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A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees

James E. Cresswell

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Abstract Honey bees provide important pollination services to crops and wild plants. The agricultural use of systemic insecticides, such as neonicotinoids, may harm bees through their presence in pollen and nectar, which bees consume. Many studies have tested the effects on honey bees of imidacloprid, a neonicotinoid, but a clear picture of the risk it poses to bees has not previously emerged, because investigations are methodologically varied and inconsistent in outcome. In a meta-analysis of fourteen published studies of the effects of imidacloprid on honey bees under laboratory and semi-field conditions that comprised measurements on 7073 adult individuals and 36 colonies, fitted dose–response relationships estimate that trace dietary imidacloprid at field-realistic levels in nectar will have no lethal effects, but will reduce expected performance in honey bees by between 6 and 20%. Statistical power analysis showed that published field trials that have reported no effects on honey bees from neonicotinoids were incapable of detecting these predicted sublethal effects with conventionally accepted levels of certainty. These findings raise renewed concern about the impact on honey bees of dietary imidacloprid, but because questions remain over the environmental relevance of predominantly laboratory-based results, I identify targets for research and provide procedural recommendations for future studies.

Keywords Agrochemicals · Ecosystem services · Pollination · Pollution · Sustainability · Toxicology

J. E. Cresswell (✉)
School of Biosciences, University of Exeter, Hatherly Laboratories, Prince of Wales Road, Exeter EX4 4PS, UK
e-mail: j.e.cresswell@ex.ac.uk
URL: http://biosciences.exeter.ac.uk/staff/index.php?web_id=james_cresswell

Introduction

Chemical insecticides are important to crop productivity in intensive farming systems, where they preserve about one-fifth of crop yield (Oerke and Dehne 2004). Insecticide use is widespread and routine in the major staple crops (e.g. cereals, soybeans, maize) and in many fruit and vegetable crops (Ragsdale 2006). When sprayed, insecticides can cause unintended harm by killing beneficial insects, such as pollinators and the natural enemies of the crop's pests, both inside the target field (Croft 1990) and outside by drift (de Jong et al. 2008). One ameliorative measure is to avoid spraying and, instead, to apply the insecticide as a seed dressing. Plants take up the chemical on germination and distribute it systemically, so that the insecticide is delivered specifically to pests that consume crop tissues. However, some beneficial insects still can be harmed by this approach, because systemic insecticides can be present at 'trace' levels (defined here as a range of 1–10 μg insecticide kg^{-1}) in the pollen and nectar of plants such as sunflower, *Helianthus annuus* L. (Bonmatin et al. 2003; Schmuck et al. 2001), oilseed rape, *Brassica napus* L. (Bonmatin et al. 2005) and *Phacelia tanacetifolia* Benth. (Wallner et al. 1999 cited in Decourtye et al. 2003), which bees consume (Rortais et al. 2005). Potentially, bees could be exposed at a large scale to trace dietary insecticides originating from crop seed dressings. For example, in the UK alone, 80 million bees could intake insecticides each year by foraging on treated oilseed rape, because the average density of honey bees (*Apis mellifera* L.) and bumble bees (*Bombus* spp.) in UK fields is one bee per 77 m^2 (Hoyle et al. 2007) and the annual crop area is approximately 6×10^5 ha (DEFRA 2009). In the United States, insecticide residues are widespread in pollen sampled from honey bee hives (Mullin et al. 2010). Consequently,

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establishing whether trace dietary insecticides harm bees is important if we are sustainably to manage pollination services to economically important crops and wild plants (Kremen and Ricketts 2000; Potts et al. 2010).

The neonicotinoid insecticides, which include imidacloprid, clothianidin, thiamethoxam and thiacloprid, are among the most important chemicals in crop protection (Elbert et al. 2008) and they are widely used in seed dressings (Sur and Stork 2003). Neonicotinoids are neurotoxins that act as agonists of insect nicotinic acetylcholine receptors and are lethal through disruption of the insect nervous system (Matsuda et al. 2001). Concerns over unintended impacts of some neonicotinoids on bees have led to government restrictions in France (Bonmatin et al. 2005; Schmuck et al. 2001) and Germany (BVL 2010), but these have largely been responses to the inadvertent release of insecticidal dust during seed drilling (Greatti et al. 2006). However, an important issue is whether systemic neonicotinoids have detrimental effects on bees through their presence at trace levels in nectar and pollen.

