AMIGA

Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems

Collaborative Project: Medium-scale focused research project

Grant Agreement No.: 289706

Deliverable 6.3 Report on pollinator monitoring and honey bee exposure

Date Delivered: 30th November 2015

Status: preliminary - Statement of confidentiality: one publication is in the process of publication (Danner et al. 2015, in review), and therefore according to the accepted standards for scientific publications, the Consortium will retain the present report confidential until the scientific paper will be accepted for publication by a peer-reviewed scientific journal.

Lead Beneficiary: Ingolf Steffan-Dewenter & Stephan Härtel (WUE)

Contributing beneficiaries: none further beneficiaries

NON TECHNICAL SUMMARY

In European countries, crop pollination by insects is estimated to have an annual value of 14.6 billion Euros. By foraging for flower resources on crops, pollinators can be exposed to genetically modified (GM) pollen. Despite the high economic value of pollination, landscape scale studies which analyse pollen use and foraging distances to evaluate potential exposure risks to GM crops are currently completely lacking. In addition, baseline data to quantify pollinator diversity in important crops before commercialising GM varieties in Europe are required to measure a potential GM crop effect by a before/after approach.

Honey bees are important pollinators in agricultural landscapes. During maize pollen shedding, maize fields were the preferred pollen resource in our experimental setting. The mean foraging distances of honey bees that collected maize pollen were 589 m ± 41 m, and ranged from 27 m to 3,040 m. 5% of all foraged maize fields were beyond 1,456 m. In the bloom of oilseed rape, rape pollen appears not as important as pollen resource as maize fields were. Nevertheless, an increasing area of flowering oilseed rape within a 2 km flight range reduced mean pollen foraging distances from 1,324 m to only 435 m.

In a second part of our report, we suggest harmonized methods to monitor pollinator diversity in different European bio-geographic regions. Further, we compiled studies on wild bee diversity and abundance in main potential GM crops across Europe as baseline data to assess future changes in pollinator richness and abundance

Our results are essential in order to interpret and link the outcome of laboratory ERA studies on GM crops for pollinators to the situation in the field. In addition, when applied to landscape management programs the foraging data of honey bees could be used to shorten foraging distances and to reduce exposure risks to GM crops and/or systemic chemical Plant Protection Products (PPPs). The outcome of our report has the potential to further sustain pollination services in European agricultural ecosystems.

POLICY RELEVANCE

The scientific results in this deliverable could serve to inform EU policy about potential honey bee exposure rates to GM crops and give a reference for base line data on wild bee diversity and abundance in European agricultural landscapes.

According to the Regulation EC No 1107/2009 of the European Parliament and the Annex II of Directive 2001/18/EC, environmental risk assessments should consider the possible environmental impact resulting from new PPPs as well as direct and indirect interactions of GM plants with non-target organisms (NTOs). Our report delivers important data for the case-by-case evaluation of potential risks arising from the deliberate release of GMOs into the environment.

1. Introduction and Objectives

Exposure risks to GM crops

Worldwide the area under genetically modified (GM) crop cultivation is still growing reaching in 2014 about 13 % of the total arable land. This global trend is not followed by European countries were the proportion of GM crop fields is < 0.1%. GM crops with high adoption rates on a global scale are maize (30% GM adoption), cotton (68%), soybean (82%) and oilseed rape (25%) (James 2014). All flowers of these commercialized GM crop varieties show a high attractiveness to insects and express the new traits also in pollen grains. Consequently crop flower visiting insect are directly exposed to GM traits when foraging for pollen.

The honey bee (*Apis mellifera* L.) is a globally distributed pollinator and plays an important role in maintaining the ecosystem service of pollination in agricultural landscapes (Klein et al. 2007; Potts et al. 2010). The global occurrence of *Apis mellifera* in all agricultural regions with GM crop cultivation makes this species unique in terms of environmental risk assessment for GM traits. Honey bees are exposed to GM products, because pollen foragers show a strong association to crops. Indeed it has been shown that crop pollen can contribute significantly to pollen harvest of honey bee colonies (Odoux et al. 2012). For bees pollen is the main protein source and essential for colony growth and development (Haydak 1970). Its quality and diversity has been identified as an important factor for honey bee health (Alaux et al. 2010; Di Pasquale et al. 2013). In contrast to nectar, it is stored in only small amounts within the hive (Seeley 1995).

