Pollen dearth and poor-quality pollen affect longevity and nursing ability of honeybees in intensive agricultural landscapes

Avant-propos du chapitre 2

La nutrition caractérise une fonction clé pour tous les organismes. Pour l'abeille domestique, *Apis mellifera L.*, le pollen représente l'élément limitant pour son développement, sa santé, et sa survie. En effet, de nombreuses études ont démontré l'importance de l'apport en pollen pour l'abeille (Haydack, 1970; Rinderer and Elliott, 1977 ; Wahl and Ulm, 1983 ; Brodschneider et Crailsheim, 2010 ; Alaux *et al*., 2010a; DeGrandi-Hoffman *et al*., 2010 ; Wang *et al*., 2014). Les colonies sont de plus en plus fréquemment placées à proximité de grandes cultures de manière à ce qu'elles soient approvisionnées par de grandes quantités de pollens. Mais ce type de ressources varie en abondance et qualité dans le temps et l'espace (Decourtye *et al*., 2010). En effet, des périodes de pénuries en pollens suivent la floraison des grandes cultures (Maurizio,1950 ; Louveaux, 1959 ; Odoux *et al*., 2012 ; Requier, 2013). L'effet de ces périodes de pénuries, fournissant à l'abeille du pollen en plus faible quantité, n'a été que très peu étudié. Dans un premier temps nous avons donc testé l'hypothèse selon laquelle ces périodes de déplétion pollinique pouvaient apporter un stress à l'abeille. Pour cela, nous avons nourri des abeilles en laboratoire avec du pollen de culture récolté par des ruches sous serre et nous avons identifié les effets des diverses carences sur la physiologie d'abeilles nourrices (glandes hypopharyngiennes et taux de *vitellogénine*), et sur leur survie.

Durant ces périodes de pénuries, la disponibilité des ressources florales de plantes sauvages prend toute son importance. Or, ces ressources varient en qualité et diversité en fonction du lieu et de la période de butinage. De nombreuses études ont démontré que malgré l'importance de l'apport protéique pour l'abeille, ces dernières ne sélectionnent pas des pollens contenant de plus fort taux de protéines (Levin et Bohart, 1955 ; Pernal et Currie, 2002). En revanche, d'après Schmidt (1984), elles consomment préférentiellement des mélanges plutôt que du pollen monofloral. Les pollens utilisés dans la seconde partie de notre étude sont de vrais échantillons de pollens récoltés par des colonies situées dans un paysage agricole à l'ouest de la France. Ces mélanges recueillis supportent le postulat de Shmidt *et al.* (1984) et plus récemment Odoux *et al.*, (2012) et Requier (2013), que les abeilles sélectionnent des mélanges quand elles en ont le choix, même en présence d'une culture à floraison massive à proximité. Nous avons donc testé dans la seconde partie de cette étude les effets des différentes variétés de pollens (qualité et diversité) récoltées par des abeilles dans un environnement d'agriculture intensive, au cours d'une saison de butinage (de Mai à Septembre) sur la santé des abeilles.

Pollen dearth and poor-quality pollen affect longevity and nursing ability of honeybees in intensive agricultural landscapes

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Abstract

Intensive agricultural systems often expose honey bees (*Apis mellifera L.*) to large temporal variations in the availability, diversity and quality of nutritional resources, with long periods of dearth following the flowering of a main resource-rich mass-flowering crop. Such nutritional irregularity is expected to affect worker health. The positive effects of pollen consumption on workers have largely been documented, but the extent to which the nutritional quality of the pollen mix available in agrosystems can modify honey bee physiology is not clear. We tested the effect of natural pollen shortage and different seasonal pollen mixes on honey bee survival and nursing capacities (hypopharyngeal gland development and *vitellogenin* expression) by feeding them with field harvested pollen diets in different amounts. A drop in pure oilseed rape (*Brassica napus L.*) pollen availability (60 % and more) resulted in a drastic reduction of survival and performance of nursing in a quantitative-dependent manner. Despite some variation in taxonomic diversity and nutritional quality, the pollen mixes harvested over the season had a similar positive influence on bee health, except for the one collected in the late July which induced poor survival and nursing capacities. This period coincided with the mass-flowering of maize (*Zea mays L.*), which produces poor-quality pollen. In intensively farmed agricultural landscapes, periods between the blooming of mass-flowering crops can be stressful

for honey bees due not only to resources depletion but also to poor pollen quality as the pollen available *ad libitum* during the mass flowering of some crops can fail to provide workers with an adequate diet for their development.

1- Introduction

Bee species can be classified into two broad categories regarding their pollen diet: specialists, who feed on a few or even a single plant species, and generalists, who forage on a large array of phylogenetically unrelated plant species (Cane and Sipes 2006). Compared to specialists, generalists usually have a better resilience to environmental changes (Biesmeijer *et al.* 2006). Honey bees (*Apis mellifera L.*), which are extreme generalist, are thus expected to adapt to the human impact on landscape, notably in intensive agricultural landscapes. However, beekeepers have frequently cited starvation and poor foraging conditions as a significant driver of colony losses (Otis, 2007; Van Engelsdorp *et al*., 2007). In addition, Naug (2009) suggested that nutritional stress due to habitat loss plays an important role in honeybee colony losses. The intensification of agriculture often causes a decrease in the diversity of floral resources due to the destruction of natural habitats and the use of monocultures over large areas, but it also affects the quantity of resources available since the flowering period of crops is usually short (Decourtye *et al*., 2010). Such changes in the landscape and thus in spatial and temporal availability of foraging ressources can impact colonies as it has long been recognized that a lack of food, in particular pollen dearth, contributes to weaken colonies (Mattila and Otis, 2006; Maurizio, 1950). Indeed, pollen nutrients (proteins, lipids, vitamins and minerals) are essential to colony development and survival (Brodschneider and Crailsheim, 2010). Several studies have shown in details how the lack of pollen can decrease the longevity (Haydack, 1970; Rinderer and Elliott, 1977, Smeets and Duchateau, 2003; Wang *et al*., 2014), metabolism (Toth, 2005; Fischer and Grozinger , 2008; Willard *et al*., 2011; Alaux *et al*., 2011), immuncompetence (Alaux *et al*., 2010a; DeGrandi-Hoffman *et al*., 2010), and the tolerance threshold of individual honey bee workers to pathogens and pesticides (Rinderer *et al*., 1974; Rinderer and Elliott, 1977; Wahl and Ulm, 1983; Bowen-Walker and Gunn, 2001; Mayack and Naug, 2009; DeGrandi-Hoffman *et al*., 2010). Pollen shortage also hinders the development of hypopharyngeal glands required for the production of brood food (Maurizio, 1950; Loper and Berdel, 1980a, b; Pernal and Currie, 2000; Degrandi-Hoffman *et al.*, 2010). A reduction of brood rearing combined with a shorter lifespan of adults can directly impact the colony

