THÈSE

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École doctorale : Sciences pour l'environnement - Gay Lussac (La Rochelle)
Secteur de recherche : Biologie de l'environnement, des populations, écologie

Présentée par :
Fabrice Requier

Dynamique spatio-temporelle des ressources et écologie de l'abeille domestique en paysage agricole intensif

Directeur(s) de Thèse :
Vincent Bretagnolle, Jean-François Odoux

Soutenue le 18 décembre 2013 devant le jury

Jury :

Président	**Didier Bouchon**	Professeur des Universités, Université de Poitiers
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UNIVERSITÉ DE POITIERS

ÉCOLE DOCTORALE GAY-LUSSAC
SCIENCES POUR L'ENVIRONNEMENT

THÈSE

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Fabrice REQUIER

Dynamique spatio-temporelle des ressources florales et écologie de l'abeille domestique en paysage agricole intensif

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Directeurs de Thèse : Vincent BRETAGNOLLE
& Jean-François ODOUX

Réalisée au Centre d'Études Biologiques de Chizé, Équipe AgriPop
et à l'INRA du Magneraud, Équipe Entomologie

Soutenue le 18 décembre 2013

Jury :
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Résumé

Dynamique spatio-temporelle des ressources florales et écologie de l'abeille domestique en paysage agricole intensif

L'effondrement des populations d'abeilles domestiques, observé à l'échelle mondiale depuis une vingtaine d'années, est particulièrement alarmant, provoquant le déclin de la filière apicole mais également celui d'un pollinisateur essentiel en milieu agricole. La conservation de l'abeille domestique et de l'apiculture en paysage agricole est donc actuellement un enjeu majeur pour de nombreux acteurs. L'intensification de l'agriculture, insufflée par la politique agricole européenne, est désignée comme une cause majeure dans le déclin de la biodiversité, dont les abeilles sauvages. De nombreuses études ont révélé que le manque de ressources florales est une cause majeure de ce déclin généralisé, mais de façon surprenante, cette cause n'est que très peu investiguée à l'heure actuelle pour l'abeille domestique. Plus généralement, l'écologie de l'abeille domestique en paysage agricole est mal connue.

A l'interface entre écologie comportementale, évolutive et paysagère, l'objectif général de cette thèse est d'étudier l'influence conjuguée de l'intensification de l'agriculture et de la dynamique spatio-temporelle des ressources florales en paysage agricole sur (i) leur utilisation et (ii) l'histoire de vie des abeilles. L'originalité de cette étude provient de l'envergure spatiale et temporelle des données empiriques collectées à l'aide de vastes dispositifs de suivis tant au niveau individuel qu'au niveau des colonies.

Les résultats obtenus confirment l'influence négative de l'intensification de l'agriculture sur l'écologie de l'abeille domestique. En effet, la dynamique des ressources dominées par trois cultures majeures que sont le colza, le tournesol et le maïs, provoque l'apparition d'une déplétion d'approvisionnement en pollen et nectar. Des ressources aujourd'hui marginalement présentes en paysage agricole intensif telles que les zones boisées et les adventices jouent un rôle prépondérant dans le régime alimentaire des abeilles, pouvant contrer l'intensité de cette déplétion d'approvisionnement. Par des mécanismes a priori adaptatifs, les colonies et les individus adaptent leurs comportements et histoire de vie en réponse à la déplétion d'approvisionnement. Cependant, ces mécanismes de compromis d'allocation des ressources ont un coût important et aboutissent à un affaiblissement des colonies, qui se traduit par la mortalité hivernale des colonies d'abeilles domestiques.

Cette étude révèle empiriquement l'importance de la dynamique des ressources dans les causes du déclin de l'abeille domestique, et évoque la présence d'une disette alimentaire en céréaliculture intensive. Ces résultats sont discutés dans l'optique de concevoir des mesures de conservation de l'abeille domestique, de l'apiculture et du service de pollinisation en paysage agricole intensif, afin de limiter les stress environnementaux pour les abeilles tels que le manque de ressources florales et l'exposition aux pesticides.

Mots clés : écologie comportementale, écologie évolutive, écologie paysagère, conservation, régime alimentaire, foraging, histoire de vie, abeille domestique, apiculture, paysages agricoles, Mesures Agro-Environnementales

Abstract

Spatio-temporal dynamic of floral resources and honey bee ecology in intensive agricultural landscapes

The collapse of honey bee populations, described around the world for twenty years, is particularly alarming because it causes the decline of beekeeping and of an essential pollinator in agricultural habitats. The conservation of honey bees and beekeeping in agricultural landscape is currently a major challenge for many operators. The agricultural intensification, instilled by the (European) Common Agricultural Policy, is considered as a major cause in the decline of biodiversity, including wild bees. Many studies have shown that lack of floral resources is a major cause of this widespread, but surprisingly, this cause of decline is very few investigated actually to honey bees. More generally, the honey bee ecology in agricultural landscapes is poorly understood.

At the interface between behavioral, evolutionary and landscape ecology, the overall objective of this PhD thesis was to study the combined influences of agricultural intensification and the spatio-temporal dynamics of floral resources on (i) the use of this resource and (ii) the life history of honey bees. The originality of this study comes from its large spatial and temporal scale as well as its joint approach at individual and colony levels.

The results confirm a negative impact of agricultural intensification on the honey bee. Indeed, three major crops i.e. rapeseed, sunflower and maize have created a temporal resource dynamic which causes a strong food supply depletion in pollen and nectar. Some resources more marginally represented in intensive agricultural landscapes such as wood and weeds play an important role in the honey bee diet, being able to restrict the intensity of food supply depletion. By adaptive mechanisms, colonies and individuals adapt their behavior and life history in response to the food supply depletion. However, these trade-off mechanisms in resource allocation have a significant cost and generate a weakening of colonies that increases overwintering mortality of honey bee colonies.

This study shows empirically the importance of the resource dynamic in the causes of honey bee decline, and highlights the presence of a food-shortage period in cereal farming systems. These results are discussed so as to build conservation measures on the honey bee, beekeeping and pollination service in intensive agricultural landscapes, in order to limit environmental stress for honey bees such as lack of floral resources and pesticide exposure.

Key-words: behavioral ecology, evolutionary ecology, landscape ecology, conservation, diet, foraging, life history, honey bee, beekeeping, agricultural landscape, agroecology, Agri-Environmental Schemes

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Cette thèse a été réalisée grâce à une subvention du ministère de l'Agriculture (bourse CASDAR) et une de la région Poitou-Charentes, dans le cadre d'un projet de recherche *Polinov*. Ce projet (Polinisateurs et systèmes de cultures innovantes, 2010-2012) regroupait un partenariat multidisciplinaire, avec de nombreux organismes tels que l'ACTA (pilotage du projet), l'INRA, le CNRS, l'ITSAP, le CETIOM, ARVALIS, l'ENS, Universités et chambres d'agriculture. Le projet *Polinov* avait trois objectifs, (i) la protection des abeilles au sens large, (ii) la durabilité de l'agriculture, et (iii) la durabilité de l'apiculture.

