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## Territorial biodiversity and consequences on physico-chemical characteristics of pollen collected by honey bee colonies

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**Abstract** – Pollen resources may become a constraint for the honey bee in cereal farming agrosystems and thus influence honey bee colony development. This survey intended to increase knowledge on bee ecology in order to understand how farming systems can provide bee forage throughout the year. We conducted a 1-year study to investigate the flower range exploited in an agrarian environment in western France, the physico-chemical composition of honey bee-collected pollen, the territorial biodiversity visited by the bee at different periods, and the relationships between these three datasets. Palynological analyses showed the importance of maize among crop pollens and that of weeds during the food shortage period. Pollen protein varied from 16% to 29% and lipids from 7% to 24%. The contribution of different habitats to pollen harvest, was from crops (62%), woods (32%), grasslands (4%), and gardens (1%).

**agrosystem / honeybee / floral biodiversity / pollen analysis / chemical analysis**

### 1. INTRODUCTION

Since 1962, agrosystems have been considerably intensified due to the Common Agricultural Policy and farming systems represent the major land use (e.g., 46% in France) (Agreste 2011). This intensification results in the standardization of land use (in particular, grasslands are replaced by cereals), in the mechanization of farming practices, and in the increasing use of inputs (fertilizers, pesticides, etc.) thus creating environmental problems (Benton et al. 2003). Agrarian landscapes are complex anthroposystems, consisting of a mosaic of different elements: natural

(forest massifs), semi-natural (forest fragments and hedgerows), and artificial (set aside lands, crops, and urban areas including gardens).

All agrosystems host a number of pollinator insects which play a crucial role in agriculture. One of the key species for pollination is the honey bee which has a very large foraging range and uses different landscape foraging habitats. Honey bees are spatial collectors and pollen their harvest could be considered as a “global” picture of the resources surrounding floral resources at any time of the year.

A large decline in honey bee populations is occurring today on a global scale (Cox-Foster et al. 2007; Glinski and Kostro 2007; Kievits 2007) which may affect a number of agricultural productions requiring insect pollination. Today the causes of this decline have not been completely identified (vanEngelsdorp et al.

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2009; Le Conte 2008), and the environmental aspects of this issue are cause for concern.

Authors have already described how a loss of floral diversity in cereal fields in modern cropping systems may have been overlooked until today (Olivereau 1996; Jauzein 2001). Fried et al. (2009) assessed the increasing evidence of biodiversity loss during the last 40 years in North-East France agricultural landscapes, where 40% of weed species had significantly declined while 10% had significantly increased, in particular nitrophilous species. In such cropping where intensive chemical weeding is currently used, pollen resources may become a constraint for honey bees, and result in weak colony development and poor survival, due to loss of immunocompetence (Alaux 2010). Resource sustainability has to be taken into account throughout the year to ensure both the development of the bee colony and its overwintering capacity. In addition, the complementary nutritional aspects of multifloral pollen provisions are the guarantee of a balanced quality food able to produce healthy honey bees. During food shortage periods alternative flower resources, such as attractive wildflower strips, can play a survival role for bee colonies (Decourtey et al. 2010).

Pollen quality has been studied for a long time, in particular for its nutritional content (Haydak 1970; Campana and Moeller 1977; Standifer et al. 1980). Authors showed that physico-chemical characteristics of pollen harvests varied during the flight period since the average pollen spectrum varies during the year. Manning (2001) reviewed how pollen chemical composition influenced bee health and in particular he investigated the effects of lipid-enhanced food on bees. Obviously, in a modern crop system no global study has investigated both how honey bees exploit the various plant habitats and how floral elements of the landscape provide quality pollen amounts to colonies.

We defined three objectives to investigate over one whole year the relationships between: (1) pollen harvested by colonies and landscape habitats, (2) the diversity of pollen harvested and their physico-chemical content, and (3) the

contribution of different landscape habitats to physico-chemical content of pollen.

We quantified pollen resources available to bees within their flight range during a year and the flower range exploited in an intensive farming system environment. The study concerned three categories of data analyses of (1) the landscape habitat composition, (2) the honeybee-collected pollen, and (3) the physico-chemical composition of the pollen harvest.

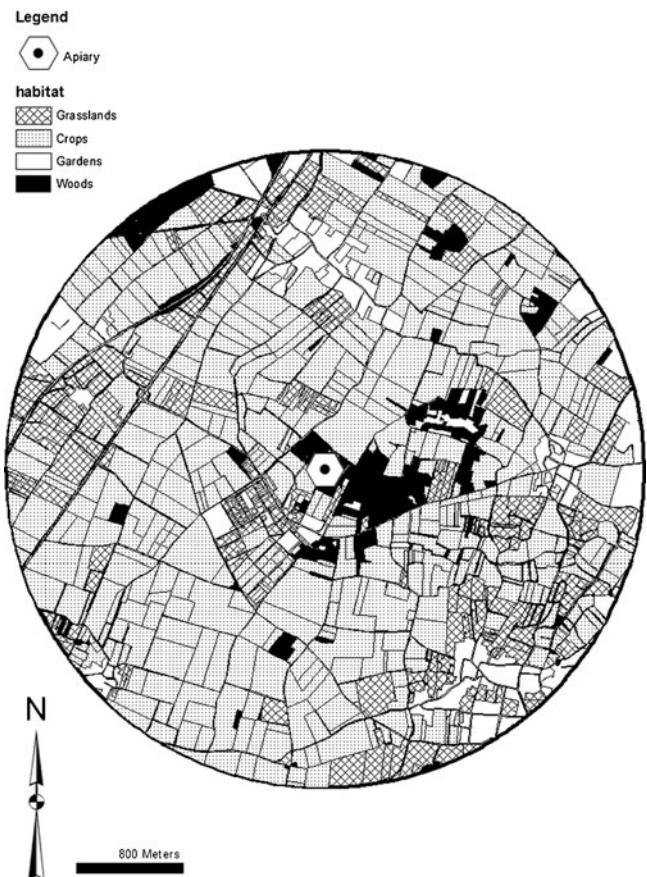
## 2. MATERIALS AND METHODS

This survey was carried out in 2006 using honey bees from the apiary of our experimental centre in an agrarian environment in western France. We considered the different categories of flowers known to supply food resources to bees. Attractive crops are characterized by varieties, sowing date, irrigation, and pesticide use, weeds which depend on farming practices (ploughing, hoeing, fertilization, herbicides, etc.), trees and shrubs, especially forest borders and hedgerows, grasslands, gardens, and also anthropic structures (road banks, industrial areas, etc.).