In intensive farming systems honey bees are often depending on flower resources of crops, but the influence of mass-flowering crops like maize, oilseed rape or sunflowers on pollen foraging behavior is not well understood. Despite increasing general evidence for negative effects of intense agriculture on pollinators, no valid empirical data for Europe exist that allow to quantify possible exposure risks of bee colonies to crop flower products in relation to landscape context or specific crops. Such data are not only needed in connection with GM crops. This kind of information is also required for estimating the exposure rates to systemic insecticides like neonicotinoids, which are also being channeled into the hive via crop flower resources.

Foraging distances of honey bees determine the spread of GM pollen across agricultural landscapes, the limitation of being exposed and potential contamination of honey and other bee products. Variation of foraging distances in landscapes with different proportions of crop areas and semi-natural habitats is completely unexplored. In the process of decision-making, data about foraging distances to crop flower resources are crucial for a case-by-case evaluation of potential environmental risks to honey bees and their services and products.

Monitoring

It has been estimated that in Europe more than 2000 bee species (Hymenoptera: Apiformes) potentially contribute to pollination services. On the other hand honey bee colonies with about 20,000 individuals in spring and up to 50,000 individuals during summer are assumed to play an overwhelmingly important role for pollination services, particularly for mass-flowering crops grown as monocultures in agricultural landscapes. Several recent studies show that the functional complementarity of wild pollinators in terms of spatial and temporal resource use (Höhn et al. 2008), resource specialisation on certain crops (Bommarco et al. 2012) and different sensitivity to harsh climatic conditions (Brittain et al. 2013) significantly contribute to the stability of crop pollination services (Garibaldi et al. 2011). Further, flower-visiting wild pollinators can facilitate the pollination efficiency of co-occurring honey bees (Greenleaf & Kremen 2006). However, a recent study indicates that despite these well-documented potential benefits of pollinator diversity, most crops are only pollinated by a small fraction of total pollinator species pools (Kleijn et al. 2015). Therefore, an assessment of pollinator diversity and abundance in major crops in different bio-geographic regions of Europe is essential for an ecological risk assessment. New cropping systems including the cultivation of GM varieties might change the occurrence of pollinators and the security of pollination services in agricultural landscapes. Impacts of GM crops might include not only direct toxic effects but also lower pollen production or flower attractiveness. By using harmonized monitoring methods (Westphal et al 2008) to gather baseline data to quantify pollinator diversity in important crops before commercializing GM varieties in Europe, we provide data to measure a potential GM crop risk by a before/after approach.

The overall aim of this deliverable is related to task 6.4 and 6.5 of WP6 of the AMIGA project. In this report we address the following two objectives:

- We quantified the exposure of honey bees to potential major GM crops

- We assessed the pre-market status of pollinator diversity in focal crops by using harmonised sampling methods on a local to landscape scale as well as across different European biogeographic regions

This report mainly summarises findings of the following articles:

Danner N, **Härtel S**, **Steffan-Dewenter I** (2014) Maize pollen foraging by honey bees in relation to crop area and landscape context. Basic and Applied Ecology 15: 677-684.

Danner N, Molitor AM, Schiele S, **Härtel S**, **Steffan-Dewenter I** (2015) Season and landscape composition affect pollen foraging distances and habitat use of honey bees. Ecol. Appl.: in review.

Schindler M, Diestelhorst O, **Härtel S**, Saure C, Schanowski A, Schwenninger HR (2013) Monitoring agricultural ecosystems by using wild bees as environmental indicators. Biorisk 8: 39-51.