population (Blaschon *et al*, 1999; Schmickl *et al.*, 2003; Keller *et al*, 2005; Mattila and Otis, 2006, 2007; Girard *et al*., 2012). Pollen resources in an agricultural farmland are continuously available, but with considerable variation over time during a season (Maurizio, 1950; Louveaux, 1959; Odoux *et al*., 2012; Requier, 2013). For example, amounts of pollen collected by colonies in an agrosystem in western France follow a very irregular pattern, with clear shortage periods (Steffan-Dewenter and Kuhn, 2003; Decourtye *et al*., 2010; Odoux *et al*., 2012; Requier, 2013). These temporal variations in the harvest of pollen also depend a lot on the apiary location (Louveaux, 1959; Duelli and Obrist, 2003). Furthermore, pollen quality and diversity can also affect the size of hypopharyngeal glands and worker longevity (Schmidt *et al*, 1984, 1987; Tasei and Aupinel, 2008; Di Pasquale *et al*., 2013). And, the diversity of pollen species harvested by honey bee colonies shows also considerable temporal variation (Dimou and Thrasyvoulou, 2007; Decourtye *et al*., 2010; Wratten *et al*., 2012).

In order to better understand how the availability of pollen resources (quantity, quality and diversity) in an agricultural landscape can influence honey bee health, we fed workers with environmentally-relevant diet. We first tested the effect of pollen shortage by providing bees with controlled amount of pollen encountered in the environment during a period of low food supply. Whether a threshold amount of pollen is required for developing nurse physiology or the response is gradual could also be determined. Then, by feeding workers with representative pollen mixes harvested in an intensive agricultural area across different seasons, we assessed the influence of diet diversity and quality over time and determined whether some time frame are critical for honey bees or, conversely, if there is no stress period. The impact of the different diet on the survival and nurse physiology (Toth and Robinson, 2005; Toth *et al*., 2005) was determined. For that purpose, we measured the development of hypopharyngeal glands and the level of vitellogenin. Nurses secrete 60 to 80 % of the brood diet from their hypopharyngeal glands, providing a secretion rich in protein for larvae (in Winston, 1987). Vitellogenin is the main storage protein in the haemolymph and precursor for many other proteins in the honey bee (Amdam *et al*., 2003). This lipoprotein synthesized by the fat body was found to act as an antioxidant to promote longevity in both queen and worker bees (Seehuus *et al*., 2006; Corona *et al.*, 2007). In addition, *vitellogenin* is used by nurses for the production of brood food (Amdam *et al.*, 2003, Seehuus *et al*., 2007).

2- Materials and methods

Bee rearing

The experiment was performed on 1-day-old workers. They were obtained from three colonies by placing brood combs with late-stage pupae into an incubator at 34°C and 50 – 70 % of humidity. Bees that emerged within 10 hours were collected and mixed before placing them in cages (10.5 cm x 7.5 cm x 11.5 cm) at 34°C and 50 – 70 % of humidity in an incubator. They were provided *ad libitum* with candy (Apifonda® + powdered sugar) and water throughout the experiment. Fresh pollen diets were prepared according to environmentally-relevant diet and were supplied to the bees from day 1 for 9. Each day, pollen diets were weighed to determine the amount of pollen consumed per bee, per day and per modality. If a bee died during the pollen feeding period, it was removed and the amount of pollen was adjusted daily to the number of surviving bees.

Influence of pollen shortage on nurse worker physiology and survival

To assess the influence of pollen shortage experienced by colonies in agrosystems, we fed bees with controlled amount of pollens. Pollen quantities were estimated from a recent study, which analyzed pollen collection in an agricultural landscape of western France (Requier, 2013). From April to October, the weight of pollen collected by colonies followed a bimodal seasonal trend, marked by a low food supply period between two oilseed mass-flowering crops (May for oilseed rape and July for sunflower). This dearth period was characterized by a median value of pollen weight loss of 66 %. On average, the pollen supply of colonies was then reduced of 40 % compared to the peak during the oilseed rape blooming period. Considering this variability of pollen availability under field conditions, we determined bee nursing capacities and survival along a decreasing gradient of consumed pollen quantities: 100 %, 40 %, 30 %, 20 %, 15 %, 7 % and 0 %. The pollen quantity consumed under the *ad libitum* feeding condition was recorded as the "100 %" modality (average of 3.6 mg / bee / day). Groups of 44 one-day old bees were reared in cages and fed with the pollen diet. The trial with each diet modality was replicated ten times. The pollen of oilseed rape (*Brassica napus*, commercial F_1 hybrid 'NK Fair'®, Syngenta, Basel, Switzerland) was chosen because of its high occurrence in the cereal farmland systems (Odoux *et al*., 2012). The pollen was collected on plants grown under two 24 m long x 8 m wide plastic tunnels with the openings covered with insectproof screening to prevent any contamination by pesticides or other pollutants on two hives introduced in the tunnel at the onset of flowering and continuously fitted with bottom pollen traps that were emptied daily. The pollen pellets obtained were stored at - 20°C until use.

Influence of "seasonal" diversity and quality of pollen mixes on nurse worker physiology and survival

In this experiment, we tested the effect on worker health of pollen mixes collected by colonies in an agricultural landscape of western France. The different pollen mixes found across different seasons were obtained from experiments conducted in 2006 (Odoux *et al*., 2012). Workers were fed with 6 pollen mixes collected by colonies from early May to late September in an apiary located at 46 ° 09′ 13″ N; 0 ° 41′ 20″ O. The pollen composition of each mix is presented in Figure 1. Each pollen mix was provided *ad libitum* to the bees (10 cages of 47 bees per diet). The protein and lipid levels of the different mixtures were determined after the pollen was dried for 24 h at 75°C (Louveaux, 1959) and the protein and lipid contents were expressed as percent of dry matter (Figure 1). The protein content was determined by Kjeldahl analysis (N x 6.25) using a Vapodest 45 (Gerhardt) and according to the procedure ISO 5983-2 norm (ISO 5983, 1997). After treating pollen with with hydrochloric acid (HCl 6N), total lipids were extracted with a chloroform / methanol mixture (2:1, v / v) (Folch *et al*., 1957).

The presence of pesticide residues in the different pollen diets was determined by Phytocontrol laboratory (Nimes, France) with gas and liquid chromatography (limit of quantification of 0.01 mg / kg and limit of detection of 0.005 mg / k (AFNOR15662, 2009 ; Table 1). The samples were analyzed for 348 pesticides (Table S1).