### 2.1. Land use monitoring

The different land uses were recorded within a 2,500-m radius around the hives, which was further than the mean foraging distance of  $1,743 \pm 95$  m observed by Steffan-Dewenter and Kuhn (2003). The proportions of landscape elements were computed using GIS Software (ArcGIS, version 9.3.1). Woods covered 26% of the area; sunflower, 26%; cereals (wheat and barley), 20%; grassland, 11% (including legumes); gardens and orchards, 5%; rapeseed, 5%; maize, 4%; proteageneous peas, 2%; set-aside fields, 1%; and sorghum, <1%. Four classes of environment were found: (1) woods and semi-natural elements of landscape, (2) crops (cereals, maize and sorghum, peas, sunflower, rapeseed, etc.), (3) grassland, and (4) gardens. These classes covered 26%, 58%, 11%, and 5%, respectively (Figure 1). However, the foraging activity area varied throughout the year because of the cropping system cycle. In order to assess the effective surface for bee foraging, we considered the field area as described in section 2.4.

**Figure 1.** Experimental area and land use. The apiary is in the middle of the area ( $46^{\circ}09'13''$  N;  $0^{\circ}41'20''$  O).



## 2.2. Pollen sampling and palynology

In order to focus only on the seasonal variation of pollen collection, we set up pollen traps on ten colonies of equal strength, randomly chosen within an apiary of 60 hives. We sampled pollen throughout the whole year except during ten winter weeks. We considered a unique mixed sample representing the pollen collected weekly. The colonies (*Apis mellifera* L.) were equipped with bottom pollen traps continuously in service. Their hole size was 5.3-mm diameter and represented a common model of pollen trap, though considered less efficient than 4.8 mm (Bienkowska and Pohorecka 1996). According to Louveaux (1958) and Lavie and Fresnaye (1963), when using metal grid with 5-mm holes the trapping rate is assessed at 10% of the total pollen harvest (temporary variations  $\pm 5\%$ ). After each harvest, impurities were removed with forceps and mixed

samples were weighed (Sartorius,  $d=0.01$  g). Each pollen mix collected from the ten hives was coarsely homogenised in a large tin. Then, from four different places of this tin, we sampled  $4 \times 6.25$ -mL pollen. These 25-mL samples were labelled, frozen at  $-18^{\circ}\text{C}$ , and kept until the palynological and physico-chemical analyses were performed.

The pollen samples were poured into a dish from which four 1 g pre-samples were taken and mixed together. These mixed pellets were diluted in water, homogenized and kept 1 h at room temperature in an Erlenmeyer vessel. The suspension was stirred for 5 min with a magnetic stirrer, and two microscopic slides were prepared, each with one drop of the suspension. The microscopic preparations were dried and cleaned with alcohol and then covered with a drop of glycerine gelatine mixture stained with fuchsine, according to the technique described by Louveaux et al. (1978).

At least 1,000 pollen grains were observed per slide. An Olympus B-52 microscope  $\times 400$  with a camera connected to a computer was used to identify and count pollen grains of the different taxa (Software 2I system-Paris Pégase Pro V4). The end point of this study was the assessment of the volume of each pollen species more representative than classical grain numbering. Brian (1957) argued for considering the relative importance of the volume of pollen grain in a food study, and showed high differences between the proportions by number and by volume. Floral species ratios within a sample were estimated by considering the pollen grain as a sphere with a specific diameter. A preliminary assay conducted on pure pellets of four floral species ( $n=19$  pollen samples) focussed on the relationship between the weight of a pellet, the pollen grain volume, and the number of pollen grains in the pellet. It was concluded that pollen grain weight was correlated to grain volume (Spearman coefficient,  $P<0.001$ ). The weighed amount of each pollen species collected each week was computed by multiplying the percentage by volume of each floral species assessed in the microscopic slides and the weight of pollen harvest. Specific amounts are expressed in grams per day per hive in section 3.

The palynological method did not always allow the discrimination of very close taxa. In such cases, a reference genus was assigned to several pollen types, e.g., *Brassica* reference covered cabbage, *Raphanus*, some oil seed rape varieties, and other various wild crucifers.

According to our local botanical database available by internet at <http://www.guenievre.magneraud.inra.fr/entomologie>, each taxon was assigned to one of the four habitats. When a species is present in several habitats, it is assigned to the habitat where it is more abundant.

### 2.3. Physico-chemical characterization of pollens

For the physico-chemical characterization, the pollen samples were ground with a household mixer. The dry matter content was determined after heating at 75°C during 24 h (Louveaux 1959). The protein content was determined by Kjeldahl method ( $N \times 6.25$ ) according to ISO 5983 norm, using a Vapodest

45 Gerhardt automat. Reducing sugar determination was carried out after deproteinization with the colorimetric method (Elsner and Ganzmüller 1931).

For the total lipid determination, Folch et al. (1957) method was applied. In order to extract also the internal lipids contained in the pollen grain (Roulston and Cane 2000), a disruption of the pollen wall (exin) was necessary, using hydrochloric acid (HCl 6N) hydrolysis. Following the acid hydrolysis, an extraction with a chloroform/methanol mixture (2:1, v/v) was carried out for each pollen sample.

The physico-chemical analyses results are expressed in percent of the dry matter.