Westphal C, Bommarco R, Carré G, Lamborn E, Morison N, Petanidou T, Potts SG, Roberts SPM, Szentgyörgyi H, Tscheulin T, Vaissiére BE, Woyciechowski M, Biesmeijer JC, Kunin WE, Settele J, **Steffan-Dewenter I** (2008) Measuring bee diversity in different European habitats and biogeographical regions. Ecological Monographs 78: 653-671.

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2. Material and Methods

2.1. *Maize pollen foraging by honey bee colonies (Danner et al. 2014)*

2.1.1 Study Region

The study was performed in the region around Bayreuth in northern Bavaria, Germany. This region is characterized by a mix of intensively managed cropland, extensive grasslands and differently sized forest fragments. Cultivation of barley and maize is accounting for most of the cropland area (24 % and 16 % respectively) within the study region. We selected 11 circular landscapes with a radius of 1,500 m (Steffan-Dewenter & Kuhn, 2003), the maximum possible maize acreage gradient within the study region and independent gradients of grassland and crop area. In each landscape maize crop fields were located at distances from below 100 m up to 1,500 m from the centre.

2.1.2 Observation hive and bee material

To observe honey bee waggle dances we used four glass-sided observation hives with two Zander brood frames each (comb area 3,056 cm²). Colonies were built three weeks before starting with observations using young, mated queens (*Apis mellifera carnica*) obtained from the LAVES Institute for Apidology in Celle, Germany. All queens derived from a single mother and were mated at the same queen-mating station to assure minimal genetic differences between colonies. Brood frames with similar amounts of brood cells, honey and pollen stocks (assessed via Liebefeld method) were transferred to observation hives along with queens and approximately 4,000 workers per hive.

2.1.3 Experimental design

Four observation hives were placed in the centre of four out of 11 selected landscapes. Hives were moved to four other landscape centres each night after termination of flight activity on a day with suitable weather conditions for foraging. All colonies were observed at least once on each observation day. We considered a series of circuits as a single dance, each circuit consisting of a straight waggle run and the return run. For each dance we recorded the duration of a series of circuits, the corresponding number of circuits, the average angle of the waggle runs relative to the vertical, the time of day and the colour of pollen carried by the dancing bee. Only dances of pollen-carrying foragers with a minimum of five consecutive circuits were decoded.

2.2. *Oilseed rape exposure and effects of landscape composition*

2.2.1 Study Region

The study region is situated in a 40 km radius around Würzburg, Germany. The landscape is dominated by agriculture with cultivation of wheat and barley (together about 50 % of agricultural land). Cultivation of mass flowering oilseed rape (OSR) accounts for about 8 % of agricultural land. Intensive wine-growing is established on sun-exposed hills next to the river Main. Semi-natural habitats (SNH) are present at varying extent and typically represented by flower rich calcareous grassland, extensive meadows and hedges.

2.2.2 Observation hive and bee material

16 glass-sided observation hives, each with two Zander brood frames were used for observations. 16 colonies were built using artificial swarms with young, mated queens (*Apis mellifera carnica*). All queens derived from a single mother and were mated at the same queen-mating station to assure minimal genetic differences between colonies. Brood frames with similar amounts of brood cells, honey and pollen stocks were transferred to each observation hive along with a queen and approximately 4000 worker bees.

2.2.3 Experimental design

We selected 16 circular landscapes with 2 km radius and a minimum distance between landscape centres (observation hive position) of 4 km. Landscape selection aimed to maximize an OSR area gradient and an independent SNH area gradient over all landscapes (Pearson´s product-moment correlation; $r = -0.16$, $n = 16$, $p = 0.55$). OSR and SNH area gradients reached from 0 to 13 percent and 0 to 14 percent of total landscape area, respectively.

2.3. *Monitoring agricultural ecosystems by using wild bees (Schindler et al. 2013)*
Here we describe general requirements and suitable methods for monitoring wild bees in agricultural ecosystems. More detailed information on the application of the suggested harmonised methods in terms of GMO monitoring with wild bees are also transferred to the related guidance document VDI 4332.