Pollen sample	Pesticides	Class	Quantity (mg/kg)
	Aclonifen	herbicide	0.032
	Cyprodinil	fungicide	0.012
	Flusilazole	fungicide	0.013
Early May	Metolachlor	herbicide	0.014
Late May	Flusilazole	fungicide	0.015
	Flusilazole	fungicide	< 0.01
Early June	Pyrimethanil	fungicide	< 0.01
Late July	/	/	/
Late August	Trifluraline	herbicide	0.06
Late September	Trifluraline	herbicide	< 0.01

Table 1: Pesticide residues in the pollen samples used for diets.

The presence of pesticide residues in the different pollen diets was assessed with a limit of quantification of 0.01 mg / kg and a limit of detection of 0.005 mg / kg.

Finally, to estimate the diversity gradient of pollen mixes, we calculated the Shannon Weaver index for each mix, as $H' = -\sum p_i \log_2 p_i$, where p_i was the amount of each pollen species represented in the mixture (Shannon and Weaver, 1949).

Physiological analysis

For both experiments, 3 bees per cage were flash frozen in liquid nitrogen at day 10, and the heads and abdomens were stored at – 80°C. The right and left hypopharyngeal glands were dissected on ice in 100 µl of physiological serum (0.9 % NaCl w / w) and analyzed with an optical microscope coupled to a camera (CF 11 DSP, Kappa). The maximum diameter of 15 randomly chosen acini per gland was used to determine the development of hypopharyngeal glands (n = 30 acini per bees). This was performed with the Saisam 5.0.1 software (Microvision®). The 3 abdomens from each cage were pooled. RNA was extracted and the expression level of *vitellogenin* was determined by quantitative PCR using a StepOne-Plus Real-Time PCR Systems (Applied Biosystems®) and the SYBR green detection method as in Di Pasquale *et al*. (2013). The influence of pollen shortage and seasonal mixes on bee survival was studied on remaining bees (41 and 44, respectively). Dead bees were counted daily and removed from the cages until the last worker died (60 or 90 days, respectively).

Statistical analysis

Since the data on hypopharyngeal gland size were not normally distributed, the effects of pollen shortage and seasonal mixes on these parameters were determined using a Kruskal-Wallis test followed by Wilcoxon tests with Bonferroni correction. *Vitellogenin* expression followed a normal distribution, we therefore used a one-factor ANOVA followed by Tukey post-hoc tests. For analysing the influence of feeding modalities on bee survival, the number of dead bees per day and cage throughout the experiments (70 or 90 days) were transformed in a survival table. A cox proportional hazards regression model was then used to compare the different modalities, with R functions (coxph) and the package [survival] (Cox, 1972). Finally, differences in the consumption of seasonal mixes were assayed using a Kruskal-Wallis test followed by Wilcoxon tests with Bonferroni correction. All analyses were performed on the statistical software R (2013).

3- Results

Influence of pollen shortage on nurse worker physiology and survival

Worker survival was highly dependent on the amount of pollen consumed. The more pollen bees received, the longer they lived with all quantitative modalities being different from each other (Cox proportional hazards regression model, $p < 0.001$, Figure 2).

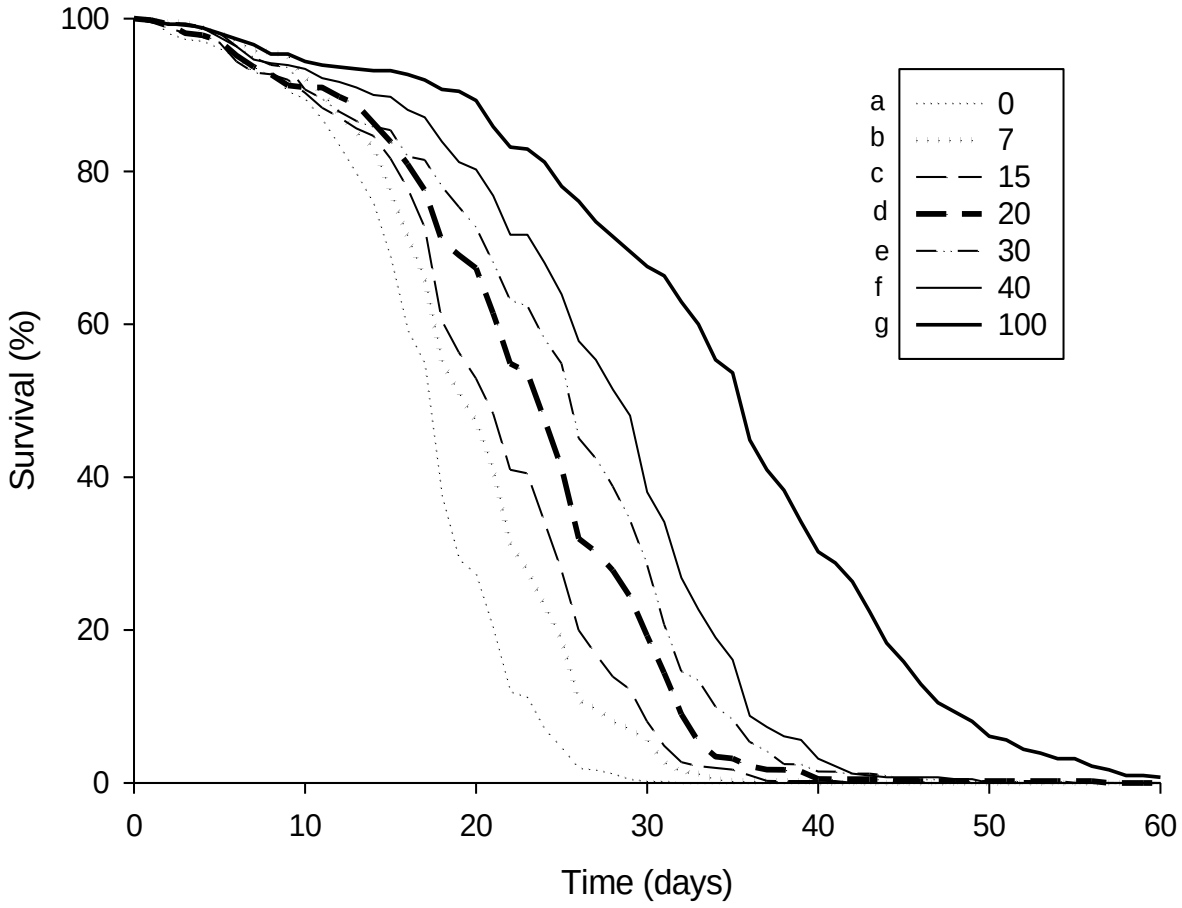

Figure 2: Effect of pollen shortage on worker survival. Data show the percentage of surviving workers over 60 days (n = 10 per modality) in function of quantities of pollen consumed (in %). Different letters denote significant differences ($p < 0.001$, Cox proportional hazards regression model).