### 2.4. Diversity and resources indexes

Diversity was always calculated on the habitat criterion, for landscape and pollen as well. The Shannon diversity index ( $H'$ ) presented was calculated as  $H' = -\sum pi \log_2 pi$  where  $pi$  was the habitats relative area for landscape diversity, or pollen abundance from each habitat for pollen diversity (Shannon and Weaver 1949). It allowed the assessment of the diversity gradient for habitat area or pollen samples.

Based on pollen analyses, the available pollen resources index has been expressed each week for the four habitats. This index is the sum of the theoretical frequencies of every taxa calculated using the area (in  $ha \cdot 10^3$ ), multiplied by a coefficient taking into account a theoretical frequency of each taxon in the landscape. The blooming periods were established using our botanical database ([www.poitou-charentes.inra.fr/entomologie](http://www.poitou-charentes.inra.fr/entomologie)). A special calculation has been performed for the crops: rapeseed, winter cereals, sunflower, and maize where weeds were taken into account. The frequency of each weed species was provided by ARAF and HYPPA weed databases from UMR1210 “Biologie et Gestion des Adventives” INRA Dijon ([www2.dijon.inra.fr/bga/hyppa](http://www2.dijon.inra.fr/bga/hyppa) and [www2.dijon.inra.fr/bga/araf2009](http://www2.dijon.inra.fr/bga/araf2009)) which takes into account the preceding crops.

### 2.5. Statistics

The statistical analyses were checked by the R software (the R Foundation for Statistical Computing,

version 2.11.1) and the Access software (Microsoft Corporation).

All variables were analyzed using generalized linear models, concerning landscape, physico-chemical components, and flora foraged during each week.

A multivariate analysis was performed from weekly samples by hierarchical cluster analysis (HCA-Ward) of  $H'$  identifying different classes characterized by their diversity level. Homogeneous chronological periods were determined, based on these classes and taking into account the highlighting of some identified species as well. Based on the  $H'$ , principal component analysis (PCA) was performed on habitat data during the main periods from weeks 16 to 32.

### 3. RESULTS

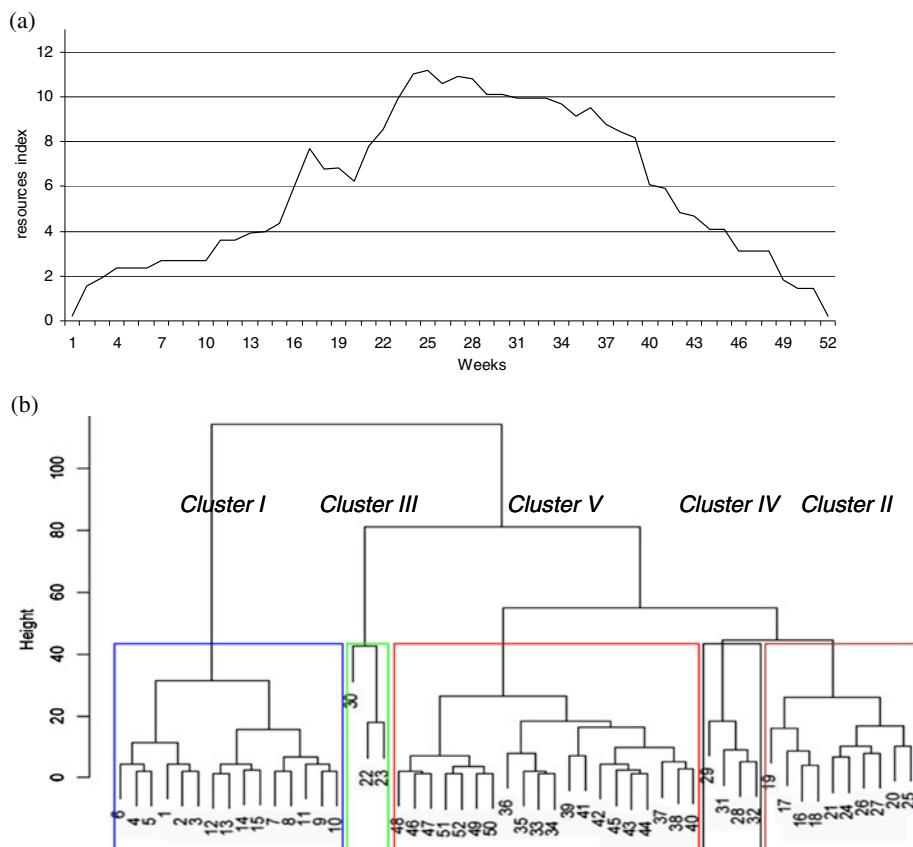
#### 3.1. Diversity of landscape and harvest

Figure 2a shows that maximum resources, including the weed blossom mass present in each crop, were observed between weeks 22 and 39, namely in summer (coefficient,  $>5$ ). According to the pollen diversity illustrated in Figure 2b, five clusters can be distinguished: cluster I, weeks 1–15 ( $H'=2.00$ ); cluster II, weeks 16–21 and 24–27 ( $H'=1.92$ ); cluster III, weeks 22, 23, and 30 ( $H'=1.84$ ); cluster IV, weeks 28–29 and 31–32 ( $H'=1.83$ ); and cluster V, weeks 33–52 ( $H'=1.38$ ). The cluster includes all taxa collected identified by palynological analyses. In total, 92 floral species were found throughout the year corresponding to 22% of the species recorded in our local melliferous plants database. In order to assess in detail the species contribution, the total estimated weights of the major flowers within each complete cluster are given in Table I. The amounts of each taxon in grams have been computed per habitat. Crops and woods were the major pollen suppliers throughout the year, accounting for 92% of the total harvest, 61.25% and 31.36%, respectively. The contribution of grasslands and gardens were only 4.5% and 1%, respectively. The main species for crops habitat were *Zea mays*, *Papaver*, *Sinapis*, and *Sorghum*

whereas for woods habitat were Rosaceae, *Hedera*, *Cornus*, and *Acer*. The contribution of *Papaver*, *Z. mays*, and *Sorghum* took place between weeks 22 and 32, and that of Rosaceae, *Cornus* and *Acer* between weeks 16 and 27. Between weeks 33 and 52, *Sinapis* and *Hedera* were the major pollen suppliers.