Two kinds of detrimental effect of dietary insecticides can be measured: lethal and sublethal effects. A lethal effect is manifested as an increased rate of mortality among dosed bees compared to undosed controls. A sublethal effect is manifested in either dosed colonies or individuals by modified performance in aspects of growth, fecundity, longevity, or behaviour (Desneux et al. 2007). For colonies of honey bees, indices of performance include rate of increase in colony mass (Faucon et al. 2005), activity at either the hive entrance (Decourtye et al. 2004b) or a remote feeder (Schmuck et al. 2001), and quantity of brood produced (Faucon et al. 2005). For individual bees, indices include learning ability (Decourtye et al. 2003), rate of food uptake (Ramirez-Romero et al. 2008) and level of locomotory activity (Lambin et al. 2001).

There are significant uncertainties about the magnitudes of both lethal and sublethal effects on honey bees caused by trace dietary neonicotinoids. In laboratory studies of lethal effects, the single oral dose that kills 50% of dosed individuals, or LD_{50}, is about one hundred times higher than the estimated daily ingestion of neonicotinoids by nectar-foraging honey bees (Rortais et al. 2005), which may suggest that systemic neonicotinoids in the field are not lethal to honey bees. However, single doses may be unrealistic, because mass flowering crops, such as oilseed rape, bloom over several weeks and foraging bees are likely to ingest nectar repeatedly. Honey bees have suffered increased mortality after multiple ingestions of trace dietary neonicotinoid at field-realistic levels (Suchail et al. 2001), but the generality of these findings is contested (Schmuck 2004). In laboratory studies of sublethal effects, the emergence of a clear picture of the potency of imidacloprid previously has been hindered by the wide variety of performance indices that have been measured and inconsistency in experimental outcomes among studies. Below, I report a quantitative survey of the literature on laboratory and semi-field studies of lethal and sublethal responses of honey bees to doses of a neonicotinoid, imidacloprid. Trace dietary intake of each of several neonicotinoids is known to affect honey bees (e.g. El Hassani et al. 2008), but I focused on imidacloprid because it is extensively used in agriculture and, among the neonicotinoids, its effect on honey bees has been the most studied.

The effects of imidacloprid on honey bees have previously been a topic of review (Maus et al. 2003), but only qualitative methods were employed. Here, I subjected data from published laboratory studies to some standard quantitative techniques of meta-analysis (Glass 1976), which is a well-established statistical methodology used to find generalities in collections of studies that have varied or conflicting outcomes (Gurevitch and Hedges 1999). After excluding anomalous studies, I fitted dose–response relationships to estimate the expected magnitudes of the effects on honey bees of field-realistic doses of trace dietary imidacloprid. The meta-analysis shows that while imidacloprid at field realistic levels is not lethal to honey bees, it has sublethal effects. This finding raises the question of why similar sublethal effects have not been detected by field trials in which honey bee colonies were exposed to neonicotinoids (Cutler and Scott-Dupree 2007). In any experiment that culminates in statistical testing, a treatment effect can go undetected because the test lacks sufficient discriminatory power to detect it, which is called a type II error. I therefore evaluate the statistical power of field and semi-field trials that have reported no sublethal effects of neonicotinoids on honey bee colonies.

Materials and methods

I collected data from published reports of laboratory and semi-field investigations into the effects of imidacloprid on honey bees. From each report, I extracted the dosing regime, the levels at which imidacloprid was dosed, and the quantitative measures of responses by bees.

Dosing regimes were classified as either ‘acute’ or ‘chronic’. Under acute regimes, bees were normally dosed by oral ingestion of a single volume of syrup containing a defined concentration of imidacloprid, but in a minority studies the dose was topical. Under chronic regimes, bees had ad libitum access to a syrup feeder with a defined concentration of imidacloprid for at least 6 days before responses were measured. Doses were converted to consistent units using a table of mass-volume relationships in sucrose solutions (Weast and Astle 1982). Where provided (Decourtye et al. 2003; Faucon et al. 2005; Schmuck et al.

