Brief Communication

Establishing realistic exposure estimates of solitary bee larvae via pollen for use in risk assessment

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Abstract

Bees foraging in agricultural habitats can be exposed to plant protection products. To limit the risk of adverse events, a robust risk assessment is needed, which requires reliable estimates for the expected exposure. The exposure pathways to developing solitary bees in particular are not well described and, in the currently proposed form, rely on limited information. To build a scaling model predicting the amount of protein developing solitary bees need based on adult body weight, we used published data on the volume of pollen solitary bees provide for their offspring. This model was tested against and ultimately updated with additional literature data on bee weight and protein content of emerged bees. We rescaled this model, based on the known pollen protein content of bee-visited flowers, to predict the expected amount of pollen a generalist solitary bee would likely provide based on its adult body weight, and tested these predictions in the field. We found overall agreement between the models’ predictions and the measured values in the field, but additional data are needed to confirm these initial results. Our study suggests that scaling models in the bee risk assessment could complement existing risk assessment approaches and facilitate the further development of accurate risk characterization for solitary bees; ultimately the models will help to protect them during their foraging activity in agricultural settings. Integr Environ Assess Manag 2022;18:308–313. © 2021 The Authors. Integrated Environmental Assessment and Management published by Wiley Periodicals LLC on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

KEYWORDS: Pesticides, Plant protection products, Pollinators, Proteins

INTRODUCTION

In recent years, there has been a growing concern that nonmanaged bee populations are in decline, potentially compromising pollination security in agricultural and non-agricultural landscapes (Gallai et al., 2009; Grab et al., 2019). Although many drivers are likely to be associated with this trend, the exposure of bees to plant protection products (PPP) could be one of them (Goulson et al., 2015). Consequently, bees need to be protected from potentially adverse events; risk assessment (RA) schemes are in place for the registration of PPPs before their placement on the market (APVMA, 2017; EFSA, 2013; USEPA, 2014).

Historically, due to the technical and logistic difficulties of testing noncommercially raised species, the established pollinator RA procedures (e.g., EFSA and EPA) have relied on the honeybee (Apis mellifera) as surrogate species. In contrast to most bees, A. mellifera is highly social (Danforth et al., 2019), which can have important consequences for both the RA process and its outcome, in particular for the expected exposure of different developmental stages and scenarios because they do not directly provide pollen to their offspring (Boyle et al., 2019). Although the currently implemented A. mellifera-centered pollinator RA schemes probably protect solitary bees (SB) as well (Boyle et al., 2019; Thompson & Pamminger, 2019), it is unclear if this also extends to exposure routes not directly addressed in current pollinator RAs (Boyle et al., 2019). One alternative exposure route is related to developing SB, which, in contrast to honeybees, often feed on a single provision of unprocessed and potentially PPP-contaminated pollen mixed with varying degrees of nectar (Boyle et al., 2019). Although nectar can also be contaminated with PPP residues, recent evidence suggests that the main driver of PPP residue in SB larvae provisions is likely pollen (Kyriakopoulou et al., 2017). Consequently, an accurate estimate of SB larvae pollen provisions is critical to evaluate the risk that SB larvae face from PPP residues during this time. However, the currently proposed estimates for pollen consumption of SB larvae rely mainly on limited information from a restricted number of
Clear linear association between the two log 10 transformed random intercept to the log 10 transformed bee weight complicated the data by an LM to the log 10 protein provision (mg) and bee adult dry weight (mg). The new model fit the data better indicated by the improved $R^2$ values compared with the original model (LM: $F = 166.5; \text{df} = 16; R^2 = 0.91; p < 0.001$; Figure 2).

$$\log_{10} \text{pollen provision (mm}^3) = 0.87 \times \log_{10} \text{bee dw (mg)} + 0.44.$$ (1)