Remerciements

Une thèse peut se résumer à trois années de travail intense et fastidieux, mais ce serait faire l'erreur d'oublier qu'une thèse est également et essentiellement un ensemble de trois années d'épanouissement personnel, procuré par une quête de connaissances tant sur le plan intellectuel, professionnel que personnel. Ce constat est d'autant plus réaliste lorsqu'on a la chance de joindre passion et travail, faisant défiler les journées et ces trois années beaucoup trop vite. Pour m'avoir permis de réaliser cette étude exceptionnelle je lance un « bouquet » de remerciements à mes deux encadrants Vincent B. et Jean-François. Vous m'aviez présenté ce projet de thèse comme une expérience pionnière lorsque je suis arrivé, par une logistique et structure d'encadrement originale. En effet, un premier encadrant chercheur écologue-ornithologue et un second ingénieur apidologue, le tout dans deux structures différentes espacées géographiquement mais également administrativement (à savoir respectivement le CNRS et l'INRA), pouvaient laisser augurer certaines difficultés logistiques. Cette situation ne s'est finalement pas révélée limitante, mais au contraire, cette complémentarité a été très enrichissante. Je suis très heureux d'avoir pu bénéficier de cette liberté d'action que vous m'avez laissée, tant dans l'orientation de mon projet de thèse que dans sa réalisation. Un grand merci à vous deux pour m'avoir encadré et conseillé pendant ces trois années de thèse, ce qui aura été très formateur. Vous m'avez également offert l'opportunité de créer et de réaliser de nombreuses expérimentations, d'encadrer des étudiants et de présenter mes travaux en congrès scientifiques internationaux mais aussi, localement, au grand public, ce qui me tenait particulièrement à cœur. Enfin, merci à vous de m'avoir intégré au sein d'une telle collaboration interdisciplinaire d'organismes de recherche.

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L'élaboration d'une thèse ne se résume pas que par le travail, il faut aussi se nourrir correctement pour survivre. Dans ce domaine, j'ai été copieusement servi ! Un très grand merci au Chef Fabrice du Magneraud et au Chef Christophe du CEBC pour leurs bons petits plats qui ont vraiment su titiller mes papilles gustatives.

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Et pour finir, une farandole de remerciement pour tous ces amis, potes qui m'ont tant soutenu et encouragé pendant cette étape de ma vie quelques fois difficile. Je commencerai par mes potes de Nogent, Yo (alias Mr l'expert) et Anne (Pimprenelle) les premiers à m'avoir poussé dans la voie de cette thèse. Merci beaucoup à vous deux, la science est joyeuse et folle avec vous, et merci à Hélène et Pénélope, toujours présentes aussi. Un très grand merci à mes potes tourangeaux, en particulier Charlie, Mélanie C. et Sophie qui, depuis 8 ans, m'offrent leur aide et leur soutien sans compter. Bien sûr, je remercie les supers potes messins Mika et Sandrine pour ces bons week-ends de détente et de rigolade. Continuons dans les bons moments de convivialité en remerciant la team St Mard, j'ai nommé Thibaud D. (promis je répare ma cheville pour la reprise des footings !), Cyril (le roi du château !) et Sandrine (alias Sardine mais j'aime pas trop !), mais aussi la grande Aurélie (et Poukie, promis, c'est moi qui cuisine maintenant !), et bien sûr Harmonie (tellement de mercis qui se résument en un grand *Nuut !*). Que de gentillesse et de générosité dans ce groupe, qui font tellement de bien en fin de thèse.

J'espère que ces quelques lignes, trop courtes et non-exhaustives tant ces trois années furent pour moi riches et épanouissantes, vous feront partager le bonheur que m'a procuré la réalisation de cette thèse.

Cette thèse est dédiée à Sylvain G. alias 'Boboche', très grand ami qui, à mon plus profond regret, n'aura pas eu le temps de devenir docteur…

Avant-propos

Par choix personnel, ce manuscrit de thèse a été structuré sous le format « thèse sur articles », comprenant cinq articles (dans le corps de texte, annexes exclues), qui se succèdent selon une structure classique (introduction, matériel & méthodes, résultats et discussion) :

- une partie « Introduction » comprenant *un article de revue* qui fait une synthèse du cadre et de la problématique de cette thèse. Ce travail de synthèse étant novateur, le choix a été d'en faire un article, dont les conclusions aboutissent à la problématique de la thèse. Un bref récapitulatif des objectifs de thèse suit cet article afin de bien définir les questions abordées dans cette thèse.
- une partie « Matériel et méthodes » comprenant *deux articles de méthodes*. Chacun de ces deux articles abordent les techniques, outils et méthodologies utilisés pour réaliser le monitoring de l'abeille domestique aux deux niveaux biologiques analysés dans ce travail, individu et colonie. L'innovation méthodologique et/ou technique nous a incités à formater cette partie de thèse sous un format d'article.
- une partie « Résultats » comprenant *deux articles scientifiques originaux*, répondant aux questions de thèse.
- une partie « Discussion » faisant une synthèse générale des travaux de thèse. Cette dernière partie n'est pas structurée sous format d'article. Cette discussion comprend un bref bilan des résultats obtenus, discutés en relation avec la littérature scientifique et des données supplémentaires, pour aboutir aux implications de cette thèse dans le cadre de la conservation de l'abeille domestique, de l'apiculture et du service de pollinisation en milieu agricole. Enfin, une ouverture sur des perspectives de recherche a été abordée.
- Une partie « Annexes » comprenant un chapitre d'ouvrage et cinq articles supplémentaires, en co-auteur et publiés durant cette thèse.

Afin d'améliorer la fluidité de lecture du manuscrit, de courts énoncés en en-tête d'article ont été ajoutés, afin de résumer très brièvement et de resituer chacun des articles dans le contexte général de la problématique de thèse.