2001), I used the analytically determined concentration of imidacloprid in the syrup, rather than the nominal concentration, but analytically determined values were reliably estimated by nominal doses in the range 1.5–48 μg kg^{-1} (regression analysis: determined dose = (1.04 × nominal dose) + 1.45, r-squared = 0.97, $n = 8$). Responses were classified as either 'lethal' or 'sublethal'. The unit of lethal responses is percentage mortality among the bees of a treatment group. As a common unit for sublethal responses, I calculated the 'response ratio', R, which is the ratio of mean performance in the dosed and control groups (Hedges et al. 1999) and $R = 1$ indicates no effect of dosing. Normally, the performance of the dosed group was taken as the numerator of the ratio, but in a few cases I used the dosed group to provide the denominator, such as when imidacloprid was associated with a slower behavioural response. When several sublethal responses were measured on the same group of colonies, I calculated R for each response and then used the mean of these R values.

The two binary categorizations, acute vs. chronic doses and lethal vs. sublethal responses, produced four categories of data. In each category, I fitted a dose–response relationship by least-squares regression. I applied two standard techniques of meta-analysis (Gurevitch and Hedges 1999): (1) weighting of the data to account for variation among studies in statistical reliability; and (2) bootstrapping by Monte Carlo resampling to determine confidence intervals for the fitted relationships. In fitting weighted least-squares regressions, the quantity minimized is $\Sigma_i (w_i \times d_i^2)$, where d_i is the difference between the ith observed response and that predicted by the model and w_i is the weight accorded to that datum (Crawley 2007). Weighting makes statistically reliable studies count more than less reliable studies and, conventionally, each datum is weighted in proportion to the reciprocal of its sampling variance (Crawley 2007; Gurevitch and Hedges 1999), but to do so in my dataset would, in effect, censor studies with the greatest sampling variation. For greater equitability, I therefore weighted each datum in proportion to the reciprocal of its standard error.

To find bootstrap confidence intervals for each of the four dose–response regression relationships, I used Monte Carlo resampling to simulate 1000 new instances of each datum. When resampling lethal responses, I used the original sample sizes and the fate of each simulated bee was randomly determined with the probability of death equal to p, where p was the observed proportion of bees suffering mortality in the original treatment group. In resampling sublethal responses, each value of the response ratio, R, was determined from two random samples from normal distributions, one for the control group and one for the dosed group, whose means and standard deviations took the appropriate observed values, but with one proviso. In a few cases, the Monte Carlo procedure produced unrealistically large values of R, which arose when statistical error allowed a very small value for the ratio's denominator. In these cases, the value of the denominator was constrained to be no less than one tenth of the observed mean of the ratio's numerator. Where the original study had made multiple measurements on the same colonies, I calculated the mean response ratio for each simulated colony after each measurement had been bootstrapped. Within each of the four categories of data, the Monte Carlo results were assembled into 1000 data sets and a dose–response relationship was fitted to each by regression. The 95% bootstrap confidence interval was given by the 2.5 and 97.5 percentiles among the 1000 fitted values at each dose.

I fitted three different forms of dose–response curve by regression. For lethal responses in acute dosing regimes, the observed data suggested a sigmoidal relationship (Fig. 1a), so I fitted a four parameter logistic, $mortality = a + [(b - a)/(1 + \exp(c - \ln(\text{dose})/d))]$, because it allows a non-zero y-intercept to accommodate 'background' mortality in zero-dose (control) subjects and it required no a priori assumptions about the symmetry of the curve. For lethal responses in chronic dosing regimes, the doses required for minimum and maximum mortality were seldom tested, so the sigmoidal dose–response relationship lacked its asymptotes (Fig. 1b). I therefore fitted a linear relationship: $\text{mortality} = a \times (\text{dose}) + b$. For sublethal responses indexed by the response ratio (Fig. 2a, b), I fitted an exponential decay from unity: $R = 1 - a \times \exp(b \times \ln(\text{dose}))$, which assumes an equal performance in control and experimental groups at zero dose and allows for either an accelerating or decelerating decline in R with increasing dose. For lethal responses, the fitted relationships were used to estimate the expected LD_{50} for acute dosing regimes and, in chronic regimes, the LC_{50} (the expected concentration of dosed imidacloprid associated with death of 50% of the bees feeding on it). For sublethal responses, the fitted relationships were used to estimate the RD_{50} for acute dosing regimes (the expected dose at which the response ratio was $R = 0.5$) and, in chronic regimes, the RC_{50} (the expected concentration of dosed imidacloprid at which $R = 0.5$). All meta-analyses were implemented using **R** statistical software (Ihaka and Gentleman 1996).

