Modelling long-term effects of IGRs on honey bee colonies

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Abstract: Systems have been developed to monitor the direct effects of insect growth regulator (IGR) pesticide exposure on honey bee development, but there has been little work on the longer-term impact of exposure on the colony. A honey bee population model provided the opportunity to investigate the effects of short-term mortality of brood and of sublethal changes in behaviour of the surviving adults on honey bee populations. The model showed that brood mortality alone has limited effect on colony size. There were two mechanisms that could have greater influence on productivity. Precocious foraging in affected adult bees, and hence early loss of brood-rearing (nurse) capabilities, had a much larger effect than expected. Increasing mortality rates by 30% to simulate sublethal effects on lifespan, rather than reduced brood-rearing capability, gave a significantly smaller effect. In order to simulate an effect with the 'shortened lifespan' mechanism as large as that with the 'premature ageing' mechanism, the mortality rate of affected adults had to be increased by 500%. A significant finding from the model is that application of IGRs in spring and early summer could have substantial effects on colony size and viability. Sublethal effects such as precocious foraging can have worse effects than massive brood mortality, as it severely reduces the ability to rear the next generation of nurse bees.

Keywords: IGR; honey bees; long-term; productivity

1 INTRODUCTION

Honey bees (*Apis mellifera* L.) provide an ideal managed system to determine the impacts of pesticide exposure on social insects. They are also economically and environmentally important in their contributions to the pollination of crops and wild flowers. Honey bees are an excellent candidate terrestrial invertebrate monitoring species owing to the ability to move colonies between sites and obtain comparable control data from the same or sister colony. Systems have been developed to monitor the direct effects of insect growth regulator (IGR) pesticide exposure on honey bee development,[1] but there has been little work on the longer-term impact of exposure on the colony. A honey bee population model developed at CSL[2] provided the opportunity to investigate the effects of short-term mortality of brood and of sublethal changes in behaviour of the surviving adults, such as decreased lifespan and precocious foraging,[3] on honey bee populations. This approach also allowed the effect of the timing of exposure on the size of the impact to be modelled. Thus, this project aimed to develop a better understanding of the link between the lethal and sublethal effects of IGRs and impacts at the population level in honey bees.

2 MATERIALS AND METHODS

2.1 Effects on honey bee colonies

The full details of the field trials are given in Thompson *et al*.[4] In summary, fenoxycarb 250 g kg^{-1} WP (Insegar) and diflubenzuron 480 g L^{-1} SC (Dimilin Flo) were obtained from UAP Welburn, York. Test solutions were prepared by diluting the IGRs with 500 g L^{-1} aqueous sucrose to rates equivalent to their maximum application rate (0.6 kg Insegar/200 L = 750 mg AI L^{-1}; 0.3 L Dimilin Flo/500 L = 288 mg AI L^{-1}) on flowering crops. The bee colonies were divided into groups of five colonies, and on day 0 the groups were dosed. Groups of colonies were fed fenoxycarb or diflubenzuron in sucrose, and a control group was fed untreated sucrose. Doses were administered by removing frames of stores from the colonies and placing a glass beaker containing 500 mL treated or control sucrose within the brood chamber. The beaker contained a cork float to allow access to the sucrose. After 1 week the beaker was removed, the volume of any remaining feed was recorded and the frames were replaced.

On the day of dosing, a single frame was selected from the centre of each colony and, using a plastic overlay sheet to locate each cell, 100 brood cells containing eggs were selected. Each cell was marked, and, on day 17 after marking, the cell was uncapped and the pupa removed to observe any abnormalities. This procedure was repeated after 1, 2, 3 and 5 weeks for the 100 eggs. Percentage data were arcsine transformed prior to statistical analysis using ANOVA with treatment $P < 0.05$.

All colonies were generally assessed prior to the day of test item application, every 2 weeks after the

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application until November and then monthly until a year after treatment. Each assessment included counts of the number of adults and brood cells as well as any behavioural or physical abnormalities. The data were analysed using a generalised linear model.