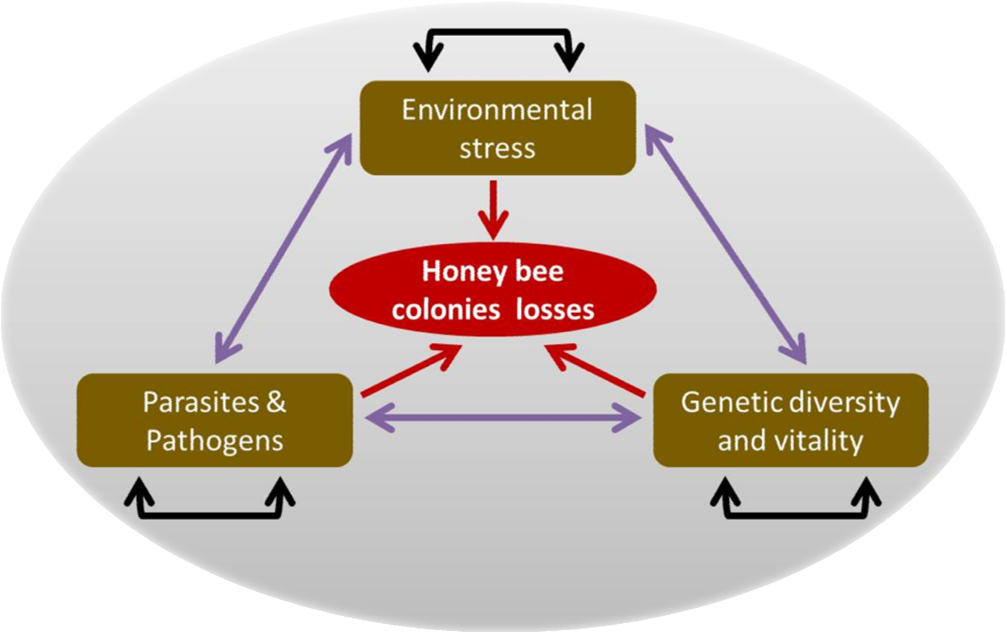
Table des matières

INTRODUCTION

Cadre et problématique

Déclin des abeilles, stress environnementaux et écologie de l'abeille domestique

Cet article de revue énonce le cadre et la problématique de cette thèse, à savoir le déclin des abeilles et par ce fait du service de pollinisation, l'actuelle méconnaissance dans l'écologie de l'abeille domestique et dans l'action réelle des stress environnementaux surgissant en paysage agricole intensif.



Review paper in prep.

Title: A review of honey bee ecology in agricultural habitats: floral resources have been overlooked as a possible cause of honey bee colony loss in intensive cereal farming systems

Authors: Requier F., Odoux J. F., Decourtye A., Bretagnolle V.

A review of honey bee ecology in agricultural habitats: floral resources have been overlooked as a possible cause of honey bee colony loss in intensive cereal farming systems

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Key-words: Beekeeping sustainability, agricultural landscape, *Apis mellifera* L., honey bee colony losses, ecology, habitat use, life history, conservation measures, Agri-Environmental Schemes.

Introduction

European agricultural landscapes have been deeply modified over the last 50 years, under the predominant influence of the Common Agricultural Policy (CAP). CAP was set in order to increase the food production to meet the growing needs of human population growth (Godfray et al., 2010). Intensification of agricultural systems has resulted in a global increase of cultivated areas, a decrease of semi-natural habitats (forests or hedgerows, but also permanent natural grasslands) and a homogenization of land-use associated with the reduction of cultivated species (Benton et al., 2003). In addition, in order to increase crop yields, the use of fertilizers and chemical pesticides was eventually generalized (Tilman et al., 2001). As a general consequence, there has been a major biodiversity loss in rural landscapes (Steffan-Dewenter and Tscharntke, 1999, Tscharntke et al., 2005, Tilman et al., 2001), with the decline of many taxa (i.e. birds, insects and plants, see Sotherton, 1998, Luoto et al., 2003, Kamp et al., 2011) including species providing essential ecosystem services to ensure the functional ecology of ecosystems (Tilman et al., 2001). In particular, the pollination service provided by insects is currently threatened because of the widespread decline of insect pollinators in agricultural landscapes (Potts et al., 2010a). Flower-visiting insects provide vital pollination service to crops and wild plants (Klein et al., 2007, Ollerton et al., 2011), and insect pollination for agriculture and biodiversity is estimated at several hundred billion euros per year (Gallai et al., 2009). Though there is a general trade-off between food production and biodiversity conservation, including ecosystem services (Butler et al., 2007, Power, 2010, Phalan et al., 2011, Gabriel et al., 2013), wild and managed bees are the primary pollinators of most agricultural crops (Klein et al., 2007, Ricketts et al., 2008, Rader et al., 2012, Garibaldi et al., 2013) as well as wild plants (Biesmeijer et al., 2006, Potts et al., 2010a), therefore ensuring the conservation of floral biodiversity (Ashman et al., 2004, Aguilar et al., 2006, Biesmeijer et al., 2006).

Not only honey bee population and pollination service decreased, but also beekeeping economy (Ellis et al., 2010, Potts et al., 2010b). Given the very strong decline in bee populations, a vast research effort eventually emerged to discover the cause of honey bee colony losses (Anderson and East, 2008), and to a lower extant, wild bees decrease as well. Land-use change (i.e., habitat loss, fragmentation, habitat degradation and decrease of resource diversity and abundance) was pointed as a major cause of the decline of wild bees in agricultural landscapes (Steffan-Dewenter et al., 2002, Kremen et al., 2002, Larsen et al., 2005, Hendrickx et al., 2007, Ricketts et al., 2008, Goulson et al., 2008, Winfree et al., 2009, Brown and Paxton, 2009), in particular the loss of floral and nesting resources. Pesticides, e.g. insecticides with lethal or sub-lethal effects (Desneux et al., 2007, Goulson, 2013), as well as herbicides, were also suggested to affect pollinators either directly or indirectly by decreasing floral resource availability (Gabriel and Tscharntke, 2007, Holzschuh et al., 2007). Additional identified causes include parasites (Meeus et al., 2011), pathogens (Whitehorn et al., 2013, Huth-Schwarz et al., 2012) and genetics (Cameron et al., 2011, Huth-Schwarz et al., 2012). Despite highly intensive research efforts however, particularly on honey bee colony losses, no single driver could be identified (Cox-Foster et al., 2007, Anderson and East, 2008, Potts et al., 2010a). Honey-bee colony losses is now better described as a multifactorial syndrome (Potts et al., 2010a). Three main groups of stressors are said to be involved: ‘Parasites and pathogens’, ‘Lack of genetic diversity and vitality’ and ‘Environmental stress’ (habitat loss, depletion of flower resources and exposure to pesticides, Neumann and Carreck, 2010, Potts et al., 2010a).