2.4. *Measuring bee diversity in different bio-geographic regions*
We systematically evaluated the performance of six sampling methods (observation plots, pan traps, standardized and variable transect walks, trap nests with reed internodes or paper tubes) that are commonly used across a wide range of bio-geographical regions in Europe and in two habitat types (agricultural and semi-natural). We focused on bees since they represent the most important pollinator group worldwide. Several characteristics of the methods were considered in order to evaluate their performance in assessing bee diversity: sample coverage, observed species richness, species richness estimators, collector biases (identified by subunit-based rarefaction curves), species composition of the samples, and the indication of overall bee species richness (estimated from combined total samples).

2.5 *Crop flower visits of wild bees: data base analysis*
Our data sets record the relative visitation rate of bees to crop flowers. We used data from 90 studies and 1,394 crop fields that used standardized protocols to examine the abundance and identity of wild bees visiting flowers of 20 different crops that depend on bee pollinators for maximum yield. We determined species abundance distributions of wild bee communities on insect-pollinated crops by pooling data within studies, that is, from fields sampled in the same year, region and crop species. We only included studies that directly observed individual bees on crop flowers, identified all individuals to species level and that were based on data from at least four fields that were 1 km or more apart. This yielded a total of 90 studies with an average of 15.7 fields per study that were on average 41.7 km apart.

3. Results

3.1 *Maize pollen foraging by honey bee colonies (Danner et al. 2014)*

The study shows that honey bees rely on maize fields as pollen resource in summer when other pollen resources become scarce. Compared to other habitat types, it turned out that flowering maize fields has the highest attractiveness to pollen foraging honey bees (Fig. 1).

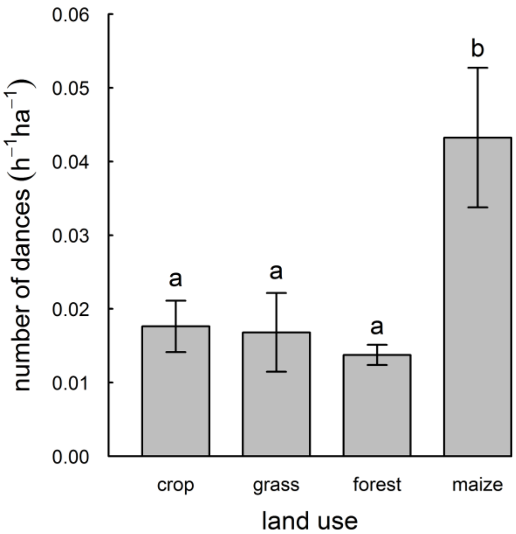

Figure 1: Pollen habitat preferences of bee foragers' in landscapes with maize. Habitat preferences based on dance frequency measured as number of dances per hour observation time and hectare of the corresponding land-use type (different letters indicate significant differences between groups: Tukey post hoc test and "BH" correction $P < 0.05$; n = 614 dances. (Reprinted from Danner et al. 2014)

Foraging distances for pollen in landscapes with different proportions of maize acreage vary between maize pollen and non-maize pollen foraging trips (Tab. 1).

	Foraging distances		
	Total	Maize	Non-Maize
N	662	126	536
Min	52	52	105
Max	4048	2546	4048
Median	595	404	648
Mean	689	521	728
SD	449	345	462
95%	1426	1149	1489

Table 1: Descriptive statistics of foraging distances in metres for maize pollen and non-maize pollen foraging recruitments. N = No. of decoded dances, SD = Standard deviation, 95% = 95% Quantile

3.2. *Oilseed rape exposure and effects of landscape composition (Danner et al. 2015)*

The study demonstrates in spring that an increasing area of flowering oilseed rape within 2 km scale reduced mean pollen foraging distances of honey bee colonies from 1324 m to only 435 m (Fig. 2a). In summer, increasing cover of semi-natural habitat areas close to the colonies (within 200 m radius) reduced mean pollen foraging distances from 846 to 469 m (Fig. 2b). Frequency of pollen foragers per habitat type, measured as the number of dances per hour and hectare, was lower for non-flowering crops than for semi-natural habitat, grassland, oilseed rape fields and settlements (Fig. 3a). In landscapes with a small proportion of semi-natural habitats a significantly higher density of pollen foragers on semi-natural habitat was observed, indicating stronger limitation of pollen resources in simple agricultural landscapes (Fig. 3b, 3c).

