Towards the comparative ecotoxicology of bees: the response-response relationship

James E. Cresswell*, Ian Laycock

Biosciences, College of Life and Environmental Sciences, University of Exeter, Hatherly Laboratories, Prince of Wales Road, Exeter EX4 4PS, United Kingdom
*Corresponding author, Phone: 44 1392 263779, Email: j.e.cresswell@ex.ac.uk

DOI: 10.5073/jka.2012.437.010

Abstract

Background: When an ecological system is exposed to an anthropogenic toxin, each species has an idiosyncratic sensitivity, but it is reasonable to expect some generality in response, especially among related species such as bees. If two species are similarly sensitive to a toxin their dose-response relationships will be similar. We propose a method to facilitate comparison between dose-response relationships, namely the response-response relationship, which can be applied to any biomarkers whose responses to the same pollutant are measured across a similar range of doses. We apply the method to bumble bees (*Bombus terrestris*) and honey bees (*Apis mellifera*) exposed to a dietary pesticide, imidacloprid, and we investigate both lethal and sublethal biomarkers.

Results: We found cross-species similarity in dose-dependent responses, but only in certain sublethal biomarkers. In honey bees, sublethal biomarkers were more sensitive than mortality. In bumble bees, fecundity was the most sensitive biomarker.

Conclusion: Our results provisionally suggest the existence of cross-species generalities. The greater sensitivity of sublethal biomarkers than mortality suggests that testing protocols which are overly focussed on mortality may underestimate the ecological impacts of toxic pollutants.

Keywords: *Apis mellifera*, *Bombus*, dose-response, imidacloprid, neonicotinoid

1. Introduction

Ecological systems are often richly complex, but scientists are constrained logistically to study only a few of their facets. When an ecological system is exposed to an anthropogenic toxin, each species has an idiosyncratic sensitivity to the pollutant. However, it is reasonable to expect that some generalities about the impacts of pollutants can be made and transferred from those organisms that have been studied to those that have not[1]. In the case of bees, for example, it will be valuable to know whether the impacts of pollutants on non-*Apis* bees can be inferred from the results of studies on honey bees (*Apis mellifera* L.), which are the focal species of current toxicological regulatory testing[2]. Based on overall toxicity, a previous comparative study[3] indicated broad similarity in the sensitivity of honey bees, bumble bees and solitary bees to a wide range of pesticide pollutants. Here, we investigate the detailed dose-dependence of various biomarkers, including sublethal endpoints.

The sensitivity of a species to a toxicological stressor is evident in the dose-response relationship, or exposure-response relationship[4]. Conventionally, the dose-response relationship is a simple graph that relates the magnitude of the stressor (e.g. the dietary concentration or the ingested amount of the pollutant) to the organism's response, which is quantified by a change in a specified biomarker, or endpoint (e.g. a physiological or behavioural variable, reproductive success, or mortality). If two species are similarly sensitive to a toxin, their dose-response relationships will be similar. Consequently, the recognition of toxicological generalities relies in part on our ability to recognize similarities among dose-response relationships. Here, we propose a method to aid comparison.

It is possible to compare two dose-response relationships simply by overlaying the plots on the same pair of axes. This is feasible when we compare two biomarkers whose responses are measured across a similar range of doses of the same pollutant. However, a new plot based on the same data facilitates the rapid evaluation of the differences between the two relationships. The plot is a response-response relationship (see Methods) and its shape is diagnostic for the relative sensitivity of the two responding biomarkers.

We need not be limited to comparing the sensitivity of different species. It is also valuable to compare the sensitivity of different biomarkers in the same species. For example, mortality is typically the focal biomarker for toxicological studies in bees and its cardinal value is the LD_{50}, the dosage required to kill half of the exposed individuals. The LD_{50} is conventionally used to compare potency among pollutants. However, if we are interested in ecologically important impacts, then we should also inquire about a toxin's effects on reproduction, because population dynamics are influenced by birth rates as well as death rates[5]. It is therefore valuable to know whether fecundity is a more or less sensitive biomarker than mortality, because this determines whether the overall demographic toxicity of the pollutant is correctly indicated by its LD_{50}. We can establish the relative sensitivity of these demographically important variables from a suitably arranged response-response relationship.