#### 3.2. Habitat origin of taxa collected

The total weight of pollen collected in the traps during 1 year was 4,817 g/c colony. Considering the four habitats defined above, the portion of pollens from the cropped area, woods, grasslands, and gardens was 62.0%, 32.2%, 4.5%, and 1.3% of the total harvest, respectively. Within the cropped area, crops accounted for 27.2% and weeds for 34.8%. Within the whole year, pollens from the cropped area (crops plus weeds) represented 3,003 g/c colony (60% in clusters III and IV), the gardens 55 g (57% in cluster II), the grasslands 221 g (65% in clusters II and III), and the woods 1,537 g (75% in clusters II and III). However, Figure 3 shows the important contribution of wood pollen from weeks 11 to 27 and 36 to 45. The spring collection contained high amounts of Rosaceae type *Prunus avium* (up to 79% and 22 g/day), *Cornus* (up to 46% and 21 g/day), *Acer* (up to 50% and 11 g/day), *Sambucus* (up to 7% and 3 g/day), *Quercus* (up to 15% and 3 g/day), and *Fraxinus* (up to 31% and 2 g/day). The autumn period was mainly characterised by the crucial contribution of *Hedera* (up to 77% and 13 g/day). The cropped areas were mainly foraged in summer, for *Zea* (up to 77% and 36 g/day), *Sorghum* (up to 37% and 18 g/day), and *Helianthus* (up to 70% and 9 g/day). *Brassica napus* never represented more than 29%. Weeds were foraged for *Papaver* (up to 66% and 36 g/day), *Sinapis* (up to 98% and 17 g/day), *Daucus/Ammi*, *Cichorium* (up to 27% and 48%, respectively, and 5 g/day), *Hypericum*, *Plantago*, (up to 15% and 13%, respectively, and 2 g/day), *Mercurialis* (up to 55% and 1 g/day), *Reseda*, *Caryophyllaceae*, *Polygonum*, *Persicaria*, *Cenopodium/Amaranthus* (max 1 g/day). These taxa represented a large part of the pollen harvested in summer, whereas *Veronica* (up to



**Figure 2.** Available pollen resources in the landscape, and diversity of harvests by honeybees. **a** Pollen index throughout the year. **b** Clustered weeks according to Shannon diversity index ( $H'$ ) of the pollen harvest.

89% and 2 g/day), was the main pollen resource at the end of winter. A significant contribution of grasslands occurred in spring, with *Onobrychis* (up to 8% and 4 g/day), *Vicia* (up to 32% and 4 g/day), *Taraxacum* (up to 45% and 3 g/day), *Trifolium* (max 2% and 1 g/day), and later *Medicago* (up to 25% and 3 g/day). The garden flowers (*Elaeagnus*, *Viburnum*, *Platanus*, *Berberis*, *Aesculus*, etc.) were visited throughout the year but in small amounts (max 1 g/day) which are not visible in Figure 3.

### 3.3. Physico-chemical content

The sugar content of pollen pellets varied from 14.6% to 41.1%. High values occurred in summer during weeks 22–28 (31.2% to 41.0%)

and weeks 33–36 (27.4% to 33.6%) (Figure 4a). On the contrary, low values (<25%) appeared after week 37 during autumn. The pollen trapped per day and per hive, brought the highest amounts of sugar to colonies during weeks 22–23, i.e., 26.1 and 23.2 g, respectively, 34.4% and 41.0% sugar (Figure 4b). Another period with high sugar provisioning was weeks 28 to 30 (9.8 to 15.7 g and 24.4% to 31.2%). The sugar-low pollen was observed in autumn, after week 40, and brought less than 3.8 g (14.6% to 25.6%). The taxa concerned by high-sugar pollens were *Papaver* and *Rubus* in spring, and *Cichorium* and *Sinapis* in summer (Table 1).

The protein content varied from 16.7% to 29.9% (annual average, 24.5%). The highest level

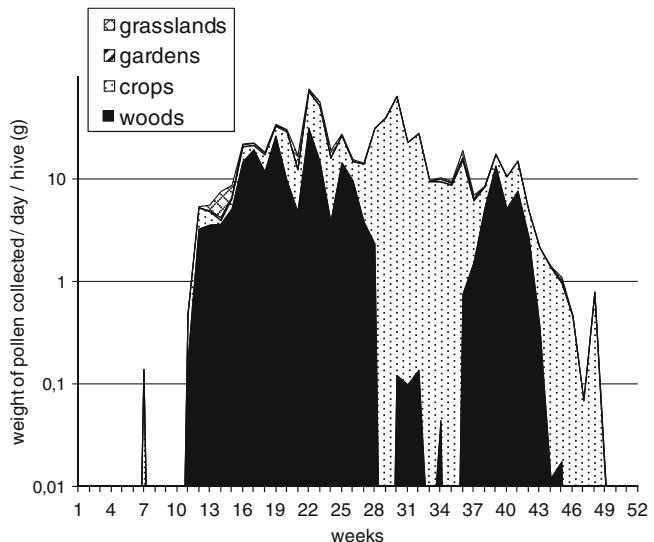
**Table I.** Taxa contribution to pollen harvest for the different clusters (in grams per hive per cluster), in annual weights per habitat in descending order