Determination of field-realistic doses

Imidacloprid has been assayed in nectar from sunflowers (Decourtye et al. 2003; Schmuck et al. 2001), in nectar from oilseed rape (Bonmatin et al. 2005) and in the honey sacs of honey bees foraging for nectar on *P. tanacetifolia* (Wallner et al. 1999 cited in Decourtye et al. 2003). Based on these reports, the field-realistic range of imidacloprid concentrations is assumed to be 0.7–10 μg L^{-1}. Given this range in concentrations, I estimated the field-realistic range

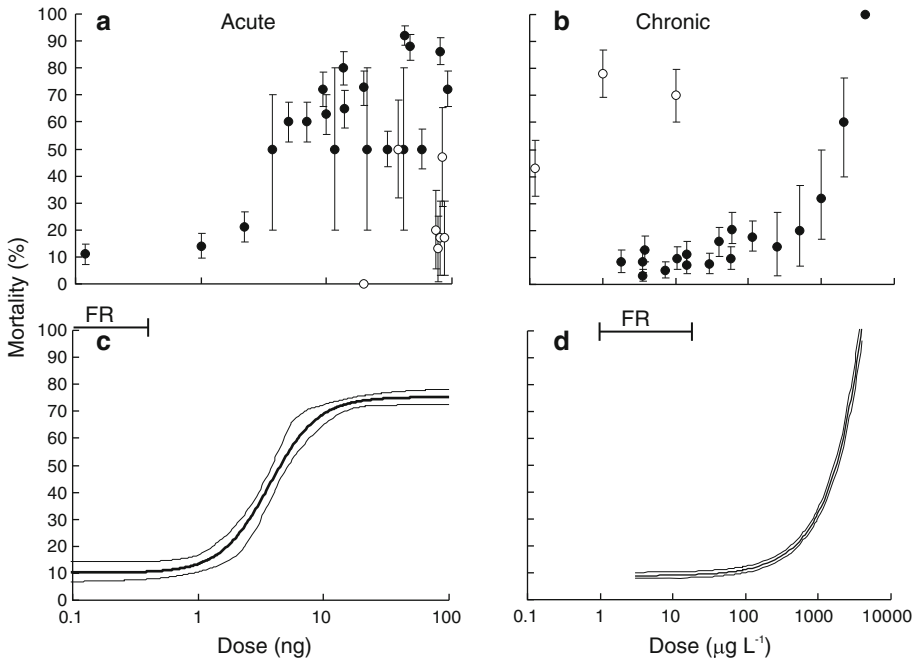

Fig. 1 Observed mortality rates (*y*-axis: percentage killed) in groups of honey bees that intake imidacloprid under: **a** acute dosing regimes: *x*-axis = ng imidacloprid; *closed symbols* indicate data from 2700 adult worker bees (Decourtye et al. 2003, 2004a; Schmuck et al. 2001; Suchail et al. 2000, 2001), *open symbols* indicate excluded data (Nauen et al. 2001); or **b** chronic dosing regimes: *x*-axis: concentration of imidacloprid in feeder syrup in μg L^{-1}; *closed symbols* indicate data from 2490 adult worker bees (Decourtye et al. 2004a; DEFRA 2007), *open symbols* indicate anomalous data (Suchail et al. 2001); *error bars* indicate 95% confidence intervals. Fitted dose–response relationship from weighted least-squares regression based on data in the *panels above*: **c** the *thick line* indicates mortality = 10.2 + [(75.3 − 10.2)/1 + exp (0.567 × (0.194 − ln (dose)))]; *r*-squared = 0.81; **d** mortality = (0.023 × dose) + 8.8; *r*-squared = 0.92; the *thin lines* indicate the bootstrapped 95% confidence intervals. The *capped horizontal lines* labeled '*FR*' indicate the field-realistic range of imidacloprid in nectar