There is indirect evidence that floral resource availability may act as a limiting factor to bee populations, since the conservation of semi-natural habitats through, e.g. Agri-Environment Schemes (AES, Whittingham, 2007) was shown to restore wild pollinator populations through an increase in resources (Corbet, 1995). Indeed recent studies have demonstrated positive impacts of AES on the abundance and species richness of bees (Kleijn et al., 2004, Kleijn and Sutherland, 2003, Scheper et al., 2013, Sepp et al., 2004). This suggests that some causal factors that led to bee decline may have been underestimated and we challenge the current view that honey bee colony loss results mainly from an interaction between Parasites & Pathogens and pesticides. Our general purpose is twofold: using literature search we first compare quantitatively the various causes that have been proposed to account for honey bee decline, with special reference to the environmental factor (including land-use change, habitat loss, flower availability, and pesticides). We review the literature published over the last 38 years in order to address the relative contribution of environmental stress within the multifactorial etiology of honey bee colony losses. We then argue that within environmental stressors, the lack of floral resources at least in intensive farming systems may be an important cause of honey bee decline. Our literature review highlights however the surprising fact that honey bee ecology in agricultural landscapes is poorly known, either in terms of habitat use, diet composition and resources selection. Since habitat use is an essential ecological baseline for biological conservation the current lack of knowledge on honey bee ecology impedes the calibration of evidence-based conservation measures (Dicks et al., 2010).

Material and methods

Literature search

Papers were searched using the *Web of Science* section of the bibliographical database *ISI Web of Knowledge* (http://apps.webofknowledge.com/). We searched separately for honey bee colony losses and honey bee ecology (Fig.1). We started our bibliographical analysis from year 1975, until September 2013. We integrated the three main decline causes, 'Parasites and pathogens', 'Lack of genetic diversity and vitality', and 'Environmental stress', and we differentiated them in order to evaluate their relative contribution within the cloud of multifactorial stressors. Within the last category, we further distinguished between 'Habitat loss', 'Flower availability and 'Pesticides' (Fig.1). The honey bee ecology in agricultural landscape (i.e. habitat use) was investigated according to 'Diet composition' and 'Foraging' key-word (see detailed in appendix S1).

In addition, to minimize potential publication bias associated with the 'file drawer problem' (Rosenthal, 1979), we also searched for gray literature (McAuley et al., 2000) using the Google web search engine. Both sets were finally completed with our own literature bank (see method in Scheper et al., 2013). The criteria key-words used with *TS* function in *ISI Web of Knowledge* tool extracted all papers on the search topic, separating papers which really studied the topic of interest from those which only mentioned it. We then manually selected papers regarding their consistency with the topic (key-words), focusing to review papers which synthetize a large breadth of information.

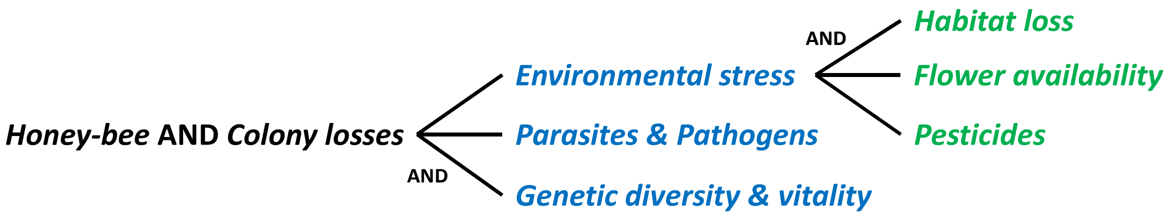

Figure 1: Classification of key-words used for bibliographical data collection about the honey bee colony losses. In blue were represented the main groups of key-words and in green the subgroups of key-words. All used key-words (*italic* words) are detailed in appendix S1.

We also distinguished the few studies on feral (unmanaged) bee colonies from the managed colonies, because habitat loss is presumably likely to further reduce nesting site availability for feral colonies only, in addition to the decrease of food resources and the increase of intoxication risk by pesticides. We also separated between empirical and experimental (laboratory) data.

Statistical analyses

All statistical analyses were performed using the R environment for statistical computing, version 2.11.1 (R-Development-Core-Team, 2009). The annual number of paper was first investigated as occurrence frequency related by the total paper published in the same year in order to relativize the potential bias of annual variation of publication.

In a first step, we modeled the temporal patterns of occurrence frequency in papers about honey bee colony losses with a function of time using Generalized Additive Models (GAMs) with *gam* function in *mgcv* R-package and a Gaussian error structure (Lin and Zhang, 1999). GAMs are modeling technics allowing temporal splines fit. Time was expressed by year scaled from 1975 to 2013 while this last year included only papers published up to September. As temporal patterns of all occurrence frequency in papers were clearly non-linear (see Results below), we used the *breakpoints* function of the R-package *strucchange* in order to identify the annual date of pattern change.

In a second step, we used Chi-squared test to compare the occurrence frequency of total papers published (1975-2013) between the three main causes of honey bee colony losses in order to test for an equal citation and investment in research into the multifactorial hypothesis of honey bee decline.

Results

A review of causal factor involved in honey bee colony losses

Honey bee populations are declining worldwide, as detected in many parts of the USA (VanEngelsdorp et al., 2008, Pettis and Delaplane, 2010, Ellis et al., 2010), Europe (Potts et al., 2010b) and Asia (Oldroyd and Nanork, 2009). Since 1975, the number of publications related to the honey bee colony losses has increased exponentially (*GAM*, $F_{4,33}$=95.15, $p<0.001$, Fig.2a), reaching almost 0.01% of the total papers published in 2013 recorded in the WOS. Start of exponential increase can be dated in 1987 (Fig.2a), i.e. the date of the first observation of collapse syndrome in honey bee colonies resulting in an increase of over-wintering mortality (Aubert, 2002, Saddier, 2008, Winfree et al., 2009, Potts et al., 2010b, Neumann and Carreck, 2010). Then, from 2006, the number of publications steadily increased, which can be related to the new description of the Colony Collapse Disorder syndrome in the USA (CCD, Cox-Foster et al., 2007, VanEngelsdorp et al., 2007, VanEngelsdorp et al., 2008, Oldroyd, 2007, vanEngelsdorp et al., 2009). The CCD consists in the appearance of associated symptoms (different from those observed in 1987), resulting in a colony depopulation of its adult workers (VanEngelsdorp and Meixner, 2010).

Despite the fact that c. 1,500 papers were published on the topic, currently no single driver can explain this phenomenon, and many factors have been proposed, that can be regrouped into three broad categories of causes (Anderson and East, 2008, Cox-Foster et al., 2007, Ratnieks and Carreck, 2010). There is a strong publication bias among the three causes of honey bee decline (χ^2=43.52, df=2, $p<0.001$, Fig.2b), with ‘Parasites and Pathogens’ being much more studied than the other two. However, many studies have highlighted interactions between several drivers to explain the increase of over-wintering colony losses (e.g. Chen and Siede, 2007, Ratnieks and Carreck, 2010, Di Pasquale et al., 2013, Alaux et al., 2010b, vanEngelsdorp et al., 2009). A consensus has emerged towards the synergic or multifactorial drivers involving the three groups occurring simultaneously or influencing each other (see also Neumann and Carreck, 2010, Potts et al., 2010a). These interactions are particularly worrying, as sub-lethal effects of one driver could make another one more lethal (Neumann and Carreck, 2010, Di Prisco et al., 2013).