The size of the hypopharyngeal glands and the expression level of *vitellogenin* were significantly affected by the quantity of pollen provided (hypopharyngeal glands: Kruskal-Wallis test, $H = 160.70$, $p < 0.001$, Figure 3A; *vitellogenin*: ANOVA test, $F = 24.51$, $df = 60$, $p < 0.001$, Figure 3B). The acini size and the level of *vitellogenin* also increased gradually with the amount of available pollen. However, bees who received the lowest amount of pollen (7 %) had glands that did not differ in size from bees that had a pollen-free diet. Similarly, low quantity of pollens (7 to 30 %) did not induce significant changes in *vitellogenin* levels as compared to bees that received no pollen at all.

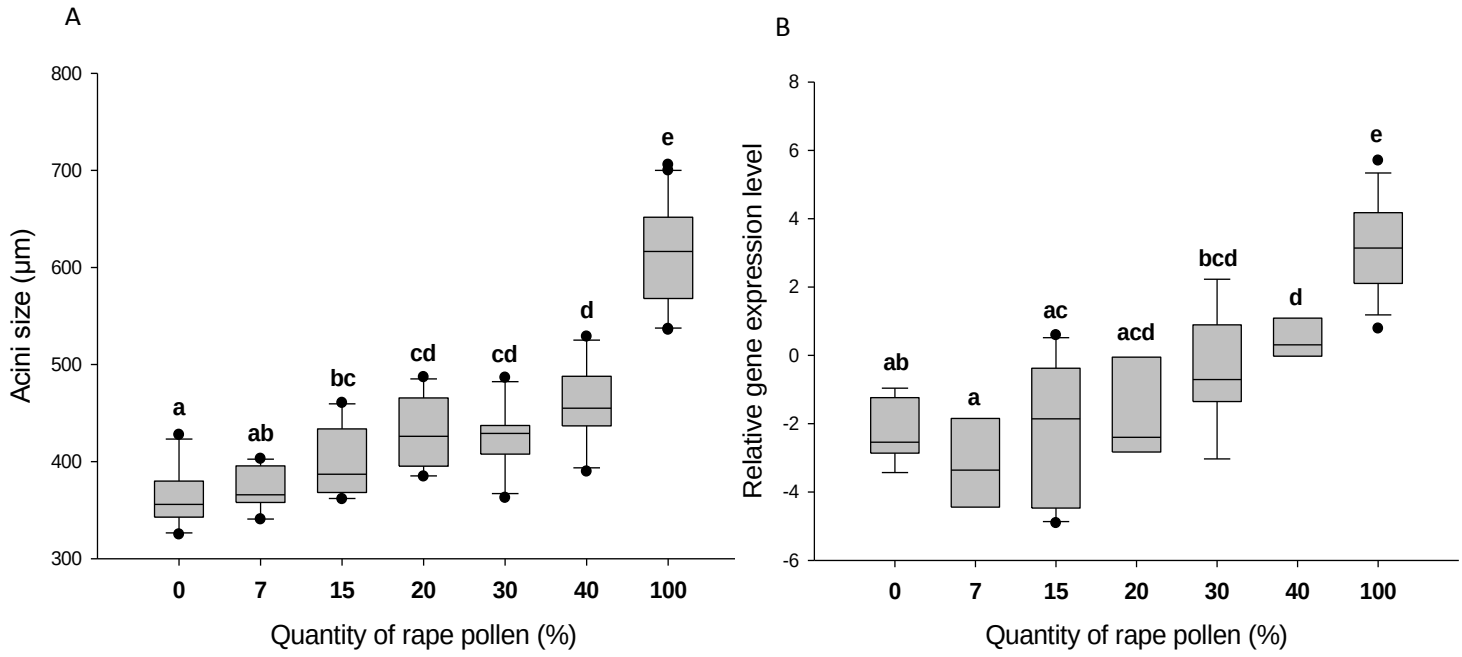

Figure 3: Effects of pollen shortage on (A) the size of hypopharyngeal gland acini, (B) *vitellogenin* expression levels, in honeybee. Box plots are shown for 30 bees (glands) and 10 pools of 3 bees (*vitellogenin*) for each diet modality. Different letters indicate significant differences between pollen quantities ($p < 0.001$, Wilcoxon tests with Bonferroni correction for hypopharyngeal glands and Tukey post-hoc tests for *vitellogenin* expression levels, respectively). Boxes show 25th and 75th percentiles range with line denoting median. Whiskers encompass 90% of the individuals, beyond which outliers are represented by dots.

Influence of "seasonal" diversity and quality of pollen mixes on nurse worker physiology and survival

The levels of proteins and lipids in the different pollen mixes ranged from 17 to 29 % and from 7 to 14 %, respectively, with the mix of "late July" having the lowest pollen quality (Figure 1). Pollen mixes from "late July" were essentially composed of crop pollens as compared to others periods, where pollen from mainly wild flower were found. The diversity of pollen mixes (Shannon index) varied among period, but there was no clear decrease in any of them. Finally, we found some pesticide residues above the detection limit in the pollen mix from the different seasonal periods. However, no residue was found in the "late July" mix.