of the amount of imidacloprid in an average nectar load to be 0.024–0.3 ng under the following assumptions: that a honey bee ingests an average nectar load of 40 mg (Ribbands 1953), that the nectar with the highest imidacloprid concentration, that of *P. tanacetifolia*, has a maximum sugar concentration of 800 g L^{-1} (Petanidou 2003); and that the nectar with the lowest imidacloprid concentration, that of oilseed rape, has a sugar concentration of 470 g L^{-1} (Cresswell 1999). The amounts of imidacloprid ingested by adult honey bees through consuming pollen are smaller than those consumed in nectar (Rortais et al. 2005), so the preceding range of field realistic dose based on nectar subsumes the range for pollen. Using the dose–response relationships fitted in the meta-analysis, I estimated the expected performance levels of honey bees after doses of dietary imidacloprid at levels measured in sunflower (acute dose = 0.13 ng, chronic dose = 4.3 μg L^{-1}) (Decourtye et al. 2003) and oilseed rape (acute dose = 0.023–0.03 ng, chronic dose = 0.7–1.3 μg L^{-1}) (Bonmatin et al. 2005).

Power analysis

The probability that a statistical test makes a type II error is denoted β and statistical power is defined as $(1 - \beta)$ (Crawley 2007). Conventionally, statisticians require that the power of a test to detect a treatment effect of a specified magnitude is $(1 - \beta) = 0.8$ (Crawley 2007) and the evaluation of statistical power is essential when interpreting tests that fail to detect an effect. The power of a particular test depends on two factors: (1) the intrinsic variability in response among the experimental subjects; and (2) the sample sizes of the treatment groups. To establish the intrinsic variability of undosed honey bee colonies, I collected data from four published investigations of sublethal effects of neonicotinoids on honey bee colonies under semi-field (Colin et al. 2004; Faucon et al. 2005; Schmuck et al. 2001) and field conditions (Cutler and Scott-Dupree 2007). I extracted the mean and standard deviations of response variables measured on groups of colonies in the undosed treatment, and their underlying sample sizes. I described the level of variability in the response variables by calculating the coefficient of variation (the ratio of the sample's standard deviation to its associated mean × 100), denoted *CV*. Based on its *CV* and sample size, I determined the power of the *j*th field trial, denoted $(1 - \beta_j)$, to detect the largest expected reduction in performance, denoted ΔP, estimated by the fitted dose–response relationships to arise from dietary imidacloprid in the field-realistic range, i.e.