2.2 Honey bee population modelling

An existing honey bee population model[1] was modified to include exposure to IGRs. The model simulates egg laying in the colony, brood development and an adult honey bee population consisting of younger nurse bees and older forager bees. Adult honey bee workers normally change over from brood-rearing work to foraging work at around 3 weeks of age.[5] In the model, the queen's egg-laying rate is determined by a variable curve function simulating seasonal variation. Adult bee mortality is also determined by a seasonally dependent function, set at a level to give a model population with a realistic seasonal cycle, and also a stable population from year to year. An extra feedback mechanism was added to the model so that the size of the brood that could be reared was limited by the number of nurse bees available (such that there were no more than 1.5 larvae per nurse bee).

Effects were determined by two model outputs: the percentage reduction in winter colony size, as reported in Thompson *et al.*,[4] and the percentage reduction in productivity/size of the colony (identified as the loss in adult bees) reported here. It was assumed that colony productivity is directly proportional to the mean number of adult bees in the colony between May and August, when most nectar production in the UK occurs. The month of IGR application was varied between model runs to determine the effects of the timing of application.

3 RESULTS

3.1 Modelling effects of increased brood mortality

As previously reported,[4] in the field study, egg mortality of 40–95% (mean 67%) was observed in the colonies over the 2 weeks after diflubenzuron application, and 45–60% (mean 52%) over the 2 weeks after fenoxycarb application. Therefore, the effects of a mean brood loss of 50 and 100% for 2 weeks were modelled in this study. This provided a representation of the effects observed in the field and an assessment of the sensitivity of the population to effects on brood production.

Modelling the effects of 50% egg mortality for 2 weeks resulted in approximately 20% loss of productivity when applied in June (Fig. 1). The modelled effects of 100% egg mortality for 2 weeks (far greater than that observed in the treated colonies) showed a maximum loss of productivity (numbers of adult bees) of 40–45% when applied in May/June (Fig. 1). This can be compared with the field-collected data,[4] where the recorded brood loss following application in June resulted in control colonies 134% (110–156%) of pretrial numbers of adult bees in July and both fenoxycarb and diflubenzuron colonies 111% (100–120% and 90–133% respectively) of pretrial levels, i.e. 17% smaller.

3.2 Modelling sublethal effects on behaviour

The most severe effects in the field study[4] were shown by fenoxycarb, a juvenile hormone analogue, which not only affected the colony viability in the short term, by leading to brood mortality and decreasing colony size, but also affected the ability of the colony to overwinter. Changes in colony size, shown here as productivity, may also be due to sublethal effects on the adult bees by decreasing longevity or by inducing precocious foraging.[6] In the model, these sublethal effects were simulated in two ways:

1. By shortening the time that a nurse bee can produce brood food and rear brood before converting to forager status (precocious foraging mechanism). This was simulated by reducing the mean number of larvae that can be reared per nurse bee (the L/N ratio).
2. By increasing the mortality of affected adults (shortened lifespan mechanism). This was simulated by increasing mortality rates by a multiplication factor.

Since fenoxycarb can persist for at least 2.5 months[7] and the effects of fenoxycarb exposure have been reported for 2–3 months after treatment,[8] sublethal

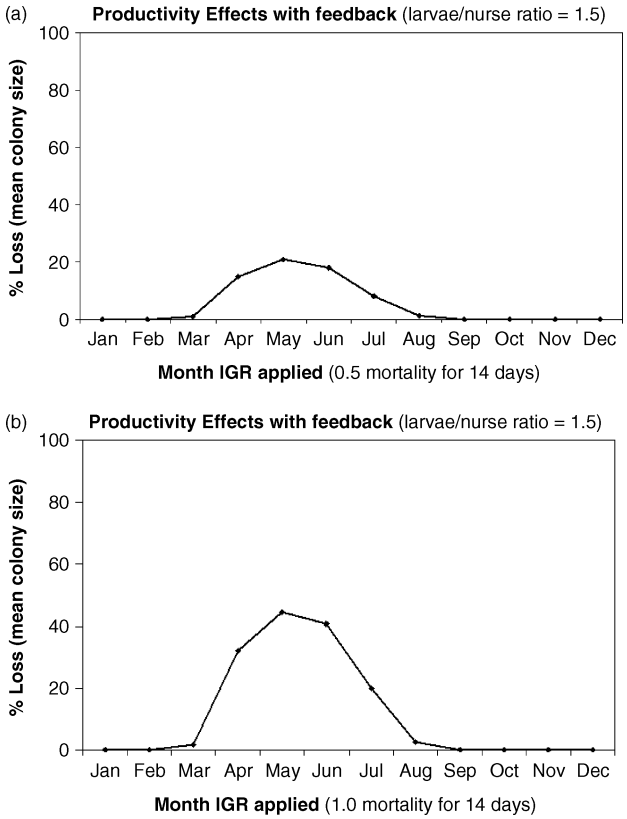

Figure 1. Effects of brood loss on the productivity/size of colonies according to the timing of exposure: (a) 50% mortality for 14 days; (b) 100% mortality for 14 days.