2. Experimental methods

Consider two conventional dose-response relationships, *A* and *B*, that are curves over the same range of dosages of the same toxin. Each point on relationship *A* is denoted (D_A,R_A) and (D_B,R_B) denotes points on relationship *B*. Each point on the response-response relationship is given by (R_A,R_B) when $D_A = D_B$.

If the two dose-response relationships, *A* and *B*, exhibit identical levels of sensitivity, then $R_A = R_B$ for any $D_A = D_B$ and the response-response relationship will be the line of equivalence, i.e. $y = x$. If instead one biomarker is more sensitive, the response-response relationship will deviate away from the line of equivalence and towards the axis that denotes the response of the more sensitive biomarker.

2.1 Case study 1: performance in honey bees vs. feeding in bumble bees exposed to imidacloprid

We compare the sensitivity of honey bees and bumble bees to dietary residues of the neonicotinoid pesticide, imidacloprid. Imidacloprid is a widely-used neonicotinoid pesticide whose residues appear in the nectar and pollen of treated crops[6]. It disrupts the insect nervous system by acting on nicotinic acetylcholine receptors[7] and it causes both mortality and various sublethal effects[8].

The dose-response relationship for honey bees was obtained from a meta-analysis of experiments testing the effects of dietary imidacloprid on honey bees[9], which established a dose-response relationship whose biomarker was average performance across a variety of sublethal biomarkers, which included learning ability of individuals[10], flight activity at the hive[11], and brood production[12,13]. Honey bee performance was defined relative to the biomarker's magnitude in undosed bees and the consensus dose-response relationship was described by: *relative performance*(%) = 100*[1 – 0.06*exp*(0.478*ln*(*dose*))], where *dose* has units of µg imidacloprid L^{-1} feeder syrup[9].

The dose-response relationship in bumble bees was established in laboratory experiments on individually caged bumble bees (Cresswell, *unpublished*) that were fed *ad libitum* on syrup (50% inverted sucrose; Attracker, Koppert B.V., Berkel en Rodenrijs, NL) containing imidacloprid at a range of ten dosages. The bees (*Bombus terrestris* L.) were obtained as domesticated colonies from a commercial supplier (Natupol Beehive, Koppert B.V., Berkel en Rodenrijs, NL). Bees were maintained in a controlled environment room (temperature 25°C, 40% relative humidity, 12:12 hours of light:darkness). In order to quantify their intrinsic variation in feeding rate due to variation in size, bumble bees were maintained on a control diet of syrup for three days before dosing began. Once dosing began, each cage was provided with a syrup feeder containing either control syrup or a syrup with one of the following nine doses of imidacloprid in units of µg imidacloprid L^{-1}: 125.00; 50.00; 20.00; 8.00; 3.20; 1.28; 0.51; 0.20; 0.08. Imidacloprid was obtained as a solution in acetonitrile (Dr. Ehrenstorfer GmbH, Augsburg, Germany) and the acetonitrile was removed by evaporation with a vacuum dryer (ScanVac MaxiVac Beta, Labogene, Lynge, Denmark) and the imidacloprid was suspended in water before being mixed into feeder syrup.

2.2 Case study 2: lethal vs. sublethal biomarkers in honey bees exposed to imidacloprid

Both dose-response relationships were obtained from a meta-analysis of experiments testing the effects of dietary imidacloprid on honey bees[9]. The dose-response relationship for sublethal effects uses relative performance as the biomarker and it is as described in the first case study. The dose-response relationship for lethal effects[9] is given by: *mortality*(%) = 10.2 + [(75.3 – 10.2)/(1 + *exp*(0.567 (0.194 – *ln*(*dose*))))].

2.3 Case study 3: feeding vs. fecundity in worker bumble bees exposed to imidacloprid

Queenless microcolonies[14] of four or five worker bumble bees were established from queenright colonies of *B. terrestris*, (Natupol Beehive; Koppert B.V., Berkel en Rodenrijs, Netherlands) in softwood boxes (internal dimensions: 120 × 120 × 45 mm). Each microcolony fed on syrups prepared as described above (section 2.1) and at the same range of doses. Each microcolony contained a pollen ball that was prepared by grinding pollen pellets collected from honey bee hives (Werner Seip Bioprodukte, Butzbach, Germany) into a powder and mixing the mass with water to form dough. Brood production was quantified after 14 days of exposure to dosed syrups.