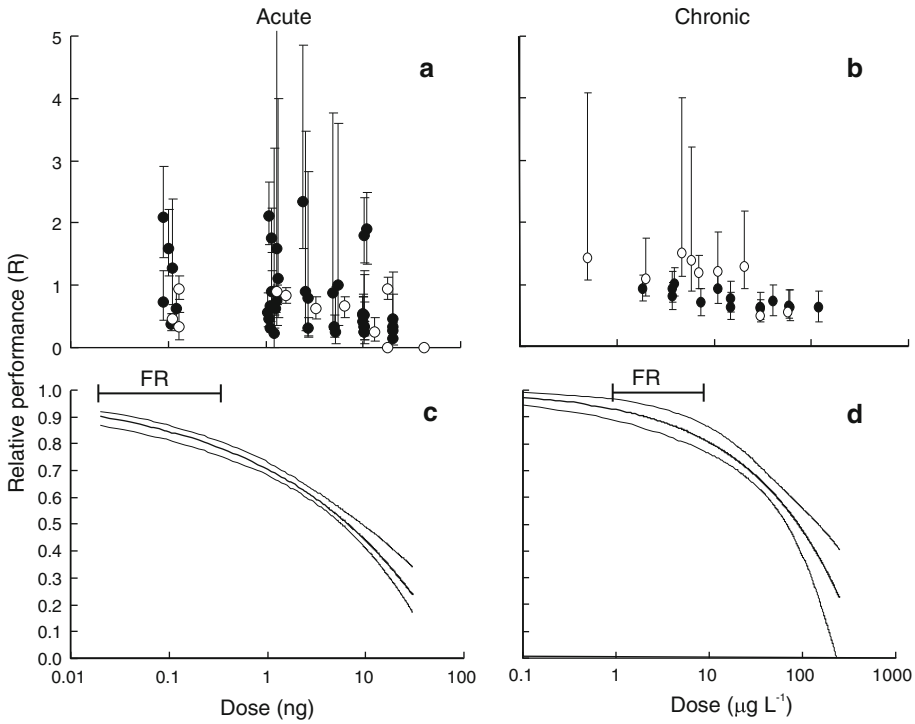

Fig. 2 Observed performance by dosed honey bees that intake of imidacloprid (*y*-axis: mean response ratio, *R%*, of dosed and control subjects) in groups of honey bees that intake imidacloprid under: *a* acute dosing regimes: *x*-axis = ng imidacloprid; data from 1409 adult worker bees (Bortolotti et al. 2003; Decourtye et al. 2004a; Guez et al. 2001; Lambin et al. 2001; Yang et al. 2008), *closed symbols* indicate topical dose, *open symbols* indicate oral dose, some data points have minor adjustments parallel to *x*-axis to allow discrimination of *error bars*; or **b** chronic dosing regimes: *x*-axis: concentration of imidacloprid in feeder syrup in μg L^{-1}; data from 474 adult worker bees and 36 colonies (Colin et al. 2004; Decourtye et al. 2003, 2004b; Faucon et al. 2005; Ramirez-Romero et al. 2005; Schmuck et al. 2001), *closed symbols* indicate measurements made on individual bees and *open symbols* indicate measurements on colonies, *error bars* indicate 95% confidence intervals. Fitted dose–response relationship from weighted least-squares regression based on data in the panels above: **c** the *thick line* indicates $R = 1 - 0.29 \times \exp(0.280 \times \ln(\text{dose})$; r-squared = 0.67; **d** $R = 1 - 0.06 \times \exp(0.478 \times \ln(\text{dose})$; r-squared = 0.35; the *thin lines* indicate the bootstrapped 95% confidence intervals. The *capped horizontal lines* labeled '*FR*' indicate the field-realistic range of imidacloprid in nectar

$\Delta P = 20\%$ (see "Results and discussion" section). I evaluated the power of statistical comparisons of performance in control and dosed colony groups conducted by a one-tailed *t*-test using the *power.t.test* command in **R**. The one-tailed test is a more powerful discriminator than a two-tailed test and its use is valid here because it is reasonable to expect imidacloprid only to reduce honey bee performance. When four tests have the same sample sizes and background variability among experimental subjects as the published field trials, the probability that they all mistakenly accept the null hypothesis when $\Delta P = 20\%$, denoted M_{20}, is given by the product of the Type II error in each test, β_j, i.e. $M_{20} = \prod_{j=1}^{j=4} \beta_j$.

Results and discussion

The meta-analysis included data from tests of the effects of imidacloprid on 7073 individual honey bees and 36 colonies, which were reported in 13 peer-reviewed articles (see legends in Figs. 1 and 2) and one report by a regulatory agency (DEFRA 2007). I did not include data from several other potentially relevant studies for the following reasons. One had a dosing regime that could not be classified as either acute or chronic by my definitions (Ramirez-Romero et al. 2008), one had levels of dose that could not be quantified with sufficient precision, because application was by aerosol (Bailey et al. 2005), and one had unreplicated treatments (Medrzycki et al. 2003). Two further studies reported results that were anomalous among the collective dose–response relationships (Nauen et al. 2001; Suchail et al. 2001; see Figs. 1 and 2). These anomalies may have been caused by variation among honey bee stocks in their physiological capacity for detoxification arising from either genetics, age, or diet (Meled et al. 1998; Smirle and Winston 1987; Wahl and Ulm 1983) and, if so, further work is required to establish fully the susceptibility of honey bees generally to dietary neonicotinoids.

Lethal effects

Based on the fitted dose–response relationships, field-realistic doses of imidacloprid had virtually no effect on rates of mortality in adult honey bees in laboratory and semi-field conditions under either acute or chronic dosing regimes (Fig. 1). If this finding generalizes to environmentally relevant field conditions, it suggests that the presence of trace dietary neonicotinoids is not, in itself, the cause of catastrophic mortality of adult honey bees, such as is associated with colony collapse disorder (CCD) (van Engelsdorp et al. 2010). This conclusion is consistent with a previous study of genomic transcription (Johnson et al. 2009), which found no association between CCD and the expression of genes associated with detoxification of xenobiotics, like insecticides (but see van Engelsdorp et al. 2009), but it also does not exclude the possibility that pesticide exposure is part of a suite of interacting stressors that can predispose bees to CCD (van Engelsdorp et al. 2009). Neonicotinoids originating from seed dressings still may pose a lethal hazard to honey bees by their presence in non-floral plant substances, such as leaf exudates in seedling maize, where concentrations are substantially above trace levels (Girolami et al. 2009).