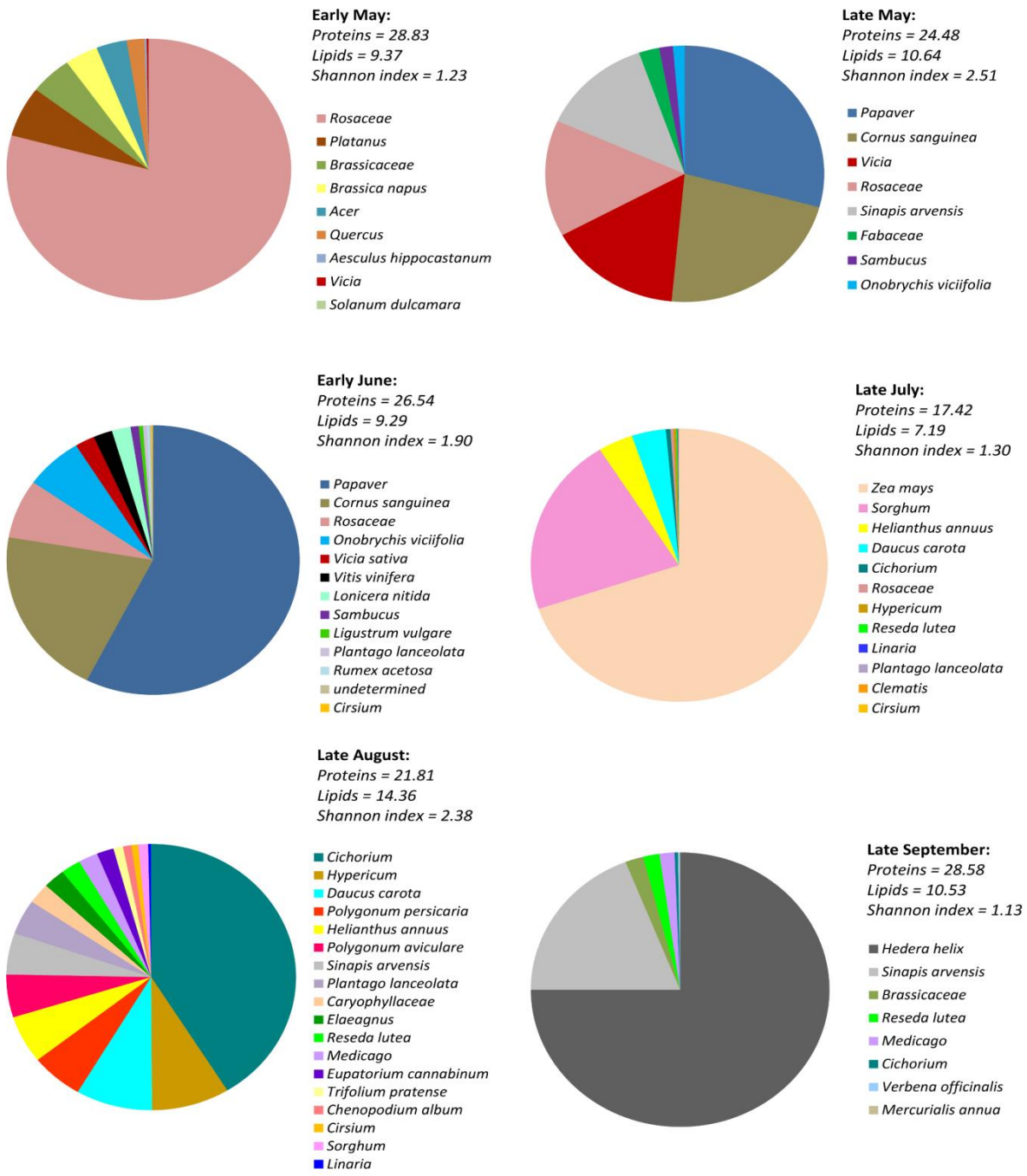

Figure 1: Composition and quality value parameters of pollen seasonal mixes harvested in an agricultural landscape of western France (46 ° 09′ 13″ N; 0° 41′ 20″ O). Pollen proteins and lipids are expressed as percentage of pollen dry matter. Shannon Weaver index were calculated for each mix, as $H' = -\sum p_i \log_2 p_i$, where p_i was the amount of each pollen species represented in the mixture

Pollen mixes were provided *ad libitum*, but they were not consumed equally by workers (Kruskal-Wallis test, $H = 46.64$, $p < 0.001$, Figure 4). Notably, the mix from "late July" was poorly consumed (less than 4 mg/bee/day), but its average consumption was not different from the mix of "early May" and "early June".

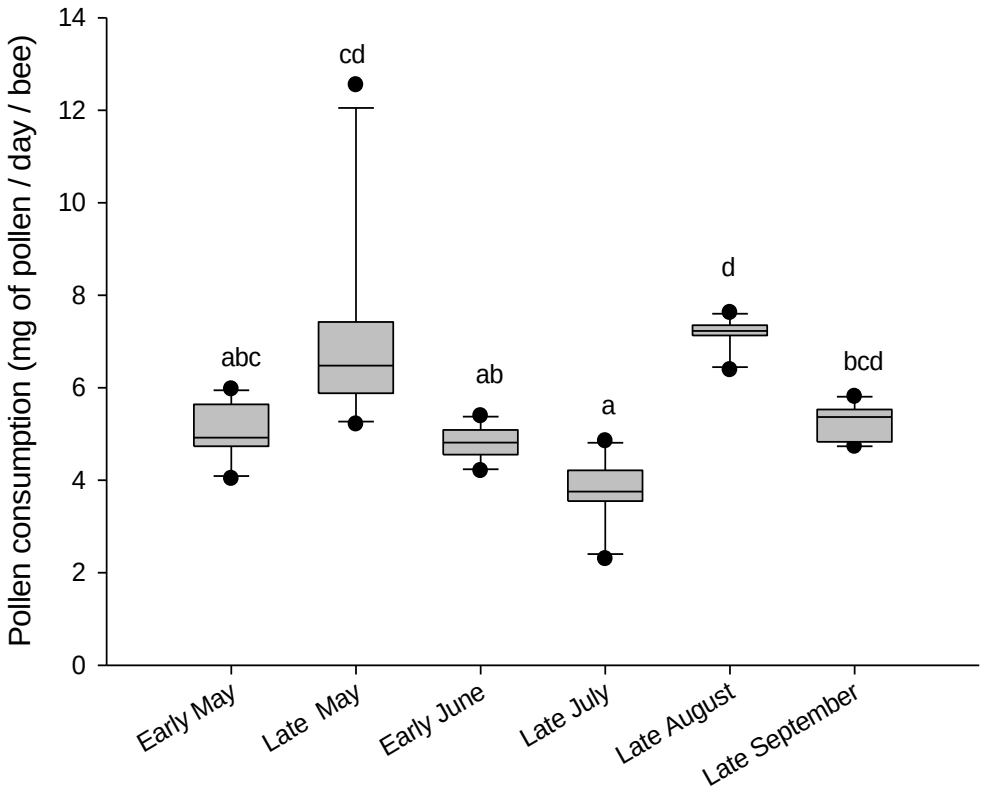

Figure 4: Amounts of pollen consumed by bees. Box plots are shown for 10 cages per modality. Different letters indicate significant differences between the amounts of pollen consumed ($p < 0.001$, Wilcoxon test with Bonferroni correction). Boxes show 25th and 75th percentiles range with line denoting the median. Whiskers encompass 90% of the individuals, beyond which each outliers are represented by circles.

Most pollen mixes induced slight changes in worker survival (Figure 5). However, workers provided with the "late July" mix exhibited a dramatic reduction of longevity as compared to all others mixes. Similarly, the hypopharyngeal glands development and *vitellogenin* expression were the lowest in the workers fed with the "late July" mix (Kruskal-Wallis test, $H = 31.35$, $p < 0.001$, Figure 6A and ANOVA test, $F = 5.01$, 62 DF, $p < 0.001$, Figure 6B, respectively. Early June, late August and September mixes gave the largest acini, but there was no difference in *vitellogenin* expression between the different mixes besides "late July". Finally, we found a strong correlation between the level of *vitellogenin* and the survival recorded as the day at which 50 % of the workers died in each cage) (Spearman coefficient = 0.554, $p < 0.001$).