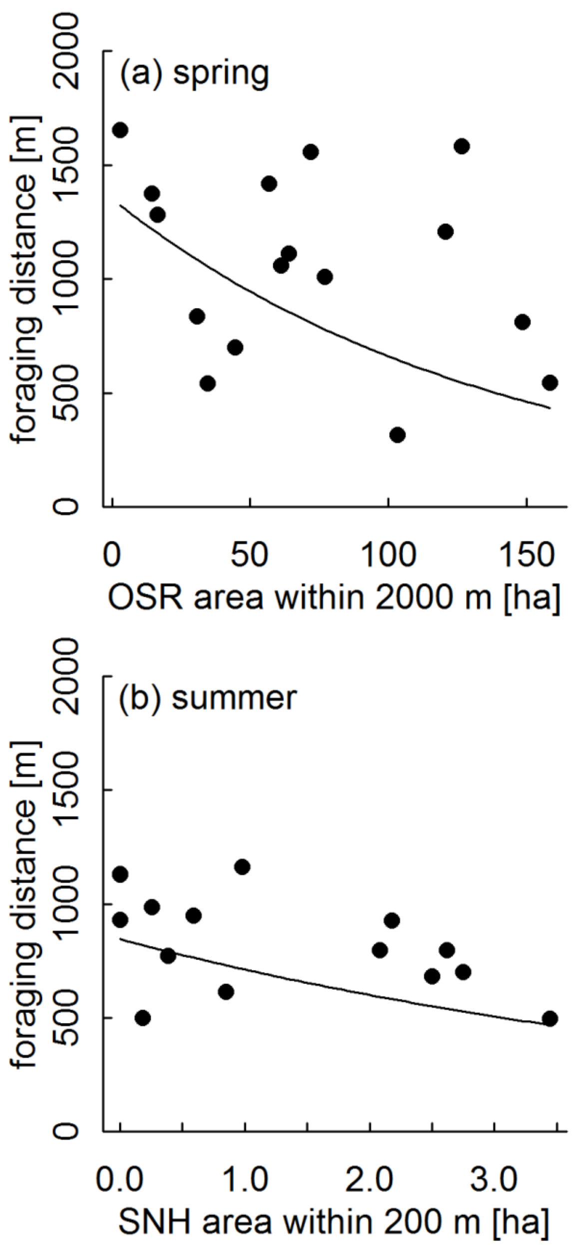

Figure 2: Landscape composition effects on pollen foraging distances. Pollen foraging distances in relation to oilseed rape (OSR) and semi-natural habitats (SNH) area in 16 landscapes are displayed in spring (a) and summer (b). Displayed dots show distance means per landscape while fitted lines derive from analysis of original data (n = 940 dances and n = 407 dances in spring and summer, respectively), back-transformed for plotting (reprinted from Danner et al. 2015).