**Predicting the corresponding protein provision**

Assuming that larvae protein requirements are an important driver in determining the pollen provisioned volume, we expected that accounting for the variation in protein content in host plant pollen would improve the model’s fit. We used host plant preferences of the SB species present in the dataset (Müller et al., 2006; Westrich, 2018) to determine the likely protein concentration (%) in the collected pollen provisions (Pamminger et al., 2019). We used the median plant genus estimates or family level information if genus level information was not available (see dataset). In case of pollen generalist bee species (i.e., bees collecting pollen from multiple plant genera), we used the median protein concentration of their reported host plant genera. Using the information, we calculated the expected volume of protein provided to SB larvae (mm$^3$) and estimated the corresponding amount of protein (mg) using the reported mean protein density estimate of 1.37 (mg/mm$^3$; Erickson, 2009). To determine if this correction improved the scaling association, we fit an LM to the log 10 protein provision (mg) and bee adult dry weight (mg). The new model fit the data better indicated by the improved $R^2$ values compared with the original model (LM: $F = 166.5; \text{df} = 16; R^2 = 0.91; p < 0.001$; Figure 2).

**MATERIALS, METHODS, AND RESULTS**

All statistics and visualizations were conducted in the R statistical environment (R, 2013) Version 4.0.3 using RStudio Version 1.4.1103 (RStudio, 2020). We used the following packages: ggplot2 (Hadley, 2016), ggapubr (Kassambara, 2020), dplyr (Hadley et al., 2021), ggrepel (Slowikowski et al., 2016), and tidyverse (Wickham et al., 2019).

**Data**

We used a published dataset (Müller et al., 2006) describing the association between adult SB species’ dry weight (mean dw [mg]) and the volume (mm$^3$) of pollen provisioned. The dataset includes information on female specimens of 14 SB species from Europe and, in four cases, measurements of male bees as well (total $N = 18$). We replicated the data by fitting a linear model (LM) with a random intercept to the log 10 transformed bee weight (predictor variable) and pollen provision volume (response variable) following the authors’ initial analysis (Müller et al., 2006). We confirmed their findings demonstrating clear linear association between the two log 10 transformed variables (LM: $F = 46.41; \text{df} = 16; R^2 = 0.74, p < 0.001$; Equation [1]; Figure 1).
In July 2021, we searched the literature for additional information, we updated the protein provisioning dataset and refit the LM (Equation [3], LM: $F = 270.8; df = 22; R^2 = 0.93; p < 0.001$; Figure 3).

$$\log_{10} \text{ protein provision dw (mg)} = 0.92 \times \log_{10} \text{ bee dw (mg)} - 0.1. \quad (3)$$

**Predicting pollen provisions**

Because pollinator exposure assessment is based on the provisioned pollen and the expected or measured PPP residues within it, we rescaled the protein prediction model (Equation [3]; by adjusting the $y$-intercept), assuming a median pollen protein concentration of 29.1% (Pamminger et al., 2019) to predict the corresponding amount of pollen (mg) a generalist SB, such as *Osmia bicornis* (Westrich, 2018), is likely to collect based on its body weight.

$$\log_{10} \text{ pollen provision dw (mg)} = 0.92 \times \log_{10} \text{ bee dw (mg)} + 0.44. \quad (4)$$

**Testing the model prediction**

To test the validity of Model 4, we predicted the expected pollen provision of a commercially available, regulatory relevant, and pollen generalist (Westrich, 2018) SB species (*O. bicornis*). Based on the reported mean body weight of male and females of 21.72 (mg dw; Kendall et al., 2019) the model predicts a pollen provision of 47.3 (95% prediction confidence interval [CI]: 43.4–51.8) mg of dw pollen. To test this prediction, we set up a field experiment in June 2020 and released *O. bicornis* (total $N = 430$ males and 215 females supplier = WAB Mauerbienenzucht; https://www.mauerbienen-shop.com/) in approximately equal proportions at three open agricultural locations (L1–L3) and in one flight tunnel (L4; host plant Phacelia spp.) in southwest Germany (Limburgerhof). The flight tunnel setup was similar to the OECD 75 and EPPO (2010) honeybee semifield test setup (EPPO, 2010; Franke et al., 2021; OECD, 2014). All locations were provided with artificial nest sites and were similar to the methods reported for *Osmia* field testing (Franke et al., 2021). After one initial week of acclimatization, we checked the nest sites twice a week for newly built cells and removed the pollen provisions before the larvae had hatched, storing them in individual Eppendorf tubes at $-20^\circ C$ until analysis. Over three weeks, we collected 161 pollen provisions and measured their wet and dry weights as well as the corresponding amount of water, sugar, and pollen in mg following established methodology (Kapheim et al., 2011). The pollen provisions were weighed (wet weight) to the closest 0.1 mg using a Mettler Toledo Laboratory Balance (XPR205DR) scale. Then, the provisions were dried at $60^\circ C$ overnight and reweighted (dry weight). The water content was calculated as the difference between the two measurements. The dried pollen was suspended in 0.5–0.75 ml deionized water (depending on the provision size) and vortexed for 2 min to ensure the transition of all soluble sugars into solution. After centrifugation to a loose pellet at 6000 rpm for 1 min using a VWR Galaxy Mini Star centrifuge, the dissolved sugars in the supernate were quantified using a hand-held refractometer (Bieno Vinum Refraktometer für Winzer und Mostereien) following the manual’s instruction. The amount of dw pollen was calculated by subtracting the measured amount of sugar in mg from the pollen dry weight. The summary statistics for all locations are presented in Table 1. We found that *O. bicornis* provided 64.23 (95% prediction CI: 47.86–85.11) median mg dw pollen in agreement with the model predictions (the 95% CI of the prediction and measurement provisions overlap see Figure 4).
DISCUSSION