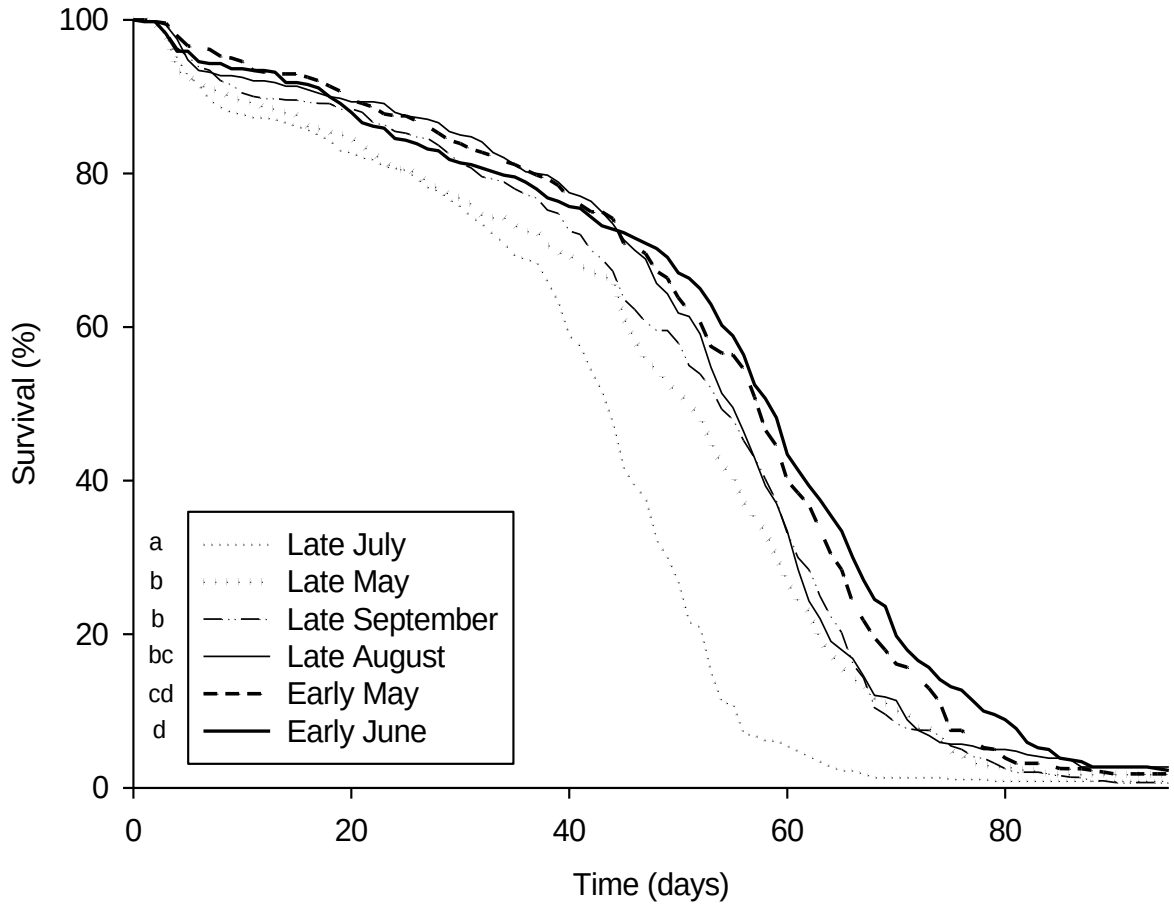

Figure 5: Influence of pollen seasonal mixes on bee survival. Data show the percentage of surviving workers over 90 days (n = 10 per modality). Different letters denote significant differences ($p < 0.001$, Cox proportional hazards regression model).

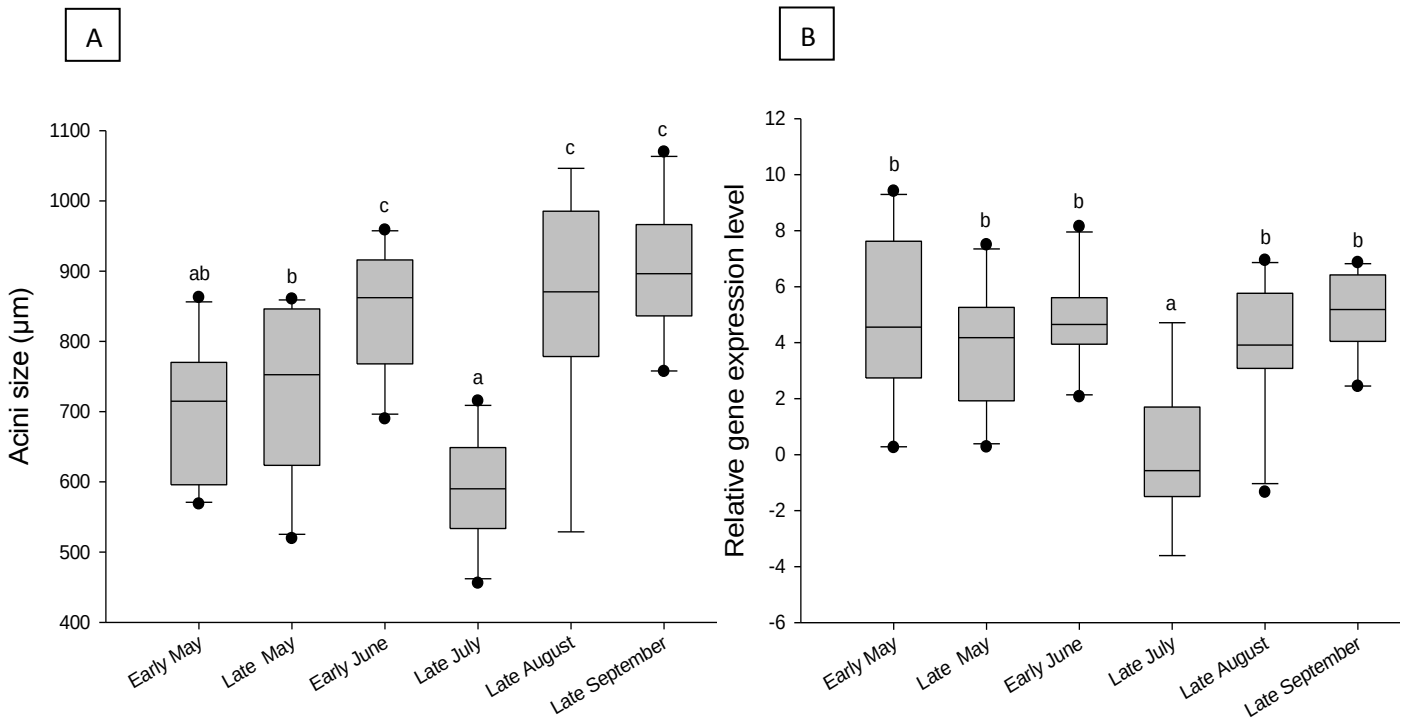

Figure 6: Influence of the seasonal pollen seasonal mix on (A) the size of hypopharyngeal gland acini, (B) the *vitellogenin* expression levels, in honeybee. Box plots are shown for 30 bees (glands) and 10 pools of 3 bees (*vitellogenin*) per diet modality. Different letters indicate significant differences between pollen quantities ($p < 0.001$ based upon Wilcoxon tests with Bonferroni correction for hypopharyngeal glands and Tukey post-hoc tests for *vitellogenin* expression levels). Boxes show 25th and 75th percentiles range with line denoting median. Whiskers encompass 90% of the individuals, beyond which each outlier are represented by dots.