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effects lasting 2.5 months from initial IGR application were simulated.

3.3 Effects of reduced lifespan

Juvenile hormone (JH) is involved in the regulation of behavioural development in honey bees.[9] JH titres increase with age, they are low in bees that work in the hive and high in bees that forage and are involved in colony defence. Jaycox *et al*.[10] showed that bees treated with a JH mimic could not develop their hypopharangeal glands and started to move out of the brood nest to guard the colony and forage, and the lifespan of treated bees was reduced by 39%. The decreased lifespan was simulated by increasing the mortality rate of adult bees emerging for 2.5 months after treatment. Three increased rates were used, 1.3-, 1.5- and 5-fold increases in mortality. The 1.3- and 1.5-fold increases decreased the mean colony size by a maximum of approximately 10% in May when overlaid on the 50% brood loss, and the 5-fold increase in mortality decreased the colony size by an additional maximum of 40% in May (Fig. 2). This means that a very large decrease in longevity would be required to result in a significant reduction in the size of the colonies.

3.4 Effects of precocious foraging

Treating bees with JH induces precocious foraging, and removal of the corpora allata (the glands that produce JH) delays bees in developing into foragers.[11] Thus, it appears likely that exposure to the JH analogue fenoxycarb may induce precocious foraging in the exposed individuals and reduce the number of nurse bees available to rear brood. To simulate the impact of reducing the number of nurse bees, the model was adapted to reduce the number of larvae that could be reared by each nurse bee from 1.5 to 0.5 for bees that emerged for 2.5 months after treatment. This showed that reducing the L/N ratio to 1 resulted in a 50% reduction in mean colony size in April, and reducing it further to 0.5 resulted in a 75% reduction in mean colony size (Fig. 3). Exposure earlier in the season had a larger impact than from June onwards. The additional influence of a 30% increase in mortality rate (equivalent to a 30% reduction in lifespan) had very little additional influence on colony size (Fig. 4).