In this study, we developed and tested two scaling models to predict the protein (Equation [3]) and pollen provisions (Equation [4]) of developing SB based on the corresponding adult dry weight for use in pollinator RA.

The protein prediction model (Equation [3]) revealed that the LM fits the log10 transformed data well ($R^2 = 0.93$) across a wide weight range (4.6–43.1 mg dw). This suggests that this equation might apply to most bees independent of their social organization because the observed relationship is likely driven by conserved protein needs of developing bees, which in turn, depend mainly on their size (larger bees need more protein) and not their social organization. However, it is unlikely that this holds true for the presented pollen prediction model. In contrast to SB species, social bees preprocess pollen to varying degrees and often provide it continuously to their offspring during their larval stage (Danforth et al., 2019; Gould & Gould, 1988; Westrich, 2018). Because in many cases it is unknown to what degree these alternative feeding patterns change the direct pollen exposure of developing social bees, it is unlikely that the observed relation between adult SB size and larvae pollen needs can be extended directly to social bees without accounting for species or group-specific differences.

SB often provide their offspring with a single provision of unprocessed pollen of known host plant origin (Danforth et al., 2019; Westrich, 2018), which makes it possible to extrapolate their pollen needs directly from their protein requirements whenever the pollen protein concentration of the host plant(s) is known (Pamminger et al., 2019). In this case our pollen provision model is based on the median protein concentration found in the pollen of bee-pollinated flowers, which seems acceptable as a starting point to predict the needs of pollen generalist bees such as O. bicornis (Westrich, 2018). Using this model, we were able to predict the pollen needs of the known pollen generalist O. bicornis (Westrich, 2018), supporting the validity of the model as an extrapolation starting point for O. bicornis pollen needs (see Figure 3). However, considering that the measured values were above the predicted values, more independent data are needed to better calibrate the model, in particular under different environmental conditions and landscape configurations. Although the presented model seems promising at predicting the needs of pollen generalists such as O. bicornis, it might be less accurate to predict the needs of pollen-specialist bee species, particularly ones preferring pollen with extremely high or low protein concentrations (Westrich, 2018). In such cases, it might be beneficial, as with the procedure outlined in this paper, to rescale the protein model (Equation [3]) considering the host plant preferences and the associated pollen protein concentrations. Overall, it would be beneficial to gather additional protein and pollen collection data for bees on the ends of the weight spectrum (e.g. large Xylocopa sp. and small Lasiosglossum sp.) to see if the model predictions hold.

When looking in more detail at the O. bicornis provisions sampled at the four locations, we find some variation in provision size and relative composition (Table 1). Considering the small number of locations (tunnel $N = 1$, field $N = 3$), we did not conduct a formal statistical analysis but rather describe the observed patterns qualitatively, which must be interpreted with care, given the low sampling size. Overall, the tunnel location (Phacelia spp. only) provided the

![Figure 4](image-url)

**Figure 4** depicts the relationship between bee dry weight and the expected pollen provisioning for the developing larvae in mg (linear model 4 and the associated 95% confidence interval (CI; black dotted line). We compare these expectations with the measured pollen provisions of *Osmia bicornis* in 2020 (in red; median observed = 64.23 mg and associated 95% CI).