4- Discussion

Despite the extreme flexibility of their pollen foraging behavior and the considerable breadth of their pollen diet, it is often believed that the development of intensive agriculture can have negative impacts on honey bee colonies. However, the extent to which the modifications of the floral landscape affect workers is not easy to analyze. Indeed, there are many other sources of variability, beyond resource availability, that can affect bees in the field, such as climate, pests and diseases, genetic, and pesticides (Van Engelsdorp *et al.,* 2009; Van Engelsdorp and Meixner, 2010). Here, by feeding bees in controlled conditions with environmentally relevant diets in standard conditions, we were able to assess the effects of some components of the nutritional stress linked to agrosystems on honey bee health, all other things being equal.

Influence of pollen dearth on nurse worker physiology and survival

Periods of resource shortages are commonly observed in intensive agricultural landscapes between the blooming periods of major mass-flowering crops such as oilseed rape and sunflower (Steffan-Dewenter and Kuhn, 2003; Requier, 2013). It is well-established that the availability of pollen is important for worker survival (Haydack, 1970; Di Pasquale *et al*., 2013; Wang *et al*., 2014), but we found here that natural shortages of up to 60 % and more can quickly reduce worker survival. As an example, a loss of 10 % of pollen caused an average decrease of 2 - 3 days in bee longevity, indicating that longevity is highly dependent on nutrients quantity and not governed by a threshold model (Figure 2).

Nursing capacities were also affected by pollen shortage. Our results indicate that those capacities are not regulated through quantitative thresholds of pollen availability. This was observed with the development of hypopharyngeal glands (Figure 3A). There was no minimal

amount under which, gland did not develop at all and above which glands almost fully developed. Although 7 % of the *ad libitum* consumption did not succeed in initiating the development of glands compared to the controls. The development of hypopharyngeal glands is highly dependent on protein intake (Maurizio, 1950; Pernal and Currie, 2000) so it might not be surprising to find that the more workers consumed pollen, the more proteins were directed to the acini. Fernandes-da-silva and Zucoloto (1993) also tested the effect of pollen on the development of hypopharyngeal glands in the stingless bee *Scaptotrigona depilis Moure* (*Hymenoptera, Apidae*). By testing three amounts of pollens (0.25 g, 0.50 g and 0.75 g for 8 days), they found that the two lowest amounts were not sufficient for a good development of glands. Based on these results, they calculated that at least 0.80 mg pollen / day / bee would be necessary to provide sufficient gland development, and this figure was equivalent to the 20 % modality in our tests. Yet in honey bees, access to only 20 % of the full pollen intake resulted in a poor development of hypopharyngeal glands.

Similarly, the *vitellogenin* expression tended to increase gradually with the amount of pollen available. Bitondi and Simoes (1996) tested the effects of 0 %, 15 % and 50 % of pollen diet on *vitellogenin* expression level in Africanized honey bees. They found that workers that received 50 % of pollen had more *vitellogenin* than those that received only no or only 15 %, of pollen. This is consistent with our results since the workers that received 15 % and 40 % of pollen expressed significant differences in vitellogenin expression. However, contrary to hypopharyngeal glands, 40 % of pollen was the minimum required for obtaining an expression significantly higher than a pollen-free diet. This indicates that some tissues, like *fat bodies*, the main site of *vitellogenin* synthesis (Amdam *et al.*, 2005) are quantitatively more demanding in nutrients than others (such as hypopharyngeal glands) for initiating a nursing physiology.

Influence of "seasonal" diversity and quality of pollen mixes on nurse worker physiology and survival

Despite major changes in pollen composition and quality of the mixes among the seasons, survival and nursing capacities were weakly affected by the different diets, provided they were found *ad libitum*. However, the "late July" mix consistently caused a significant reduction of longevity and nursing capacities. Those negative effects could be attributed neither to the presence of pesticide residues since this pollen mixture was the only one that was not contaminated by any of the ones assayed (at a detection limit of 0.005 mg / kg), nor to its diversity index, which was above two others diets. It turned out that this mix had the lowest

percentage of lipids and proteins, both of which are essential for worker health (Haydack, 1950; Pernal and Currie, 2000; Manning, 2001), and this mix was also the least consumed. The "late July" period corresponded to the flowering of maize (*Zea may*s) in north western of France, as confirmed by the presence of 69.8 % of maize pollen in the mix Maize produces pollen of poor nutritional quality that is deficient in histidine, although the amount of other essential amino acids can be high (Pernal and Currie, 2000; Höcherl *et al*., 2012). Its high prevalence in the Late July mix likely accounts for the low quality of this diet. Jacobs (2004) showed that consumption of corn pollen had a negative impact on the longevity of *Apis mellifera L.* bees reared in experimentation cages. Similarly, Höcherl *et al*. (2012) observed that bees fed with maize pollen show a reduction in brood rearing ability and shortened lifespan. However, they did not find an effect on bee immunocompetence. The availability of different floral resources is expected to compensate for the low quality of specific pollens (Di Pasquale *et al.* 2013). However, the predominance of maize pollen did not allow such phenomenon. Either the flowering landscape did not provide enough qualitative resources (forcing bees to forage mostly on maize), or this pollen, despite its low nutritional quality, is too attractive for colonies during this period of late July.

The variability of nutritional landscape in agrosystems can expose bees to dearth periods and / or deficiency in diet quality. As a consequence, such nutritional stress can directly impact bee longevity and nursing capacities. In field conditions, it could lead to a higher turn-over between cohorts of bees and a suboptimal brood rearing by malnourished nurses (Loper and Berdel, 1980b). The colony productivity can then be affected. Providing bees with a great diversity of floral resources, such as grass strips, fallow land, or a semi-natural environment, represents one way to overcome any nutritional stress across the seasons (Decourtye *et al*., 2010).