PARASITES ANS PATHOGENS

'Parasites and pathogens' is the most frequently cited cause of honey bee decline (61% in frequency of occurrence, Fig.2b), including the ectoparasitic mite *Varroa destructor*, viruses and bacteria. In many cases, it has been considered as the sole factor involved in honey bee colony losses (Rinderer et al., 2010, Nguyen et al., 2011, Faucon et al., 2002), in particular a key factor in over-wintering mortality (Dahle, 2010). Also, *Varroa* would be at the origin of the disappearance of many feral honey bee colonies in the USA and Europe, where only colonies managed by beekeeping can survive thanks to curative treatments (Kraus and Page, 1995, Moritz et al., 2007, Jaffe et al., 2009). *Varroa* is an invasive species in the USA and Europe since its introduction from Asia by the beekeeping industry (Sammataro et al., 2000). Despite the application of *Varroa* control methods on managed honey bee colonies, colony losses still occur, suggesting that *Varroa* alone cannot explain the recent decline in honey bee populations (Stokstad, 2007). Other studies have then highlighted the interaction between *Varroa* and viruses (Chen and Siede, 2007, Francis et al., 2013, Dainat and Neumann, 2013, Berthoud et al., 2010), suggesting that the infestation of *Varroa* could facilitate the susceptibility to viruses. The viral pressure includes mainly Deformed Wing Virus (DWV, transmitted by *Varroa*), Acute Bee Paralysis Virus (ABPV), Chronic Bee Paralysis Virus (CBPV) and Kashmir Bee Virus (KBV). These viruses have a sub-lethal effect on individuals in the colony, causing morphological or behavioral disorders. In interaction with other factors, they reduce the fitness of the colony and increase over-wintering mortality of colonies (Martin et al., 2010, Berthoud et al., 2010, Carreck et al., 2010a, Carreck et al., 2010b). The over-wintering mortality can be also increased by the action of bacteria (Evans and Schwarz, 2011), or microsporidian *Nosema ceranae* (Higes et al., 2008, Cox-Foster et al., 2007, Cornman et al., 2012, Paxton et al., 2007).

GENETIC DIVERSITY AND VITALITY

The second group of drivers is actually the least cited for explaining the honey bee colony losses (7.7% frequency of occurrence, Fig.2b). The honey bee is native of Africa, Europe and Asia, with no less than 26 subspecies and numerous ecotypes, and presents a large variability in behavioral, morphological and molecular traits (Meixner et al., 2007, De la Rúa et al., 2009, Meixner et al., 2010), highlighting a very high level of genetic diversity (De la Rua et al., 2013). However, European beekeepers have completely replaced the native honey bee populations by introduced strains (selected for their great honey production or low aggressiveness, see Meixner et al., 2007, Soland-Reckeweg et al., 2009). Current European honey bee populations are either managed colonies on one hand, or rare remnants of feral colonies beset by *Varroa* on the other hand (Kraus and Page, 1995, Moritz et al., 2007, Jaffe et al., 2009). A significant loss of genetic diversity in honey bee populations has been detected in Europe (Moritz et al., 2007, Jaffe et al., 2009), despite the multiple matings of honey bee queens, because queens currently come from very few selected lineages (‘races’; see Meixner et al., 2010). This is unfortunate since genetic diversity is well known to increase resistance against diseases (Tarpy, 2003), regulation homeostasis (Oldroyd and Fewell, 2007), thermoregulation (Graham et al., 2006), and above all, the fitness of the colony (Mattila and Seeley, 2007, Tarpy et al., 2013).

ENVIRONMENTAL STRESS

The third group ‘Environmental stress’ accounts for about 31.3% of publications on honey bee colony losses (Fig.2b), being composed actually of three different subgroups, ‘Habitat loss’, ‘Flower availability’, and ‘Pesticides’. These three subgroups occur unequally in the literature (χ^2=25.95, df=2, $p<0.001$, Fig.2d), with ‘Pesticides’ class showing over 56% of occurrence frequency.