| Habitat           | Taxa                  | Cluster I<br>(weeks 1–15) | Cluster II<br>(weeks 16–21 and 24–27) | Cluster III<br>(weeks 22–23 and 30) | Cluster IV<br>(weeks 28–29 and 31–32) | Cluster V<br>(weeks 33–52) |
|-------------------|-----------------------|---------------------------|---------------------------------------|-------------------------------------|---------------------------------------|----------------------------|
| Crops             |                       |                           |                                       | 255.4                               | 485.1                                 | 18.7                       |
|                   | <i>Zea mays</i>       | 143.7                     |                                       | 495.3                               |                                       |                            |
|                   | <i>Papaver</i>        | 175.8                     |                                       | 16.1                                |                                       |                            |
|                   | <i>Sinapis</i>        |                           |                                       | 129.9                               |                                       |                            |
|                   | <i>Sorghum</i>        |                           |                                       |                                     | 93.9                                  |                            |
|                   | <i>Helianthus</i>     | 44.8                      |                                       | 31.0                                | 110.4                                 |                            |
|                   | <i>Daucus/Ammi</i>    | 49.4                      |                                       | 23.7                                | 71.5                                  |                            |
|                   | <i>Cichorium</i>      | 2.6                       |                                       | 1.9                                 | 29.0                                  |                            |
|                   | <i>Brassica napus</i> | 10.3                      | 97.8                                  |                                     |                                       |                            |
|                   | <i>Hypericum</i>      | 10.7                      |                                       |                                     |                                       |                            |
|                   | <i>Polygonum</i>      | 56.3                      |                                       |                                     |                                       |                            |
|                   | <i>Plantago</i>       | 12.5                      |                                       | 2.9                                 |                                       |                            |
|                   | <i>Veronica</i>       | 22.9                      |                                       |                                     |                                       |                            |
|                   | <i>Reseda</i>         |                           | 0.3                                   |                                     |                                       |                            |
|                   | Minor taxa (24)       | 4.2                       | 30.7                                  | 0.3                                 |                                       |                            |
| Total crops (g)   |                       | 37.4                      | 624.6                                 | 12.2                                | 1.5                                   |                            |
| Gardens           |                       |                           | 25.1                                  | 977.2                               | 5.5                                   |                            |
|                   | <i>Platanus</i>       |                           |                                       |                                     | 39.6                                  |                            |
|                   | <i>Elaeagnus</i>      |                           |                                       |                                     | 526.5                                 |                            |
|                   | <i>Berberis</i>       |                           |                                       | 5.0                                 |                                       |                            |
|                   | <i>Viburnum</i>       | 3.3                       |                                       | 0.3                                 |                                       |                            |
|                   | <i>Aesculus</i>       |                           |                                       | 3.0                                 |                                       |                            |
|                   | <i>Laurus</i>         |                           |                                       | 1.6                                 |                                       |                            |
|                   | <i>Tamaris</i>        |                           |                                       | 0.9                                 |                                       |                            |
|                   | Minor taxa (4)        | 0.6                       |                                       | 0.8                                 |                                       |                            |
| Total gardens (g) |                       | 3.9                       |                                       | 31.7                                | 5.0                                   |                            |
| Grasslands        |                       |                           |                                       |                                     |                                       | 0.5                        |
|                   | <i>Vicia</i>          |                           |                                       |                                     |                                       | 14.8                       |
|                   | <i>Onobrychis</i>     |                           |                                       | 35.8                                |                                       |                            |
|                   | <i>Taraxacum</i>      | 43.6                      |                                       | 12.1                                |                                       |                            |
|                   | <i>Medicago</i>       |                           |                                       | 2.0                                 |                                       |                            |
|                   | <i>Fabaceae</i>       | 0.7                       |                                       | 11.4                                |                                       |                            |
|                   |                       |                           |                                       |                                     | 0.7                                   | 28.4                       |
|                   |                       |                           |                                       |                                     |                                       | 0.2                        |

Table I (continued).

| Habitat              | Taxa             | Cluster I<br>(weeks 1–15) | Cluster II<br>(weeks 16–21 and 24–27) | Cluster III<br>(weeks 22–23 and 30) | Cluster IV<br>(weeks 28–29 and 31–32) | Cluster V<br>(weeks 33–52) |
|----------------------|------------------|---------------------------|---------------------------------------|-------------------------------------|---------------------------------------|----------------------------|
|                      | <i>Borago</i>    | 1.5                       |                                       | 6.0                                 |                                       |                            |
|                      | <i>Poaceae</i>   |                           | 3.2                                   | 5.9                                 | 5.9                                   | 1.2                        |
|                      | <i>Trifolium</i> |                           | 5.7                                   | 0.3                                 |                                       | 2.2                        |
| Total grasslands (g) | Minor taxa (5)   | 1.0                       | 71.6                                  | 71.4                                | 0.7                                   | 32.0                       |
|                      |                  | 45.3                      |                                       |                                     |                                       |                            |
|                      |                  |                           | 561.3                                 | 70.4                                | 12.6                                  | 0.3                        |
| Woods Rosaceae       |                  | 7.4                       |                                       |                                     |                                       |                            |
|                      | <i>Hedera</i>    |                           |                                       |                                     |                                       | 257.4                      |
|                      | <i>Cornus</i>    | 0.0                       | 14.5                                  |                                     | 214.8                                 |                            |
|                      | <i>Acer</i>      | 52.6                      | 157.6                                 |                                     |                                       |                            |
|                      | <i>Sambucus</i>  |                           | 20.9                                  |                                     |                                       |                            |
|                      | <i>Quercus</i>   | 37.1                      |                                       |                                     | 27.9                                  |                            |
|                      | <i>Fraxinus</i>  | 22.1                      |                                       |                                     |                                       |                            |
|                      | <i>Liliaceae</i> | 0.2                       | 19.0                                  |                                     |                                       |                            |
|                      | <i>Ficaria</i>   | 14.9                      |                                       |                                     |                                       |                            |
| Total Woods (g)      | Minor taxa (17)  | 12.8                      | 16.7                                  | 11.2                                | 5.4                                   | 0.2                        |
|                      |                  | 110.1                     | 827.2                                 | 324.4                               | 18.0                                  | 257.9                      |
| Total (g)            |                  | 196.6                     | 1,555.1                               | 1,378.0                             | 856.1                                 | 831.2                      |