ACKNOWLEGMENTS

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Annexes du chapitre 2

1-naphtyl	Desmedipham	Fuberidazole	Picoxystrobine
2-Phenylphenol	Desmetryn	Furalaxyl	Pinoxadene
Acephate	Diafenthiuron	Furathiocarb	Piperonyl
Acetamipride	Dialifos	Halosulfuron-methyl	Pirimicarb
Aclonifen	Diallate	HCB	Pirimiphos-ethyl
Acrinathrine	Diazinon	HCH	Pirimiphos-methyl
Alachlore	Dichlobenil	HCH	Prochloraz
Aldicarb	Dichlofenthion	Heptachlore	Procymidone
Ametryn	Dichlofluanide	Heptenophos	Profenophos
Amitraze	Dichlorvos	Hexaconazole	Prometryn
Anthraquinone	Diclobutrazol	Hexazinone	Propachlor
Atrazine	Diclofop-methyl	Hexythiazox	Propamocarb
Azaconazole	Dicofol	Hydramethylnon	Propanil
Azimsulfuron	Dieldrin	Imazalil	Propaquizafop
Azinphos-ethyl	Diethofencarb	Imazaquin	Propargite
Azinphos-methyl	Difenacoum	Imidachlopride	Propazine
Azoxystrobine	Difenoconazole	Indoxacarb	Propetamphos
Benalaxyl	Diflufenican	Iodofenphos	Propham
Benfluraline	Dimetachlor	Iprodione	Propiconazole
Benfuracarb	Dimethenamid-P	Iprovalicarb	Propoxur
Benoxacor	Dimethoate	Isofenphos-ethyl	Propyzamide
Bensulfuron-methyl	Dimethomorphe	Isofenphos-methyl	Prosulfocarb
Benthiavalicarb-isopropyl	Diniconazole	Isopropaline	Prosulfuron
Bifenazate	Diphenylamine	Isoprothiolane	Prothioconazole
Bifenox	Disulfoton-sulfone	Isoproturon	Prothiophos
Bifenthrine	Diuron	Isoxaflutole	Prothoate
Biphenyl	DMST	Isoxathion	Pyraclostrobine
Bispyribac-Sodium	Dodine	Kresoxim-methyl	Pyraflufen-ethyl
Bitertanol	Edifenphos	Lenacil	Pyrazophos
Boscalide	Emamectin	Linuron	Pyridaben
Bromacil	Endosulfan	Lufenuron	Pyridaphenthion
Bromophos-ethyl	Endrin	Malathion	Pyridate
Bromophos-methyl	Epoxyconazole	Mandipropamide	Pyrimethanil
Bromopropylate	EPTC	Mecarbam	Pyriproxyfen
Bromuconazole	Ethidimuron	Mepanipyrim	Quinalphos
Bupirimate	Ethion	Mesosulfuron	Quinomethionate
Buprofezin	Ethoprophos	Metalaxyl	Quinoxyfen
Butafenacil	Ethoxyquin	Metamitron	Quintozene
Butoxycarboxim	Etoxazole	Metazachlor	Quizalofop-ethyl

Butralin	Etrimphos	Metconazole	Rotenone
Buturon	Famoxadone	Methabenzthiazuron	Sebuthylazine
Cadusaphos	Famphur	Methamidophos	Simazine
Captafol	Fempropathrine	Methidathion	Spinosad
Captan	Fenamidone	Methiocarb-sulfoxide	Spirodiclofen
Carbaryl	Fenamiphos	Methomyl	Spiromesifen
Carbendazim	Fenamiphos-sulfone(+sulfoxide)	Methoxychlor	Spiroxamine
Carbetamide	Fenarimol	Methoxyfenozide	Sulfosulfuron
Carbofenothion	Fenazaquin	Metobromuron	Sulfotep
Carbofuran	Fenbuconazole	Metolachlor	TCMTB
Carbosulfan	Fenchlorphos	Metoxuron	Tebufenozide
Carboxin	Fenitrothion	Metrafenone	Tebufenpyrad
Chlorbenside	Fenoxaprop-ethyl	Metsulfuron-methyl	Tebutam
Chlordane	Fenoxycarbe	Mevinphos	Tecnazene
Chlorfenson	Fenpiroximate	Molinate	Tefluthrine
Chlorfenvinphos	Fenpropidine	Monalide	Tepraloxydim
Chloridazon	Fenpropimorphe	Monocrotophos	terbufos
Chlorobenzilate	Fenson	Monolinuron	Terbufos-sulfoxide
Chlorothalonil	Fensulfothion-oxon	Monuron	Terbumeton
Chloroxuron	Fenthion (+sulfone+sulfoxide)	Myclobutanil	Terbuthylazine
Chlorpropham (+3Chloroanilin)	Fenthion-oxon (+sulfone+sulfox.)	Napropamide	Terbutryne
Chlorpyrifos	Fenuron	Neburon	Tetrachlorvinphos
Chlorpyrifos-methyl	Fenvalerate	Nicosulfuron	Tetraconazole
Chlorthal	Fenvalerate	Nitrofen	Tetrahydrophtalimide
Chlorthiamid	Fipronil	Norflurazon	Tetramethrine
Chlorthiophos	Flazasulfuron	Novaluron	Thiabendazole
Chlortoluron	Flonicamid	Nuarimol	Thiachloprid
Chlozolinate	Fluazifop	Oxadiazon	Thiamethoxam
Cinosulfuron	Fluazinam	Oxadixyl	Thiophanate-methyl
Clethodim	Fludioxonil	Oxamyl	Tolclofos-methyl
Clodinafop	Flufenacet	Oxasulfuron	Tolylfluanid
Clofentezine	Flufenoxuron	Oxyfluorfen	Transfluthrin
Clomazone	Fluometuron	Paclobutrazol	Triadimefon
Cloquintocet	Fluoxastrobin	Paraoxon-ethyl	Triallate
Coumaphos	Fluquinconazole	Parathion-ethyl	Triazamate
Cyanazine	Flurochloridone	Parathion-methyl	Triazophos
Cyazofamide	Fluroxypyr-methylhexyl	Pencycuron	Trichloronat
Cycloxydime	Flurtamone	Pendimethaline	Tricyclazole
Cycluron	Flusilazole	Permethrine	Tridemorphe
Cyfluthrine	Fluthiacet-methyl	Perthane	Trifloxystrobine

Cyhalofop-butyl	Flutriafol	Phenmedipham	Trifloxysulfuron
Cyhalothrine	Fluvalinate	Phenothrine	Triflumizole
Cypermethrine	Folpet	Phosalone	Trifluraline
Cyprodinil	Fomesafen	Phosmet	Triflusulfuron-methyl
DDT	Fonofos	Phosphamidon	Triforine
Deltamethrine	Foramsulfuron	Phoxim	Triticonazole
Demeton-S	Forchlofenuron	Phtalimide	Vinclozoline (+3,5dichloroanilin)
Demeton-S-methyl	Formetanate	Picolinafen	Warfarin

Table S1. List of pesticides analyzed in the pollen diets.