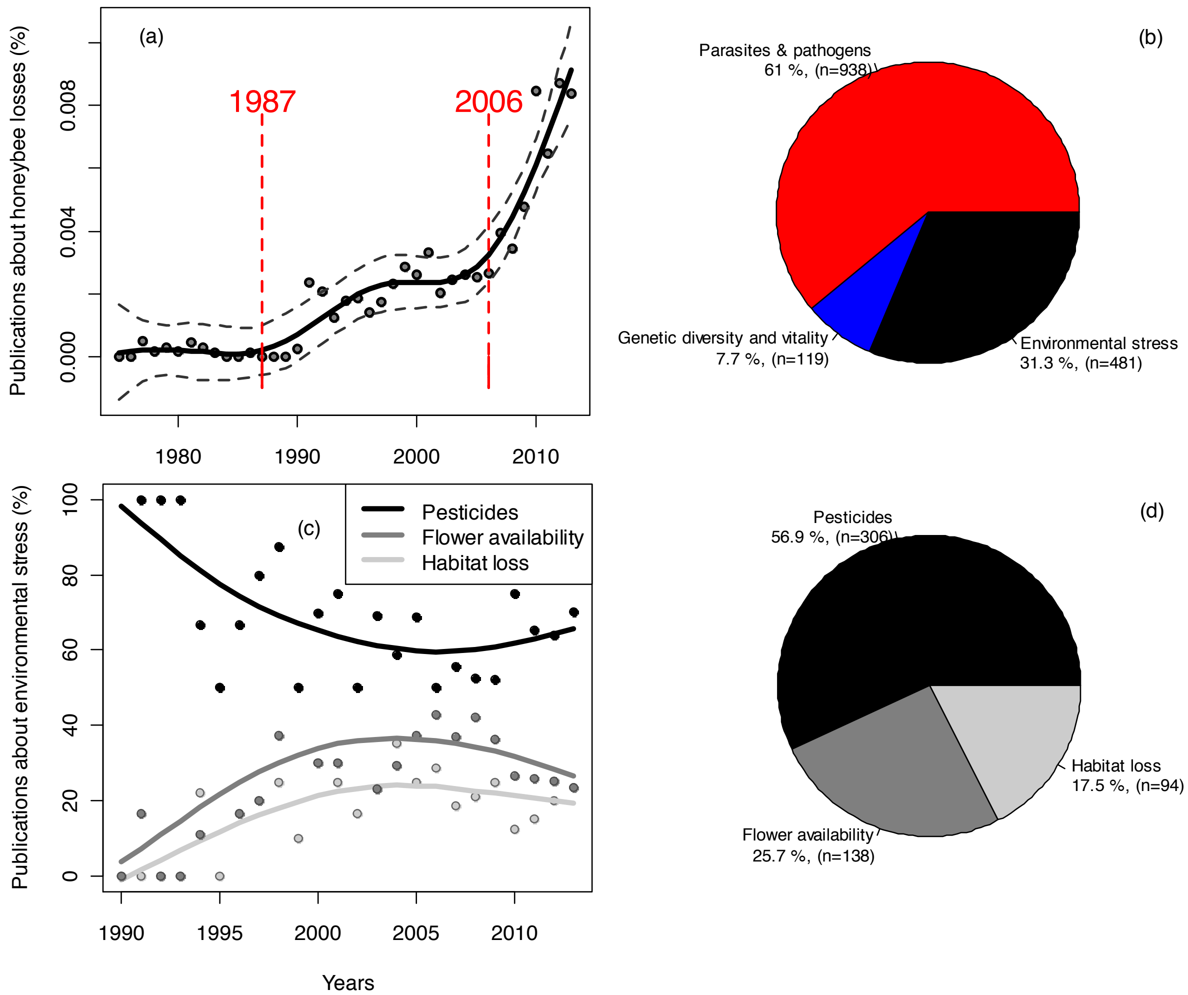

Figure 2 : Bibliometric analysis about honey bee colony losses, (a) pattern of total paper occurrence frequency about honey bee colony losses, (b) respective contribution of the three main groups in total publications regarding honey bee colony losses, (c) patterns of occurrence frequency of three ‘Environmental stress’ subgroups, (d) respective contribution of the three subgroups in the total publications of ‘Environmental stress’

Pesticides

Over 100 papers and reports have been published on pesticides effects on honey bee losses for the sole 2012 year (Osborne, 2012). The dominance of pesticides over 'Flower availability' or 'Habitat loss' has been decreasing until 2004, but since then, increased again (*GAM*, $F_{2,21}=5.7$, $p<0.01$, Fig.2c). Some scientists and many beekeepers believe that pesticides have a leading role in colony-loss (Oldroyd, 2007), probably in interaction with other stressors (Vidau et al., 2011, Alaux et al., 2010a). In cereal agricultural landscapes, honey bees are exposed to a variety of pesticides since they forage extensively on flower-blooming crops, such as oilseed rapeseed (*Brassica napus*), maize (*Zea mays*), or sunflower (*Helianthus annuus*), that are routinely treated against insect pests with insecticides (Mullin et al., 2010). Systemic pesticides in particular diffuse throughout all plant tissues and eventually contaminate nectar and pollen resources that are used by bees (Rortais et al., 2005). Pesticide exposure can induce sub-lethal effects such as disorders in movements, behavioral and spatial orientation of foragers, all possibly strongly affecting honey bee colony dynamics. Many symptoms of agro-chemicals treatments are visible on motor function of honey bees, e.g. uncoordinated movements (review in Desneux et al., 2007). Since 2001 there has been an emergence of publications on a new group of insecticides, the neonicotinoids (i.e. *imidacloprid* and *thiamethoxam*). First symptoms of poisoning appearing after *imidacloprid* exposure cause stationary behavior in bees (Medrzycki et al., 2003), but also staggering, tumbling, hyperactivity and tremors (Suchail et al., 2001) as well as a reduction in the number of flights by foragers (Decourtye et al., 2011b). The honey bee exposure to *thiamethoxam* causes behavioral disorientation which decreases the probability of homing flight to the hive (Henry et al., 2012a), and a decrease of the adult population size in the colony, which can generate the collapse of the colony (Henry et al., 2012b). (Pettis et al., 2012) further highlighted an interaction effect between *imidacloprid* and pathogens, with a significant increase in the *Nosema* pathogen infection. More recently, (Di Prisco et al., 2013) showed the occurrence at sub-lethal doses of *clothianidine* induced viral proliferation.

Habitat loss

Habitat loss in agricultural landscapes acts negatively on biodiversity (e.g. Fahrig, 2003, Benton et al., 2003). Loss of natural and/or semi-natural habitats is regarded as the primary cause of the decline of wild pollinators, through a decrease of nesting and foraging sites (Kremen et al., 2002, Larsen et al., 2005, Winfree et al., 2009, Ricketts et al., 2008, Brown and Paxton, 2009, Goulson et al., 2008, Steffan-Dewenter et al., 2002, Hendrickx et al., 2007). 'Habitat loss' is also referred as a cause of honey bee colony losses (17.5% of 'Environmental stress' studies, Fig.2d), and shows a similar temporal trend compared to 'Flower availability', i.e. increase between 1987 and 2004, and then decrease (*GAM*, $F_{2,21}=10.75$, $p<0.001$, Fig.2c). For honey-bee however, 'Habitat loss' is always interpreted indirectly through a loss of diversity in floral resources, and therefore a factor of nutritional stress (Naug, 2009). Indeed, the natural and semi-natural habitats shelter a wide variety of plants which may represent food resources for bees, unlike crop resource that provides a monospecific source (Requier et al., in revision).

Flower availability

While floral resources certainly have an impact on the honey bee longevity, especially through the over-wintering survival which is totally dependent on the level of reserves stored during the spring and summer seasons, there is no demonstrated evidence of a direct link between floral resources decrease and honey bee colony losses. For example, colonies having suffered CCD symptoms in comparison to control (without CCD symptoms) revealed no differences in individuals body mass, levels of protein and fat (vanEngelsdorp et al., 2009). However, life history, growth and survival of honey bees are closely associated with the availability of resources in their environment (Brodschneider and Crailsheim, 2010, Keller et al., 2005b, Haydak, 1970, Naug, 2009). Though, the 'Flower availability' has been rather poorly investigated (25.7% of 'Environmental stress' studies; Fig.2b), increasing between 1987 and 2004, but decreasing between 2005 and 2013 (*GAM*, $F_{2,21}$=12.44, p<0.01, Fig.2c). Despite it is well known that weeds have suffered a major decline in farmland habitats (Fried et al., 2009, Storkey et al., 2012) no study has tested the hypothesis that the decline in weed richness and abundance may have affected honey bee colony dynamics. Only a few studies suggest a possible alteration of foraging areas for honey bee by the current agricultural intensification and land-use changes (Naug, 2009, Decourtye et al., 2010), an hypothesis supported by beekeepers who mention the lack of food resources (quantity, diversity and quality) as one of the main causes of honey bee decline (VanEngelsdorp et al., 2008). Actually, only experimental laboratory studies investigated how lack in resource quantity (Toth et al., 2005, Mattila and Otis, 2006, Mattila and Seeley, 2007), diversity and quality (Di Pasquale et al., 2013, Alaux et al., 2010b) may affect honey bee health (Brodschneider and Crailsheim, 2010, Decourtye et al., 2010), but there is no empirical evidence of these effects from field studies. Deprivation in pollen food resource (pollen shortage) causes a decrease in honey bee population (Keller et al., 2005a), and a greater susceptibility of individuals to parasites or pathogens (Alaux et al., 2010b, Mayack and Naug, 2009). In addition, the amount of pollen brought back to the hive is known to affect the physiological metabolism (Alaux et al., 2011), immunocompetence (Alaux et al., 2010b), and tolerance against pathogens such as viruses (DeGrandi-Hoffman et al., 2010). In addition, pollen food-shortage reduces the sensitivity to pesticides (Wahl and Ulm, 1983), the learning and memory capacities (Mattila and Smith, 2008), the regulation of foraging ontogeny and activity (Feigenbaum and Naug, 2010, Toth et al., 2005), the social interaction (Schulz et al., 2002), the behavioral development (Schulz et al., 1998), the inspection and feeding of larvae by workers (Huang and Otis, 1991), the pheromonal regulation (Fischer and Grozinger, 2008), and the alteration of physiological responses (Willard et al., 2011). Based on experimental approaches, it was shown that variability in diversity and quality of the resource act negatively on the lifespan of honey bees (Schmidt et al., 1987, Schmidt et al., 1995, Maurizio, 1950), and on the development of the hypopharyngeal glands (Pernal and Currie, 2000). Pollen diversity, in turn, ensures the immunocompetence of honey bees (Naug and Gibbs, 2009, Alaux et al., 2010b). When *Nosema* infection is added to nutritional stress (depletion of resource), bees show a decrease in survival. Finally we can conclude on underlying the fact that there is an inexistent knowledge of the floral availability for the honey bee in modern agricultural landscape and the consequences on the diversity and regularity.