**Figure 3.** Habitat contribution to pollen collection (weekly mean per colony).



of protein was in spring, weeks 16–19 (28.6% to 29.1%), and in autumn after week 44 (26.0% to 29.9%). Lower values occurred early in spring, up to week 14 (20.0% to 24.5%), and in summer, from weeks 24 to 36 (16.8% to 24.0%) (Figure 4a). The protein amounts were the highest during weeks 16–23 in spring, and then during weeks 28–30 in mid-July (Figure 4b). The quantities provided per day and per hive ranged from 4.7 to 19.6 g and 5.8 to 11.3 g, respectively, whereas the pollen protein percentage varied from 25.8% to 29.1% in spring and from 16.7% to 18.3% in summer. The protein diet decreased impressively after week 41, in mid-October. The annual average was 4.4 g/week. The most important protein provider habitats were crops and woods, especially in spring (Figure 3), with a high contribution of *Papaver/Cornus*, (14.8 to 19.6 g and 25.8% to 26.2% protein) and also in summer with large amounts of crop pollens *Zea/Sorghum* (5.8 to 11.3 g and 16.7% to 18.4% protein) (Table I). Selected pure *Z. mays* pollen loads were analyzed and contained 15.7% of protein.

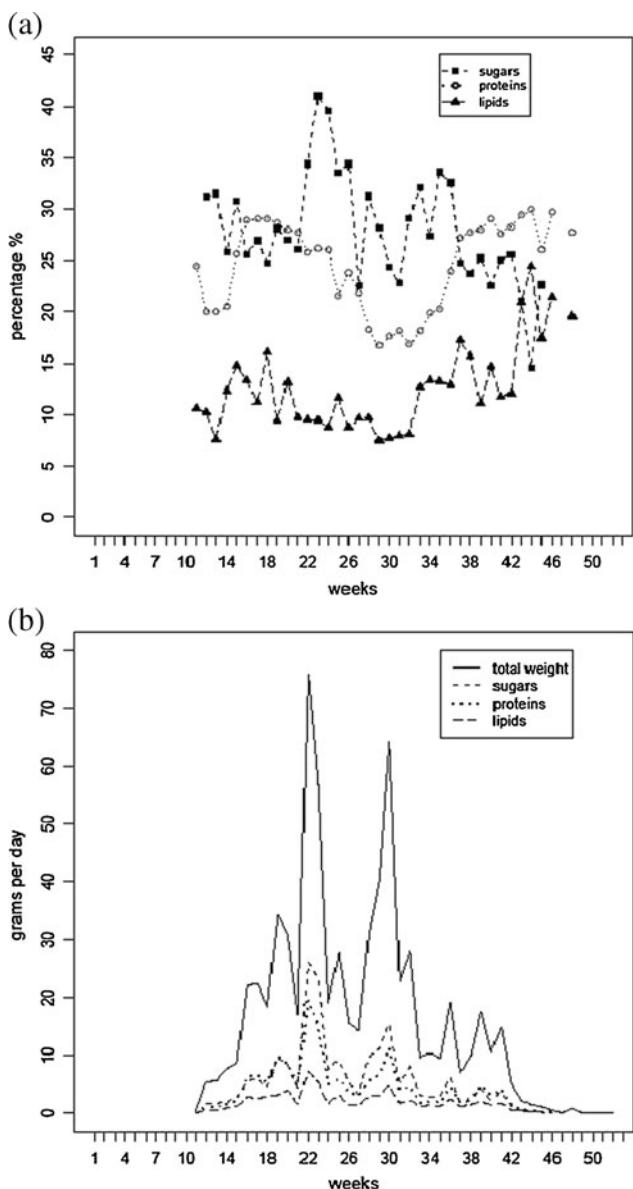
Lipids varied greatly with a 3.25 ratio between the lowest level in week 29 (7.5%) and the highest level in week 44 (24.4%) (Figure 4a). The annual mean lipid content was 12.6%. The maximum lipid amount of pollen per day and per hive was provided during weeks 22–23 (June) with 7.2 and 5.3 g (around 9.5% lipids) (Figure 4b). Weeks 24

to 27 (mid-June to mid-July) were characterized by a strong decrease in lipid amounts with 1.4 to 3.2 g (8.8% to 11.6% lipids). Until week 41 (October), lipid amounts reached up to 4 g, and pollen contained 11.7% to 24.4% lipids, and then dropped. As for proteins, crop elements were the most important habitat contributing to lipid supply (Figure 3). *Papaver* was predominant in weeks 22–23 (5.4 to 7.3 g and 9.4–9.5% lipids) and *Zea/Sorghum* in weeks 28–30 (3 g and 7.5% to 9.7% lipids) (Table I). Sunflower peak pollen represented up to 1.4 g and 9.6% lipids (week 27), and rapeseed 1.3 to 3 g and 11.2% to 16.6% lipids (weeks 15–18). The annual lipid supply was 2.0 g/week.

#### 3.4. Relationships between the landscape and the harvested pollen (floral and chemical parameters)

Concerning the relationship between the foraged habitats and the physico-chemical composition of the diet, woods, crops, and grassland pollens influenced sugar and protein amounts ( $P < 0.001$ ). Lipid supplies were also explained by pollen collected from woods and crops ( $P < 0.001$ ), and grasslands and gardens as well ( $P < 0.05$ ). Obviously, the pollen diversity expressed by  $H'$  does not explain either sugar, protein, or lipid supplies.

**Figure 4.** Physico-chemical composition of pollen harvested throughout the year **a** in percentage of samples and **b** in weekly diet of bee colony.



We observed a negative relationship between the land use diversity and the resources ( $P < 0.001$ ). The land use diversity is positively linked with the woods and gardens resources and negatively with the crops one as well ( $P < 0.05$ ). Concerning the relationship between the land use and the foraged habitats, the contribution of woods was correlated to their resources ( $P < 0.001$ ). However, the pollen diversity was linked to the amounts of pollen harvested from

woods ( $P < 0.001$ ), gardens and grasslands ( $P < 0.05$ ). The more the resources increased, the more the pollen diversity increased ( $P < 0.001$ ). Considering  $H'$ , the land-use diversity and the pollen diversity were positively correlated ( $P < 0.05$ ), and in particular during spring (Figure 5).