The fitted relationships indicate $LD_{50} = 4.5$ ng (95% confidence interval (CI) 3.9–5.2 ng; Fig. 1c) and $LC_{50} = 1760$ μg L^{-1} (95% CI 1640–1880 μg L^{-1}; Fig. 1d), which are both substantially above the field-realistic range. At first sight, the estimated LC_{50} (1760 μg L^{-1}) appears high, given the estimated LD_{50} (4 ng). In a laboratory cage, an adult honey bee consumes 30–40 μl of syrup each day (Decourtye et al. 2003; DEFRA 2007) and, given $LD_{50} = 4$ ng, 50% mortality should therefore occur at imidacloprid concentrations of 100 μg L^{-1}. However, this discrepancy probably arises because relatively high concentrations of imidacloprid produce anti-feedant effects, with consumption dropping by 50% at 250 μg L^{-1} and by 90% at 1000 μg L^{-1} (DEFRA 2007). Thus, to cause 50% mortality among a group of bees, decreased syrup consumption must be offset by increased concentration of imidacloprid, hence the correspondingly high value of LC_{50}.

Sublethal effects

Based on the fitted dose–response relationships, $RD_{50} = 6.0$ ng (95% CI 5.1–9.0 ng; Fig. 2c) and the $RC_{50} = 90$ μg L^{-1} (95% CI 68 to >100 μg L^{-1}; Fig. 2d), which are both above the field-realistic range (Fig. 2). However, field-realistic doses of imidacloprid reduced expected performance in adult honey bees under laboratory and semi-field conditions by between approximately 10 and 20% in acute regimes and between approximately 6 and 20% in chronic regimes (Fig. 2). Dietary imidacloprid at measured levels in nectar from two widespread crops is expected to reduce performance in honey bees by between 6 and 11% (oilseed rape) and between 14 and 16% (sunflower). These findings raise renewed concern about the impact of systemic neonicotinoids on honey bees that forage in agriculturally intensive landscapes.

The meta-analysis suggests that the sublethal effects of field-realistic doses of dietary imidacloprid are more pronounced under acute than chronic regimes, which implies that single doses are more potent than multiple doses, but this is probably an artefact, because the two underlying meta-analyses draw data from both different subjects and responses. Experiments with chronic dosing regimes, which permitted multiple ingestions, used both colonies and individual bees as experimental subjects, whereas experiments with acute, single dose regimes involved only individual bees. Colonies are likely to be more resilient to dosing than individuals when they contain uncontaminated food stores that bees consume instead of dosed syrup (Schmuck 2004). Even when individual bees were the subjects, experiments with chronic dosing regimes measured responses in only learning and memory (Decourtye et al. 2003), whereas experiments with acute regimes studied effects on a variety of behavioural responses, including gustatory thresholds (Lambin et al. 2001), success in returning from a remote feeder (Bortolotti et al. 2003), as well as learning and memory (Decourtye et al. 2004a; Guez et al. 2001). Not all behaviours will be affected equally, and so responses to acute and chronic dosing regimes need not have similar magnitudes, because different behaviours were measured under each.

Power analysis

Based on the power analysis, the previously deployed experimental designs for field trails that reported no effect of neonicotinoids on honey bee colonies were unlikely to detect sublethal effects of trace dietary neonicotinoid of field-realistic magnitude. Only two of the four published field trials were capable of operating at the conventionally accepted level of power: the study by Faucon et al. (2005) could detect a reduction in performance of >58% and that by Cutler and Scott-Dupree (2007) could detect a reduction of >33%. Given four hypothetical experiments with the samples sizes and background variability equivalent to the four field trials, the probability that all would fail independently to detect statistically the largest predicted field-realistic sublethal effect ($\Delta P = 20\%$) is $M_{20} = 0.93 \times 0.93 \times 0.80 \times 0.57 \times 100 = 36\%$, which is unacceptably high for concluding that sublethal effects are non-existent. The lack of power arises because honey bee colonies show high levels of intrinsic variability in the kinds of measurements that

investigators have made on them (mean $CV = 49\%$, SE = 12, $n = 4$) and much larger sample sizes ($n > 80$) than used previously are necessary to detect even the largest expected field-realistic sublethal effects of trace dietary neonicotinoids. To conduct meaningfully discriminating trials in future, investigators will need either to find ways to control this variability or to develop new, consistent measurements. There is a strong imperative for this, because some authors have argued that the null results of field trials with colonies should override positive findings of sublethal effects in laboratory studies (Thompson and Maus 2007). The high intrinsic variability among honey bee colonies also explains why some trials return response ratios significantly above unity (Fig. 2), which is possible when the dosed colony group contains hives of intrinsically higher vigour than the control group.