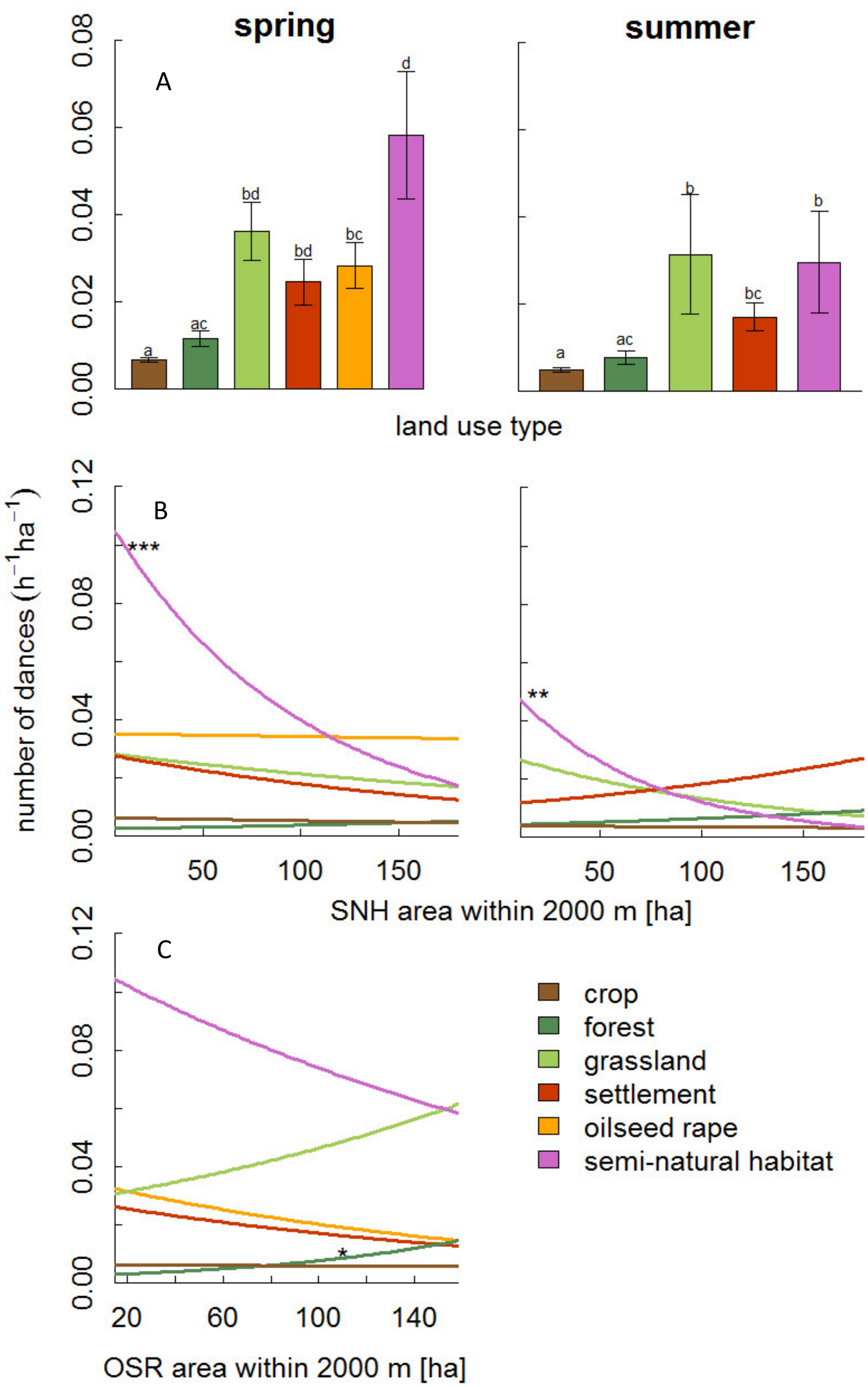

Figure 3: Pollen habitat preferences of bee forager's in landscapes with oilseed rape. Effects of semi-natural habitats (SNH) on pollen foraging preferences for different land use types in spring and summer. Barplots show mean dance frequencies +/- s.e.m. measured as number of dances per hour and hectare (3A). (3B) shows the effect of SNH and (3C) oilseed rape (OSR) area within 2000 m on dance frequency. Crop area in spring excluding OSR, in summer including former OSR fields. Different letters indicate

significant differences. Asterisks indicate slopes significantly different from zero. Model estimates were back-transformed for plotting fitted lines (reprinted from Danner et al. 2015).