Concerning land use and physico-chemical composition of the diet no relationship could be established between habitat areas and any component. In addition, resources index influenced protein

and lipid supply. Nevertheless, the land use diversity was not correlated with any component.

#### 4. DISCUSSION

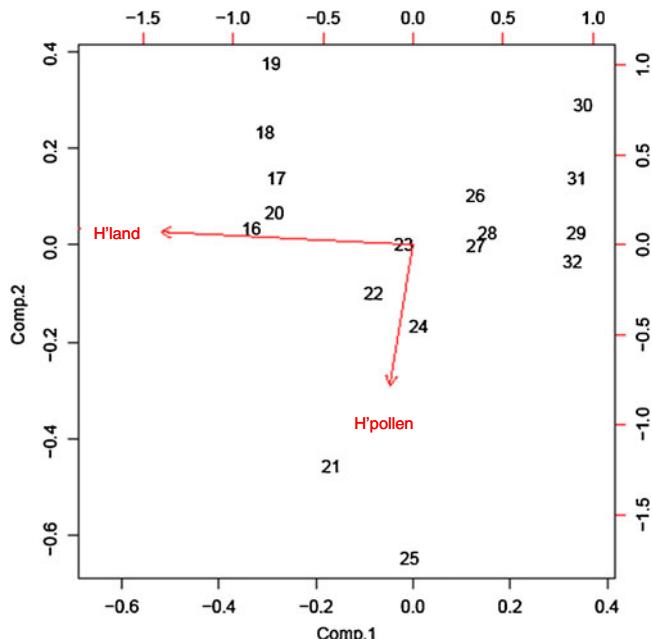
The aim of this work was not to test the factors of pollen foraging by bee colonies, but to consider the landscape elements supplying pollen. In this regard, we did not perform individual measurements on colony parameters neither their pollen collection but mixed the pollen harvests of ten colonies which were intended to reflect the available resources of a cropped environment. Authors generally carried out studies on physico-chemical characterization or nutritive value of single species bee-pollen (Maurizio 1950; Evans et al. 1987; Somerville 2001; Genissel et al. 2002) but did not take into account the mix managed by the bee colony exploiting a given landscape. Our study focused on the choice of pollens by bees in an agrarian environment where the flora was linked with agricultural practices, and within succeeding periods throughout the year. We suggest that the cropping landscape can influence the choice of the bee gatherers and that the interaction of different habitats is an insurance to compensate for shortage periods.

**Figure 5.** PCA of landscape diversity and pollen diversity harvest in clusters II to IV. The two central arrows tend towards the same side, close to the spring weeks.

Based on Winston's yearly evaluation of 15–55 kg pollen/hive (Winston 1994), we conclude that our average collection of 4,817 g means that our trap efficiency was 9% to 33% of the harvest and can be considered as correct in regard to authors' results (Louveaux 1958; Bienkowska and Pohorecka 1996). Using Lavie and Fresnaye's (1963) pollen trapping efficiency of 10%, each of our colonies collected an average 48 kg pollen/year.

#### 4.1. Relationship between the pollen harvest and the landscape

The environment of our study was typical of a cereal plain with fragmented woods and very few grasslands. Oil-seed plants (sunflower and rapeseed) covered a large part of the farming area (30.9%). Negative relationship between landscape diversity and the habitats area can be explained for crops which are by far the major land use in front of few large classes which made up index diversity calculation. The latter did not allow us to show positive correlations between pollen quantities harvested and the landscape. The pollen diversity explained by the foraging resources index revealed more adequate method of



resource assessment than only considering crop areas. The relationship between diversity of pollen and landscape is illustrated by the importance of a mixed landscape especially in spring when the pollen quantities are low. The role of grasslands in this relationship is obvious in spite of a reduced influence during the rest of the year (Figure 5).

The pollen quantity foraged from weeds was as abundant as that collected from crops. Modes of agricultural practices generate different kinds of weeds, which bloom in crops and in borders. It is noticeable that in cereal fields, *Veronica* was one of the only pollen resources for bees in early spring, when woods (*Corylus*) had finished blooming and hedgerows (*Prunus spinosa* in particular) were not yet in bloom. In late spring, when bee colonies reach their peak populations, *Papaver*, a quality pollen according to Louveaux (1968) and Maurizio (1950), ranked almost as high as maize with up to 36g/d/hive. Obviously, the abundance of this key-role plant in cereals and set-aside fields is associated with the weeding method and turnover (Cirujeda et al. 2003). Some of these weeds are native to the West of France (e.g., *Papaver*, *Sinapis*, etc.), other important ones are not (e.g., *Reseda*, *Ammi*, etc.). In mid-August, during the “inter-crop” period, the taxa *Daucus*/*Ammi*, *Cichorium*, *Hypericum*, *Plantago*, *Mercutialis*, and *Reseda* represented more than 50% of the total pollen harvest whereas at the end of summer the group of taxa: *Melilotus*, *Mercurialis*, *Reseda*, *Sinapis*, *Veronica*, and *Cichorium* reached 88% of the harvest. These results are consistent with authors who underlined the importance of forbs and their role in sustaining bee populations and beekeeping activities (Louveaux 1968; Odoux et al. 2009; Decourtey et al. 2010).

Pollen collection of *Helianthus annuus* and *B. napus* covered 10 and 6 weeks, respectively, but exceeded 25% of the weekly collection for only 2 weeks each. In the yearly pollen harvest of our experimental colonies, *Z. mays* was the major plant foraged by honey bees. It was the dominant (>50%) species during 5 weeks despite its restricted cultivation area (4%). This suggests a high attractiveness of this pollen while sunflower

was widely in bloom at this time of year. In addition, according to authors, the exin structure may have an influence on pollen grooming (Vaissiere and Vinson 1994). This abundant collect of maize pollen has already been measured (Odoux et al. 2004) and is often reported by beekeepers. Our data are consistent with observations in a Swiss poly-culture environment where bees shifted to alternative pollen sources such as *Zea* or *Trifolium* after collecting *Helianthus* pollen during a few days (Charriere et al. 2010). Pollen foraging on *Zea* have to be considered with regard to specific hazards (irrigation and pesticide use) to bees. Yet, despite the large amounts of *Zea* pollen combined with the sunflower honeyflow, the brood activity falls at this period suggesting that this food supply does not meet protein and lipid needs.