Future research targets

While the meta-analysis has quantified the expected magnitude of sublethal effects of trace dietary imidacloprid on adult honey bees under laboratory and semi-field conditions, it is difficult to predict the impacts on honey bee colonies in the field, because of the following four complications, which should be targets for future research.

The first complication is that we do not know how strongly the responses investigated in laboratory studies (e.g. effects on learning and memory) affect the health of colonies in the field. Circumstantial evidence suggests that a link will exist, because effective learning and memory is doubtless important to the functioning of honey bee colonies, where individuals are required to find and handle flowers and to navigate home (Seeley 1985). This unresolved issue is critical for establishing the environmental relevance of the findings presented above.

The second complication is that the meta-analysis draws data exclusively from studies of adult worker bees, whereas colony health depends on the success of all life stages and castes, including larvae, queens and drones, which may be either more or less sensitive to the chemical (Nitsch and Vorwohl 1992, cited in Aupinel et al. 2007). These first two complications mean that we cannot simply translate the level of reduced performance observed in laboratory studies into the likely level of impact on colonies in the field.

The third complication is that trace levels of neonicotinoids in floral forage could become diluted in the colony's food stores, because honey bees may exploit several food sources simultaneously, some of which provide untreated nectar and pollen. If, under field conditions, only a proportion of the bees' food contains insecticide residues, the detrimental effects will be correspondingly reduced (Schmuck 2004) and the colony will have a lower response than predicted from laboratory experiments, where bees are typically fed only dosed syrup. By itself, this third complication means that the level of reduced performance observed in laboratory studies overestimates the level of impact on colonies in the field. Potentially, however, nectar and pollen from an extensive area of a mass-flowering, treated agricultural crop can dominate a colony's diet (Synge 1947).

The fourth complication is that the effects of trace dietary neonicotinoids on honey bee colonies in the field may be exacerbated by the impact of other stressors that were not present in the laboratory, such as disease (Alghamdi et al. 2008), starvation (Riddell and Mallon 2006; Wahl and Ulm 1983), or the dietary presence of other agrochemicals (Iwasa et al. 2004). Honey bees are doubtless routinely exposed to all of these stressors (Mullin et al. 2010; Seeley 1985). By itself, this fourth complication means that the level of reduced performance observed in laboratory studies potentially underestimates the level of impact on colonies in the field.

Given our current inability to resolve any of the preceding four complications, it is not currently possible to be precise about the impact of trace dietary neonicotinoids on the health and fitness of honey bees in the field and these issues are targets for future research. Additionally, information about responses to the level of dose will need to be integrated with the emerging picture of the prevalence of neonicotinoids in nectar and pollen (Mullin et al. 2010) used by bees and with observations of detriment to bees under field conditions (Nguyen et al. 2009). Further research is necessary also because previous experimental field trials had low statistical power and are equivocal about the sublethal effects predicted above. In future field trials, it will be important either to increase sample sizes by using larger numbers of colonies than previously or to reduce the statistical variation within treatment groups by developing either new measurements of colony performance or stronger control of conditions (e.g. Mommaerts et al. 2010). Given that members of the neonicotinoid family vary in potency (Iwasa et al. 2004), it will also be beneficial to investigate more fully the sublethal effects of chemicals besides imidacloprid at field-realistic levels.

Based on my analysis, I also offer the following methodological suggestions for future investigations. First, and most importantly, power analysis should be used invariably by studies that report no effect of neonicotinoids on bees. Second, future studies should focus primarily on the effects of doses in the field-realistic range, rather than higher doses. Finally, more studies of the amounts of neonicotinoids in nectar and pollen are needed to establish the field-realistic range, because the available data is meagre.

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