3. Results

The daily feeding rates of bumble bees and the sublethal performance of honey bees averaged across various biomarkers are equally sensitive to dietary imidacloprid up to 125 μg L^{-1} (Figure 1). In honey bees, sublethal performance biomarkers are more sensitive than mortality to dietary imidacloprid up to 125 μg L^{-1} (Fig 2). Performance is predicted to decrease by over 50% at a dosage of dietary imidaclopid equivalent to 0.1 LD_{50} (Fig 2). In bumble bees, the fecundity of adult workers in queenless microcolonies is more sensitive than the daily feeding rate of individuals to dietary imidacloprid up to 125 μg L^{-1} (Fig 3).

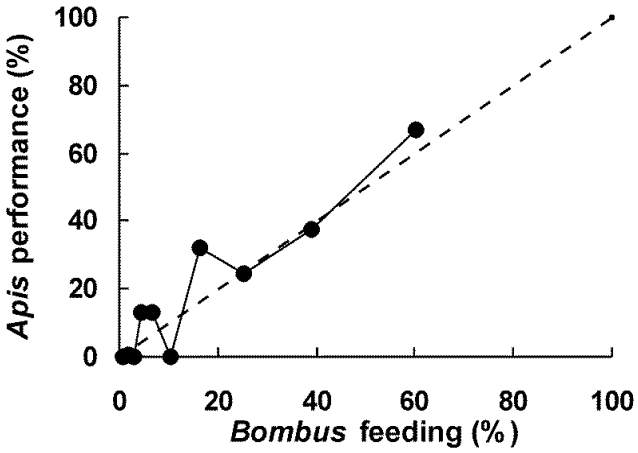

Fig. 1 Response-response relationship showing percentage reduction in the performance of honey bees (*Apis mellifera*) averaged across various sublethal biomarkers (*y*-axis) versus the percentage reduction in daily feeding rate of individual bumble bees (*Bombus terrestris*; *x*-axis) when exposed in laboratory trials to dietary imidacloprid in feeder syrups at nine doses in units of μg imidacloprid L^{-1}: 125.00; 50.00; 20.00; 8.00; 3.20; 1.28; 0.51; 0.20; 0.08. Points are interpolated for ease of inspection only.

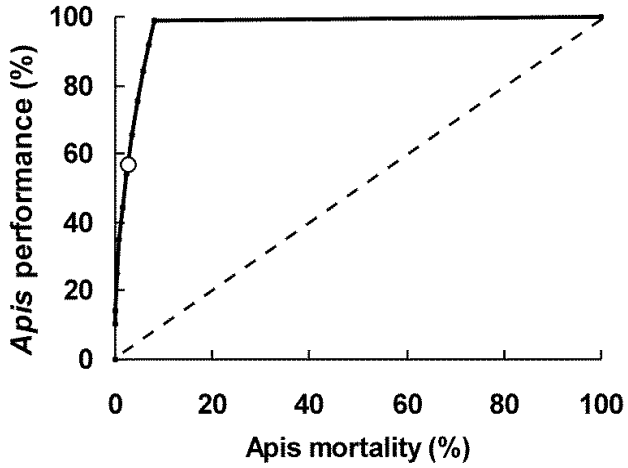

Fig. 2 Response-response relationship showing percentage reduction in the performance of honey bees (*Apis mellifera*) averaged across various sublethal biomarkers (*y*-axis) versus the percentage increase in mortality rate of honey bees averaged across various studies (*x*-axis) when individuals are exposed in laboratory trials to dietary imidacloprid in feed syrup. The abrupt inflection in the response-response relation occurs at a dosage of 350 µg imidacloprid L^{-1}. The open circular symbol indicates the point on the relationship that corresponds to $x = LD_{50}/10$, or 5% mortality.