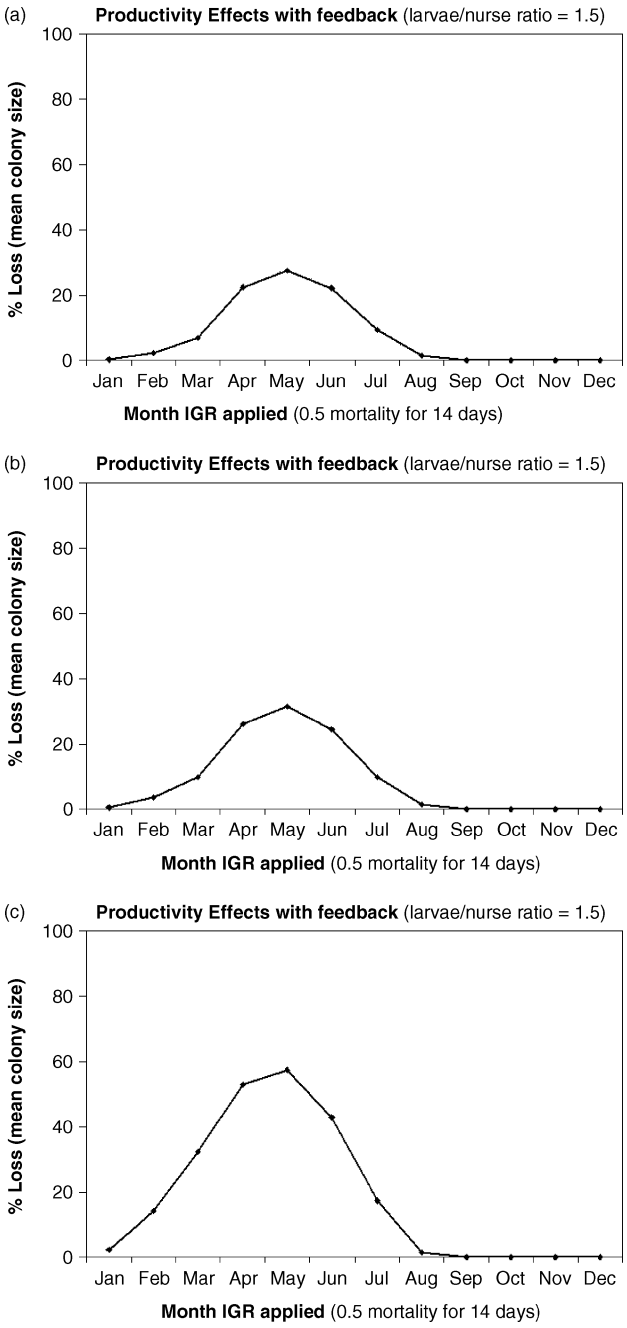

Figure 2. Effects of IGRs on the productivity/size of colonies according to month of exposure (50% brood loss applied for 14 days after exposure): (a) 1.3-fold, (b) 1.5-fold and (c) 5-fold increase in the mortality of adult honey bees (equivalent to a reduction in the lifespan of exposed individuals) for emerging adult bees for 2.5 months after treatment.

4 DISCUSSION

In spite of various assumptions, the model has shown that brood mortality alone has limited effect on colony size. There are two mechanisms that could have greater influence on productivity. The first mechanism, precocious foraging in affected adult bees and hence early loss of brood-rearing (nurse) capabilities, had a much larger effect than expected, although as yet there are no field data to indicate how much the L/N ratio might be affected. The second mechanism, shortened lifespan of affected adult bees, had a smaller effect unless it was very severe, and so is perhaps less likely to be the explanation of observed field effects in terms of overwintering size.[4] Increasing mortality rates by 30% to simulate sublethal effects on lifespan, rather than reduced brood-rearing capability, gave a significantly smaller effect. In order to simulate an effect with the 'shortened lifespan' mechanism as large as that with the 'premature ageing' mechanism, the mortality rate of affected adults had to be increased by 500%.

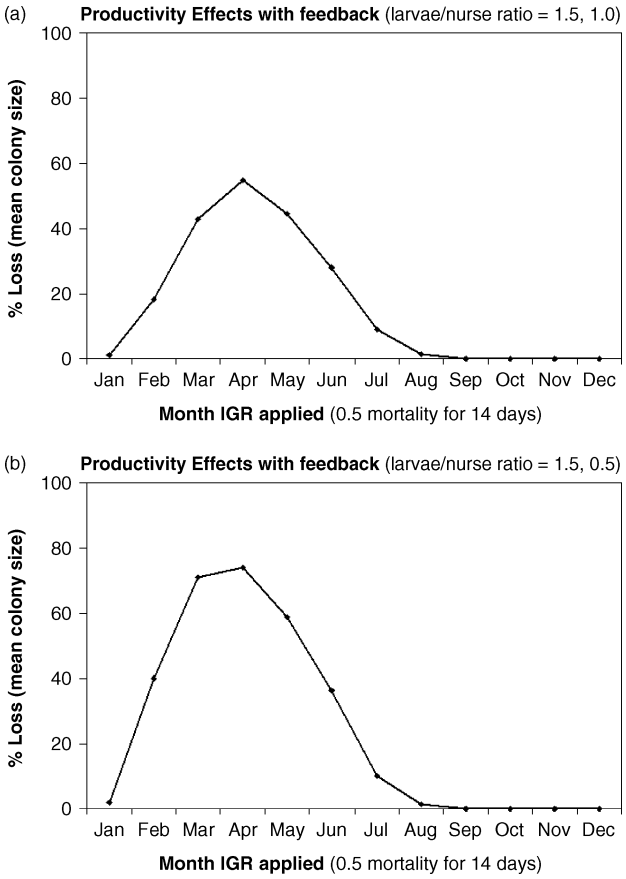

Figure 3. Effects of IGRs on the productivity/size of colonies according to month of exposure (50% brood loss applied for 14 days after exposure). Reducing the larvae/nurse ratio from 1.5 to (a) 1.0 and (b) 0.5 for emerging adult bees for 2.5 months after treatment.