**TABLE 1** Summarizing the results of the *Osmia bicornis* provision composition at the four sampled locations

| Location | N | Wet weight (mg) | Dry weight (mg) | Water (ml) | Sugar (mg) | Pollen (mg) |
|----------|---|----------------|----------------|-----------|-----------|-------------|
|          |   | Median         | SD             | Median    | SD        | Median      |
|          |   |                |                |           |           |             |
| L1       | 51| 168.3          | 76.5           | 132.3     | 60.11     | 37.2        |
| L2       | 27| 201.4          | 73.6           | 171.4     | 60.17     | 30.0        |
| L3       | 32| 161.4          | 94.3           | 132.1     | 76.57     | 28.9        |
| L4 (Tunnel) | 51| 257.1          | 107.2          | 190.9     | 81.91     | 54.1        |
| Overall median | 161| 184.9        | 151.8          | 33.6      | 76.1      | 64.2        |

Note: For all locations, we show wet and dry weight of the provisions as well as their water, sugar, and pollen content. We present median and associated standard deviations (SD).
largest provisions (median wet and dry weight) and contained the most sugar and water (nectar). In contrast, the pollen provisions observed in the tunnel setting were within the observed values of the free flying locations (Table 1). This could indicate that pollen provision might not depend as much on host plant type and abundance as it depends on nectar (in both cases their supply can be expected not to be limited in the tunnel), but more independent observation is needed to draw definitive conclusions. When looking at the nectar content, we see that, in the tunnel, the provisions are larger mostly due to their sugar and water (nectar) content. This is in line with recent findings indicating that, if given the choice, O. bicorinis bees will favor carbohydrates over protein (Austin & Gilbert, 2021). One clear limitation of our sampling procedure is that we do not know the sex of the developing larvae. Because it is known that the size of the provisions can be sex dependent in the Osmia genus (Bosch & Vicens, 2002), and that O. bicorinis sexes vary in size (Kendall et al., 2019), site-dependent variation in sex ratios might account for some of the location-specific variation in provision size. However, it is likely that the presented model can be applied to both sexes because the underlying dataset encompasses both male and female measurements for four species, and no obvious sex-specific deviations were observed in protein or pollen need (Figures 1 and 2). Although the experimental measurements support the pollen provision model, it must be noted that all of them were higher than predicted by the model. Possibly, the current model might underestimate the pollen needs, which should be clarified ideally using additional species not included in the dataset. This could be the result of variability in quantity and quality of available host plants present at the time of collecting. Such factors need to be explored and integrated when designing and testing scaling models fit for use in regulatory RAs.

In addition to pollen, bees provide their offspring with nectar. As both matrices can be contaminated with PPP residues, the total exposure SB face will depend on both components. However, given that measured PPP residues in pollen are often higher than nectar (Kyriakopoulou et al., 2017), the pollen provisions will likely drive the RA. To address the additional residues SB offspring face via nectar, it might be fruitful to explore weight-based scaling models, because energy needs are tightly linked to body size (West & Brown, 2005) as well. When taken together, these values would likely predict a worst-case scenario because not all larvae will consume the entire provision.

Scaling approaches based on body weight can be used to estimate both hazard and exposure parameters in a range of organisms, and some are currently utilized in ecological RA schemes (Davidson et al., 1986; EFSA, 2009; Mineau et al., 1996, 2001; Pamminger, 2021; Urban & Urban, 1986). Such methods offer a clear alternative to experimental investigations in cases where such approaches are not feasible (e.g. number of species) or desirable (e.g. vertebrate testing). Similarly, scaling models could be used to extend the currently implemented pollinator RA schemes to better cover SB-specific exposure scenarios, which in turn would allow a more accurate risk evaluation for SB foraging in agricultural habitats and ultimately help to better protect them.

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CONFLICT OF INTEREST

The authors are employed by Bayer Crop Science or BASF SE, manufacturers of crop protection products.

DATA AVAILABILITY STATEMENT

Data are available at: https://doi.org/10.6084/m9.figshare.14910039 and https://doi.org/10.6084/m9.figshare.14910030.

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