Woods provided a large part of pollen resources to bees in a cereal farming context. Trees and also borders and hedgerows represented around 50% of the foraged species during spring. This study revealed the major role of woods for bees all year round. In addition, the relationship between the wood area and its contribution to pollen harvest indicated an over-representation of this habitat in the spring collect during rapeseed bloom. The relationship observed in our study shows that grasslands were foraged for pollen when resources of woods and crops were low. The prairie area was more than three times bigger than that of maize and supplied only 10% of the latter. Grasslands did not clearly affect the pollen collection at any time of the year except in early spring (cluster I) where 23% of the 196 g/colony was supplied by this habitat. This can be interpreted by the management of “artificial” grasslands, using Poaceae species as the main crop and incompatible with a high biodiversity (Plantureux et al. 2005). In our study, gardens were essentially rural ones and did not cover a large area (the same as maize). *Elaeagnus*, *Platanus*, *Berberis*, *Viburnum*, *Aesculus*, etc., did not have a visible impact on the pollen harvest at any time which is opposite to observations in urban areas where ornamental species allowed bees to feed all year round (Loublier 2010)

#### 4.2. Relationship between the physico-chemical composition, landscape and pollen collected

The resources index and the habitat proportions were not correlated with any physico-chemical content of the pollen collected. Our results showed that the intake of the main components, was more related to the quantities foraged than to the rate variations of components. Protein and lipid supplies were mainly correlated with the harvest from crops and woods. The pollen harvest depends for a large part on the availability of flowers and also on the weather conditions enabling bee foraging.

The primary energy source is directly found in nectar by bees, but bees are known to agglomerate nectar to pollen in order to build their loads in case of dry weather (Clark and Lintas 1992; Chauzat and Pierre 2005). Sugar-high pollens came from wood border plants (*Cornus*, *Daucus/Ammi*, and *Papaver*) and crops (*Z. mays*, *Sorghum*, and *Helianthus*).

Pollen is the protein source for the bee, and is important for metabolism and development. According to Maurizio (1950) and Louveaux (1959), spring protein-high pollen diet was consistent with the development period of the colonies, and our loads had a higher value compared with some other authors' observations (Maurizio 1950; Roulston and Cane 2000). Fruit tree pollen (including wild ligneous Rosaceae) has been cited by authors who classed it as "excellent" from the point of view of protein content, but our data also clearly showed the role of a characteristic species of cropped landscape, *Papaver* in providing protein. During weeks 24–29 the protein deficiency was a consequence of the temporary food shortage and low values of the input diet. *Helianthus* pollen was the major species in week 27 and has been mentioned as a protein-low taxon, i.e., 15% by Pernal and Currie (2000) and Tasei and Aupinel (2008). The low protein content of *Z. mays* (from 14% to 15%, according to Stace 1996 and Somerville 2001 and confirmed in our analyses of pure *Zea* pollen) was balanced by the high quantities

collected by our bees, which resulted in high summer protein amounts (weeks 28–32). In autumn, there are discrepancies between our data and Louveaux's (1959) observations showing that the protein rate of the gathered pollen did not decrease, and this can be explained by environmental differences.

In our study, the foraged species are actually considered to be protein-rich pollen (*Sinapis* 27% and *Hedera* 28%, according to Tasei and Aupinel 2008), but the quantities collected were low so the protein amount really decreased.

The lipids brought to the hives are an essential element of bee physiology, especially for larval development and the bee's immunity system (Evans et al. 1991; Feldlaufer et al. 1993; Hornitzky 2003; Alaux et al. 2010). Indication that pollens with higher lipid levels are more attractive to honey bees (Singh et al. 1999) was not verified here in summer. The most important collected quantities were observed in spring, at the same time as protein-rich pollens i.e. during the blooming time of *Cornus* and *Papaver*. The species identified in this environment seem to produce very lipid-rich pollens with a mean of 12.6%. Australian bee-collected pollens provide opposite examples with average fat content of 2.5% (Somerville 2001). Lipid-low inflows in weeks 24–27 could have consequences on the health and development of the next nurses which may affect the lifespan of autumn workers and consequently the wintering mortality of colonies. The main flora supplying such pollens was from edges (Rosaceae) and crop areas (*Daucus/Ammi*, *Helianthus*, and *Polygonum*). The autumn amount of lipids was low in spite of the very high rate of lipids in the pollens harvested, mainly due to low temperatures after mid-October.

Based upon the need for a varied food quality, simplified landscape management generally has negative consequences on the food resources (Day et al. 1990; Geiger et al. 2010). Considering our results, the establishment of flowering set-aside fields should be recommended in summer as a priority in cereal-oilseed crop systems during the summer period, assuming that enough water is available. Therefore, this study shows the interest

as far as bees are concerned to leave fields without any farming practice for several weeks after the crop harvest, in order that colonies rear their winter workers. Finally, the weeds plants seem to be a very important pollen source, and have to be considered in the environmental ability of a landscape to feed pollinators.

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### Biodiversité territoriale et conséquences sur les caractéristiques du pollen récolté par les colonies d'abeilles

### Agrosystème / abeille/biodiversité florale / analyse pollinique / analyse chimique

### Territoriale Biodiversität und Konsequenzen für die physiko-chemischen Eigenschaften von Pollen, der von Bienenvölkern gesammelt wird.

### Agrarökosystem / florale Biodiversität / Pollenanalyse / chemische Analyse / Honigbiene

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