Review of honey bee ecology with particular reference to feeding ecology

FLORAL RESOURCES AND HONEY BEE DIET

Honey bee uses floral resources (i.e. nectar and pollen) to meet their nutritive requirements, though they also use water resources, propolis from tree resin, and occasionally honeydew from other insects (Keller et al., 2005b). The nectar is a carbohydrate source that is used as an energy fuel, and is also transformed into honey (after dehydration and chemical processes) in order to create food reserves for over-wintering. These honey reserves are essential for over-wintering survival, because honey bees do not forage in winter (at least under temperate latitudes). Pollen is an essential resource for physiological and morphological development of bees (Brodschneider and Crailsheim, 2010) and is the source of protein and lipids (Manning, 2001, Haydak, 1970). It is particularly involved in the fat body and hypopharyngeal glands construction of the workers (Maurizio, 1954), the immune system (Manning, 2001), and the growth of ovaries (Keller et al., 2005a). Pollen is stored in the colony as "bread pollen" (after mixing with honey), but in very small quantities (e.g. <1kg, Fewell and Winston, 1992) because its quality deteriorates with time (Pernal and Currie, 2000). Pollen supply follows a tense-flow according to the colony needs, mainly depending on the amount of larvae (Dreller et al., 1999).

The honey bee is a generalist insect, which uses a wide variety of plants to meet its trophic needs (Seeley and Visscher, 1985, Winston, 1994). This generalist behavior for pollen collection (polylecty), as opposed to oligolecty or monolecty in other (wild) bee species, has been supported by experimental evidence: honey bees feed preferentially on pollen mixtures rather than pollen from a single species (Schmidt, 1984). The protein content of pollen, its amino acid composition, its lipid content, vitamins and minerals vary tremendously among plant species (Weiner et al., 2010, Roulston and Cane, 2000). Honey bees show strong interest in flower-plant diversity to supply essential amino acids, which ensure good physiological and morphological development of larvae (Brodschneider and Crailsheim, 2010). Indeed, several studies have demonstrated the importance of some specific nutrients: for example, (de Groot, 1953) found that 10 essential amino acids in specific proportions are required for optimal honey bee health. Those essential amino acids cannot be synthesized *de novo* by honey bees and therefore need to be found directly in the diet. Pollen of low nutritional value, like that of dandelion *Taraxacum sp*., can be compensated by mixing it with another pollen that has a higher nutritional value for reproductive needs (Genissel et al., 2002). A mixed pollen diet increases the lifespan of honey bees as compared to a monospecies pollen diet composition (Schmidt et al., 1987, Schmidt, 1984). In addition, a diversified pollen diet might help bees to ensure their immunocompetence against pathogens (Di Pasquale et al., 2013, Alaux et al., 2010b). Finally, the richness of pollen species in the honey bee diet probably provides a buffer against deficiencies of specific nutrients (Schmidt, 1984) as well as the presence of toxic compounds in some pollen (de Mesquita et al., 2010, Marcolino de Assis et al., 2011). A honey bee colony consisting in several tens of thousands individuals needs floral resource in large quantities, both in real time but also in order to create reserves for over-wintering survival.

Availability of floral resources depends on the cropping system, farming practices, and the semi-naturals habitats. Spatial distribution, temporal floral phenology and vegetation structure are all important factors (Douteau and Charme, 2010, Briane, 1991, Janssens et al., 2006). Virtually no crops are selected taking into account their melliferous value and a therefore a continued honey flow requires a diversified landscape. So, sustaining the diversity and quantity of flower resources in the landscape might increase the chances for bees to find all beneficial nutrients (Brodschneider and Crailsheim, 2010, Decourtye et al., 2011a). However, current intensive agricultural landscapes most often provide colonies with a low variety of plant species (Rands and Whitney, 2010). In intensive agricultural farming systems, honey bees tend to be more frequently observed and more abundant in mass-flowering crops (oilseed rape and sunflower, Rollin et al., 2013). Then, foragers of honey bees may be tightly associated with mass-flowering crops, like sunflower that has pollen with a poor nutritional value (Schmidt et al., 1995). Within complex landscapes, honey bee colonies normally collect and consume pollen and nectar from a large array of plant species (around to one hundred species, e.g. Coffey and Breen, 1997, and see Appendix S2 for complete list of 56 references about honey bee diet). Surprisingly, only four studies investigated honey bee diet in agricultural landscape (Dimou and Thrasyvoulou, 2007, Koppler et al., 2007, Pernal and Currie, 2001, Odoux et al., 2012). In those latter studies however, either only pollen or nectar was studied, or quantity or diversity, and they were carried out in single landscape context and ignoring the influence of the environmental variation. Though, all studies highlighted an important floral richness for pollen resource (e.g. more than 92 species used throughout the year in an intensive agricultural landscape, Odoux et al., 2012), where the botanical origin, the diversity and quality of pollen supply are dependent on the presence of semi-natural habitats (Odoux et al., 2012, Odoux et al., 2014). These studies suggested the existence of a food-shortage period of pollen during the peak colony development, which may induce severe negative consequences on the survival of honey bee colonies. In Europe, mass flowering crops provide bees with nectar or pollen are often limited to maize, sunflower and oilseed rape at large landscape scales in cereal farmland systems (Decourtye et al., 2010). Among these three, maize provides the greatest quantities of pollen collected by honey bees, due to its long-lasting availability and good accessibility to foragers (Charriere et al., 2010, Vaissiere and Vinson, 1994, Odoux et al., 2004). On the other hand, oilseed rape and sunflower can be important nectar sources.