3.3. *Monitoring agricultural ecosystems by using wild bees (Schindler et al. 2013)*

We suggest a highly standardized monitoring approach which combines transect walks and pan traps (bowls). The combination of these two methods provides high sample coverage and reveals data on plant-pollinator interactions. We point out that comprehensive methodical, biological and taxonomical expertise is mandatory. The suggested approach is applicable to diverse monitoring goals in an agricultural context e.g. the impact of land use changes as well as monitoring potential effects of GM crops on wild bees.
An analysis of 23 studies of wild bee communities across different agricultural landscapes in Central Europe revealed a total of 293 bee species in agricultural ecosystems. Only 54 of these bee species were found in more than 10 studies (see Table 2).

taxonomic range	**infrequent (1-4 mentions)**	**occasional (5-9 mentions)**	**frequent (10-23 mentions)**	**total**
Colletidae	15	9	2	26
Halictidae	41	18	18	77
Andrenidae	31	18	18	67
Melittidae	5	1	1	7
Megachilidae	33	12	1	46
Apidae	36	20	14	70
total	161 (55%)	78 (27%)	54 (18%)	293 (100%)

Table 2: Number of bee species in agricultural landscapes (according to 23 evaluated studies, (Saure et al. unpublished, reprinted from Schindler et al. 2013).

3.4. *Measuring bee diversity in different bio-geographic regions (Westphal et al. 2008)*

The most efficient monitoring method in all bio-geographical regions, in both the agricultural and semi-natural habitats, was the pan trap method. It had the highest sample coverage, collected the highest number of species, showed negligible collector bias, detected similar species as the transect methods, and was the best indicator of overall bee species richness. The transect methods were also relatively efficient, but they had a significant collector bias. The observation plots showed poor performance. As trap nests are restricted to cavity-nesting bee species, they had a naturally low sample coverage. However, both trap nest types detected additional species that were not recorded by any of the other methods.

3.5 *Crop flower visits of wild bees: data base analysis*

Here we show that, while the contribution of wild bees to crop production is significant, service delivery is restricted to a limited subset of all known bee species. Across crops, years and bio-geographical regions, crop-visiting wild bee communities are dominated by a small number of common species, and threatened species are rarely observed on crops. Dominant crop pollinators seem to persist under agricultural expansion and many can be enhanced by targeted agri-environmental measures, suggesting that cost-effective management strategies to promote crop pollination should target a different set of species than management strategies to promote threatened bees.