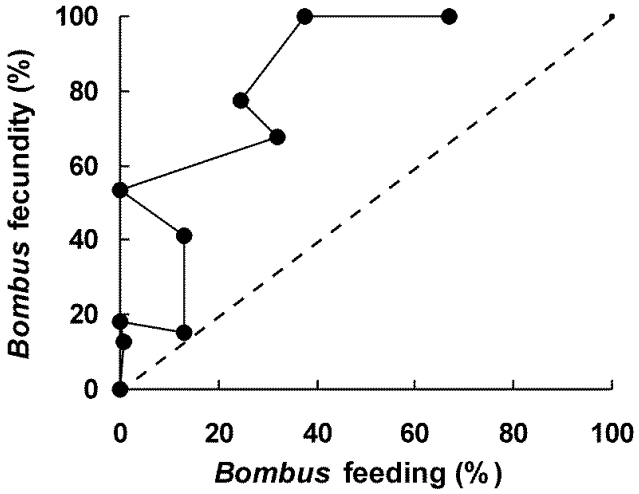

Fig. 3 Response-response relationship showing percentage reduction in the fecundity of worker bumble bees (*Bombus terrestris*) in microcolonies (*y*-axis) versus the percentage reduction in daily feeding rate of individual bumble bees (*x*-axis) when exposed in laboratory trials to dietary imidacloprid in feeder syrups at nine doses in units of µg imidacloprid L^{-1}: 125.00; 50.00; 20.00; 8.00; 3.20; 1.28; 0.51; 0.20; 0.08. Points are interpolated for ease of inspection only.

4. Discussion and conclusions

The proposition that we can find cross-species generality in toxicological sensitivity in bees receives some support from the similarity in dose-dependent responses to dietary imidacloprid between the feeding rate of bumble bees and sublethal performance biomarkers in honey bees. Arguably, this similarity emerges because the overall health of an individual is equally affected by dietary imidacloprid in both species. An individual's feeding rate probably reflects its levels of metabolic and locomotory activity. In honey bees, the sublethal performance biomarkers, such as colony activity and individual learning performance, are similarly indicative of overall health. This apparent similarity in sensitivity between honey bees and bumble bees contrasts with the substantive disparity between their LD50s for imidacloprid. However, we show below that biomarkers can differ substantively in sensitivity even within species, and this is so particularly when comparing sublethal markers and mortality. We therefore argue that the similarity between honey bees and bumble bees in sensitivity in biomarkers of overall health begins to suggest the existence of a generality, but this conclusion is highly provisional and it must be properly established by more comparisons across species and genera.

In honey bees, the greater sensitivity of sublethal biomarkers than mortality potentially has implications for the security of the conventional testing protocols used by regulators in pesticide approval, which typically focus on mortality. In Europe, regulators may give attention to the toxicity-exposure ratio, TER[15], which is calculated as: TER = LD50 / exposure. A compound is declared sufficiently non-toxic to bees if its TER $\geq$ 10, which means that, in theory, a compound could be approved if the exposure of bees is one tenth of the LD_{50}. Our findings (Fig 2) suggest that it is theoretically possible that substantive sublethal effects could emerge at an exposure of 0.1 LD_{50}. However, we note that we have demonstrated this only for an insecticidal compound, imidacloprid, whose toxicity necessarily yields TER <10. It will be valuable to investigate whether any compound that just satisfies the initial screening criterion, i.e. TER = 10, has biologically significant sublethal effects at the upper threshold exposure of 0.1 LD_{50}.

For bumble bees, fecundity was the most sensitive biomarker among those investigated here, whereas mortality was the least, if we judge by the relative magnitude of the oral LD_{50} for imidacloprid[16], which is about twenty five times greater in bumble bees than in honey bees (approximately 200 $\mu g\ kg^{-1}$ *vs*. 8 $\mu g\ kg^{-1}$). The high degree of sensitivity of bumble bee fecundity to dietary imidacloprid occurs despite the relative insensitivity of mortality as a dose-dependent biomarker, which suggests that the magnitude of the LD_{50} can mislead about the potential of a compound to have an ecological impact through demographic toxicity. A pollutant's demographic toxicity describes its impact on a target organism's population dynamics[17], which is determined by effects on both birth rates and death rates. If fecundity is more sensitive than mortality, the LD_{50} underestimates demographic toxicity.

The biological basis of the differential sensitivity of fecundity and mortality in bumble bees is largely obscure. Imidacloprid, the compound considered here, is neurotoxic to bees. It is understood that the bee is a highly integrated physiological unit with many processes under nervous control[18], but the pronounced sensitivity of fecundity relative to feeding rate, for example, is nevertheless not readily explained. We hope that future research into the mechanistic basis of these toxicological effects will pay dividends both by increasing our fundamental understanding and also by improving the prospects for the development of pesticides with low impacts on bees.

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