured honey bee colony input parameters informed output. This version of VarroaPop + Pesticide (v3.2.6.11) introduces pesticide treatments to model simulations, in which individuals can be exposed to the active ingredient by physical contact

(i.e., foraging) or ingestion. The model can be used to evaluate risks to honey bee colony survival from pesticide exposure at different times of year and with different weather and colony conditions. The complexities of *Varroa* parasitism and its effects on worker longevity and colony growth also can be included in simulations with pesticide-induced sublethal and lethal effects at each life stage. It also provides a platform for estimating the sublethal effects of pesticide exposure through future enhancements to the model.

We implement Monte Carlo simulations in order to create spatial heterogeneity and to vary in-hive pesticide distribution in exposure scenarios. We use sensitivity analyses to identify parameters that are the most influential (contributing most to output variability) as part of the continuing development of the model. This helps highlight important parameters which may require additional research, allow for the calibration of sensitive parameters to realistically simulate collected data, determine parameters which are less important in order to avoid overparameterization and to assess the relative importance of sub-routines that model elements of hive population dynamics. Sensitivity analysis has been a useful tool which has furthered understanding of other honey bee colony models (Schmickl and Crailsheim, 2007; Becher et al., 2014; Torres et al., 2015). We use the modified version of VarroaPop, VarroaPop + Pesticide, to evaluate temporally a baseline scenario without pesticide exposure and three exposure scenarios representing different application types: foliar application, seed treatment and soil application. The sensitivity analyses are employed at different temporal scales within each application type to conditionally identify important parameters.

2. Methods

2.1. VarroaPop model

VarroaPop was developed as an extension of the BEEPOP colony population dynamics model to determine the effects of *Varroa* mite parasitism on honey bee colony growth and survival. VarroaPop couples mite population growth from reproduction and immigration with colony growth based on queen egg laying rates and worker longevity (DeGrandi-Hoffman et al., 1989; DeGrandi-Hoffman and Curry, 2004; DeGrandi-Hoffman et al., 2016). *Varroa* mites affect colony dynamics by reducing the longevity of adult workers parasitized during development. An overview of the model routine is shown in Fig. 1. The flowchart at the top of the schematic diagram (Fig. 1) represents the overall daily model algorithm.

2.1.1. Queen fecundity and hive dynamics

The complete descriptions of BEEPOP and VarroaPop are available from DeGrandi-Hoffman et al. (1989), DeGrandi-Hoffman and Curry (2004) and DeGrandi-Hoffman et al. (2016). Briefly, colony growth was predicted based on the number of eggs laid per day. Egg laying was determined by the maximum number of eggs the queen can lay per day (a function of queen strength), maximum and minimum temperatures as expressed in heat units, photoperiod, and size of the adult worker population. Queen strength is initially parameterized as a continuous number between 1 and 5 which is used to linearly interpolate between values for the maximum daily number of eggs (1000–3000) and the initial sperm count (1.8–5.5 million). As a queen ages, the number of daily eggs laid by the queen declines as a quadratic function of the number of days the queen has been laying eggs. The proportion of eggs that develop into workers (i.e., fertilized eggs) was determined as a function of photoperiod, colony size, and the amount of sperm in the queen's spermatheca. Workers are categorized as house bees (workers < 21 days old unless specified otherwise) and foragers (workers > 21 days old). Workers do not perform specific tasks in the simulations. Foragers return nectar and pollen to the colony. As the queen ages, the concentration of sperm in the spermatheca is reduced and the probabilities of producing unfertilized eggs that develop into

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