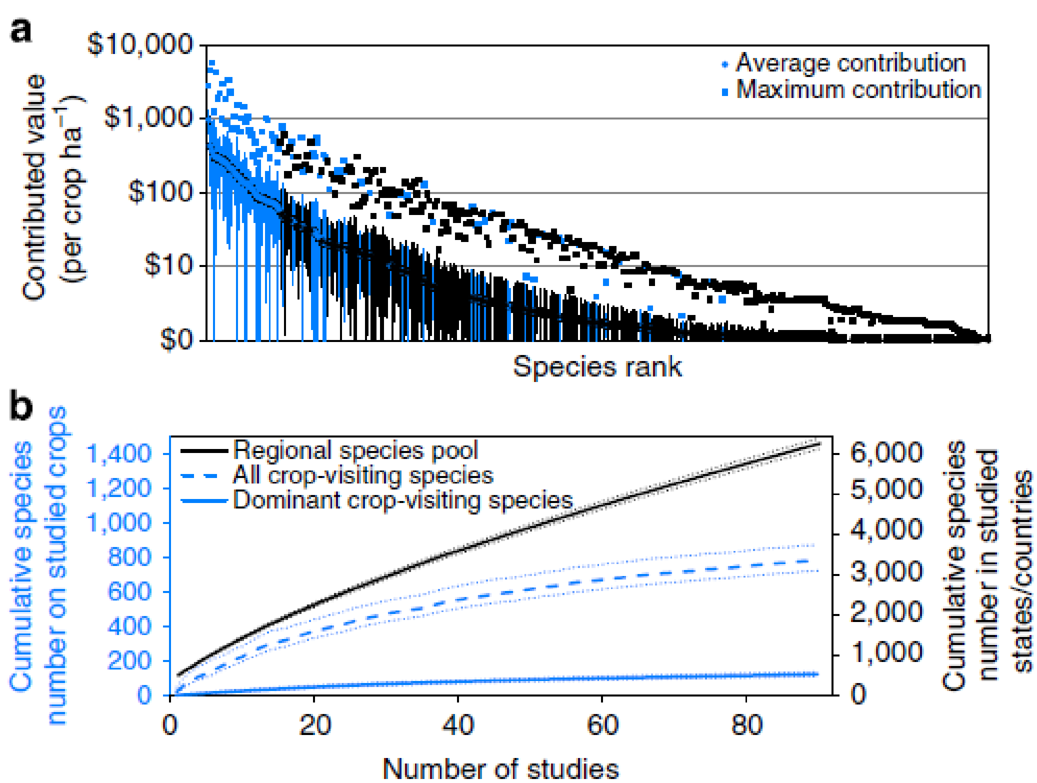

Figure 4: The relative contribution of individual species in wild bee communities to crop pollination. (a) The rank distribution of the contribution of wild bee species to crop production value in their bio-geographical area. Dominant species, contributing at least 5% of all visits within a given study, are indicated in blue. Bars indicate 95% confidence intervals. (b) The cumulative number of bee species known to exist in the countries in which the studies were done, compared with an asymptotic estimate of the number of species that visit the flowers of the studied crops (Chao1 estimator), and the number of dominant crop-visiting wild bee species. Lightly dashed lines indicate estimates ± s.e. (reprinted from Kleijn et al. 2015).

4. Discussion and Conclusions

The presented research in the framework of AMIGA WP6 delivers significant information for a better understanding of exposure scenarios of honey bees to different GM crop pollen species. The data on exposure rates are of broad interest since identified factors of the worldwide observed honey bee decline such as neonicotinoids and other systemic pesticides follow the same exposure pathways into the honey bee hive as we have analysed here for GMO exposure rates. This underpins the importance of landscape ecology research for honey bee health (Härtel and Steffan-Dewenter 2014). The exposure data are also of great value for our understanding of how GM pollen is dispersed by foraging bees in agricultural landscapes, by showing for the first time data on how GM pollen could be distributed to non-GM crop fields by honey bees. The information of the descriptive statistics in Table 1 could be used to define minimum distances between bee hives and GM maize fields to minimise the risk of GM pollen contamination of honey and other bee products. Variation of foraging distances in landscapes with different proportions of crop areas, wild flower areas promoted by agri-environmental schemes, and semi-natural habitats give useful insight of how landscapes could be managed to minimize exposure risks to crop pollen related stressors. The given data about foraging distances for crop flower resources support further the concept of a case-by-case evaluation of potential GM crop mediated environmental risks to honey bees and their pollination services.

The monitoring of wild bees is further improved by underpinning that pan traps and transect walks are the most informative sampling methods for this group of pollinators. The outstanding sampling cover of pan traps and the direct observation of plant-pollinator interactions by transect walks make these methods good candidates for further harmonisation via a guidance document. Additionally, trap nests could be used for environmental monitoring of GM pollen contamination and other pesticide residues in stored pollen and bee larvae and for the long-term monitoring of pollinator population dynamics (Riedinger et al. 2015). The suggested methods can operate efficiently in a wide range of ecological monitoring schemes in agricultural ecosystems. We like to point out that comprehensive methodical, ecological and taxonomic expertise is required for monitoring of wild bee populations across Europe. Further, the compiled data sets on pollinator diversity in major European crops and in different landscape contexts and biogeographic regions are an important resource for the documentation of long-term changes of pollinator communities in European agricultural landscapes.

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