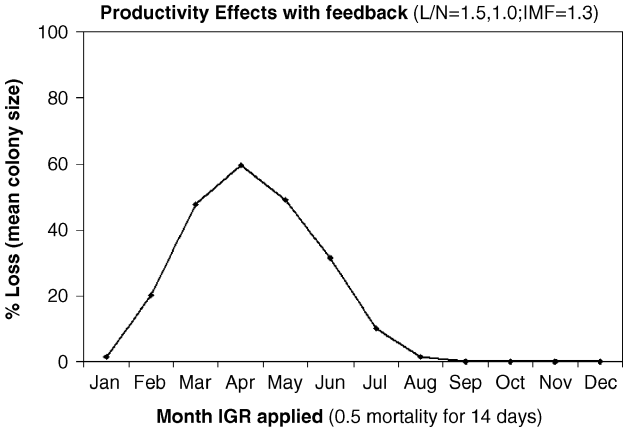

Figure 4. Effects of a 1.3-fold increase in the mortality of adult honey bees (equivalent to a reduction in the lifespan of exposed individuals) and change in the L/N ratio to 0.5 for emerging adult bees for 2.5 months on the productivity/size of colonies according to time of exposure (50% brood loss applied for 14 days after exposure).

A significant finding from the model is that application of IGRs in spring and early summer could have substantial effects on colony size and viability. Sublethal effects such as precocious foraging can have worse effects than massive brood mortality, as it severely reduces the ability to rear the next generation of nurse bees.

There are very few studies of the long-term impact of pesticides on honey bee colonies. This is a particular concern for IGRs, which are not acutely toxic to adult bees but affect brood and may also have sublethal effects on emerging adult bees, e.g. behaviour. This study has shown significant effects of some IGRs on the longer-term viability of honey bee colonies, although it should be noted that the exposure levels were far higher than they would be following application to a crop. Short-term effects on brood mortality may appear to recover within 4–6 weeks, but there may also be longer-term sublethal effects on emerging adults. The effects of IGRs on populations of non-target invertebrates is dependent on their reproductive rate,[12] and therefore these types of compound may have a more significant impact on species with lower reproductive rates, such as bumblebees which would be less affected by reduced lifespan, as they do not overwinter.

REFERENCES

1 Oomen PA, de Ruijter A and van der Steen J, Method for honey bee brood feeding test with insect growth regulating insecticides. *EPPO Bulletin* **22**:613–616 (1992).
2 Wilkinson D and Smith GC, A model of the mite parasite, *Varroa destructor*, on honeybees (*Apis mellifera*) to investigate parameters important to mite population growth. *Ecol Model* **148**:263–275 (2002).
3 Thompson HM, Behavioural effects of pesticides in bees – their potential for use in risk assessment. *Ecotoxicology* **12**:317–330 (2003).
4 Thompson HM, Wilkins S, Battersby AH, Waite RJ and Wilkinson D, The effects of four insect growth regulating (IGR) insecticides on honeybee (*Apis mellifera* L.) colony development, queen rearing and drone sperm production. *Ecotoxicology* **14**:757–769 (2005).
5 Graham JM (ed.) *The Hive and the Honey Bee*. Dadant and Sons, Hamilton, IL (1992).
6 Robinson GE and Ratnieks FLW, Induction of premature honey bee, Hymenoptera Apidae, flight by juvenile hormone analogs administered orally or topically. *J Econ Entomol* **80**:784–787 (1987).
7 Tomlin CDS (ed.) *The Pesticide Manual*, 13th edition. BCPC, Alton, Hants, UK (2002).
8 Tasei J-N, Effects of insect growth regulators on honey bees and non-*Apis* bees. A review. *Apidologie* **32**:527–545 (2001).
9 Robinson GE and Vargo EL, Juvenile hormone in the social Hymenoptera: gonadotrophin and behavioural pacemaker. *Arch Insect Biochem Physiol* **35**:559–583 (1997).
10 Jaycox ER, Skowronek W and Guynn G, Behavioral changes in worker honey bees (*Apis mellifera*) induced by injections of juvenile hormone mimic. *Ann Entom Soc Am.* **67**:529–534 (1974).
11 Huang Z-Y, Plettner E and Robinson GE, Effects of social environment and worker mandibular glands on endocrine-mediated behavioural development in honey bees. *J Comp Physiol A* **183**:143–152 (1998).
12 Stark JD, Tanigoshi L, Bounfour M and Antonelli A, Reproductive potential: its influence on the susceptibility of a species to pesticides. *Ecotox Environ Safety* **37**:273–279 (1997).

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