FLORAL RESOURCE SELECTION

Honey bees are claimed to forage selectively (e.g. Winston, 1994), though very few studies actually investigated patch or flower selection in the wild and optimal foraging theory has not explicitly been tested in the free-ranging (Van Nest and Moore, 2012). Most experiments were actually carried out using harmonic radar (Capaldi et al., 2000, Menzel et al., 2011, Riley et al., 2005, Menzel et al., 2005). In addition, groups of foragers operate only on one resource patch ("flower constancy": Gruter et al., 2011), until the energy gain is no sufficient to justify the cost of forager flight (Osborne et al., 1999, Greggers and Menzel, 1993). Relatively few foraging patches are thus used at any one time, for only few days (about 10 patches daily, Seeley, 1985). Under experimental conditions, honey-bees prefer nectar containing sucrose (Waller, 1972). They are able to assess and differentiate the sugar content to a definition of 5% (Wykes, 1952), and they prefer nectar sugar concentration between 15% and 50% (Wykes, 1952, Waller, 1972, Waddington and Kirchner, 1992, Sigurdson, 1981). The nectar accessibility also influences the floral selection, because the length of proboscis of bees (tongue) defines a range of nectar accessibility from flowers. Bees have a medium sized proboscis (6mm), which does not allow them to collect nectar from flowers with deep corollas such as *Trifolium pratense* L. and many other Fabaceae (Jablonski, 2001). Concerning pollen, food selection strategy seems to be much more complex. Indeed, the protein content in pollen does not seem to be the criteria for selection of flowers by pollen foragers (Pernal and Currie, 2001). They are in fact unable to discriminate the protein content in pollen (Maurizio, 1954, Pernal and Currie, 2002). The most protein-rich pollen is not necessarily the one selected, while in contrast, the bees can use preferentially pollens that have very low nutritional values (e.g. kiwifruit vines, Jay and Jay, 1993). The digestibility of pollen is an important aspect of its quality, and depends on its composition (pollens contain several other nutriments than protein). Moreover, appetence of pollen is influenced by the presence of phago-stimulants (Pernal and Currie, 2000). The shape and the pollen size are also important parameters for selection, e.g. large pollen grains with long echinate spines cannot be stored on the hind legs of honey-bees (e.g. cotton, Vaissiere and Vinson, 1994). Obviously, the pollen selection depends on the availability of floral resources. A lack of rich pollen in the environment will lead honey-bee colony to collect pollen with a low nutritional quality (Louveaux, 1959).

FORAGING ECOLOGY

The honey bee is a "central-place forager", food supply being carried-out with round-trip flights between the resource patch and the nest (hive, colony) by foragers (Kacelnik (Kacelnik et al., 1986, Schmidhempel et al., 1985, Dornhaus et al., 2006). More than 2,000 workers are daily available for foraging (i.e., between 2 and 10% of the total population size, Seeley, 1985), of which on average 58% forage only nectar, 25% only pollen and the remaining 17% both pollen and nectar (Winston, 1994), though percentages may be highly variable (Beekman et al., 2004). Workers'availability for the foraging task is preceded by a period of learning flight (also called orientation flight). Orientation flight allows honeybees to learn the relative position of the hive and surrounding landscape elements under the form of a mental map (Menzel et al., 2005). Honey-bees are able to forage 10 or more kilometers from their hive (von Frisch, 1967, Visscher and Seeley, 1982), rarely below 600-800 meters of their hive (Seeley, 1985). Average foraging distance is about 2000-m and the circle enclosing 95% of the colony's foraging activity has a radius of 6000-m (Visscher and Seeley, 1982, Seeley, 1985, Beekman and Ratnieks, 2000, Steffan-Dewenterand Kuhn, 2003), i.e. a hive is foraging over an area larger than 100-km^2. Beckman et al. (2004) showed that the variability of foraging distance and foraging area could be explained by the colony condition and especially by the population size. In particular, they showed that during resource scarcity, small colonies (around 6,000 individuals) forage much more closely than large colonies (around 20,000 individuals), and preferentially on pollen resources, suggesting the existence of trade-off in resource selection (Beckman et al., 2004). Steffan-Dewenter and Kuhn (2003) highlighted the influence of landscape structure on the foraging distance and foraging area of honey-bee colonies. Honey-bees forage farther in simple landscapes than in complex landscapes (Steffen-Dewenter, 2003). Pahl et al. (2011) showed that landscape structure improves the ability of honey-bee foragers to come back to the nest because it is used as spatial landmark, though it may also act as physical barriers. The foraging flights are performed after a learning step of orientation in order to build a memorial shape based on morphological landscape landmarks (Capaldi et al., 2000, Riley et al., 2005).

A food shortage period is suspected for bees in intensive farming systems, where resource patches are less abundant and more heterogeneously distributed in space (Naug, 2009, Decourtye et al., 2010) especially between rapeseed and sunflower flowering periods (Odoux et al., 2012). Agricultural landscapes still harbor semi-natural habitats, which are able to supply food resources for honeybees throughout the season, including during periods of food shortage (Decourtye et al., 2010). However, these semi-natural habitats are marginally represented in most intensive agricultural landscapes, and it is likely that agricultural intensification and land-use changes may have affected the foraging ecology of honey-bees. Unfortunately, very studies are available so far.

Conclusions

There is currently some consensus on the worldwide loss of honey bee colonies being caused by a multifactorial driver of several stress factors. Actually, there is an overwhelm number of studies oriented towards two drivers, 'Parasites and pathogens' and 'Pesticides'. Surprisingly, while loss of habitat and the lack of flower resource are presented as the main causes of the decline of wild bee pollinators and many other taxon in agricultural landscapes (Steffan-Dewenter et al., 2002, Kremen et al., 2002, Larsen et al., 2005, Hendrickx et al., 2007, Ricketts et al., 2008, Goulson et al., 2008, Winfree et al., 2009, Brown and Paxton, 2009), this possible causal factor has been remarkably poorly investigated in honey bee decline. The only studies come from experimental laboratory investigations, and no empirical field data is available to analyze whether farming system changes and agricultural intensification may have affected honey bee. In order to calibrate appropriate types of conservation measures for honey bee in agricultural landscapes, such as AES, it is necessary to know better honey bee ecological traits, such as habitat use, foraging and diet. The current lack of knowledge about honey bee ecology creates a lock in the conception of conservation measures for honey bee and beekeeping, but also for pollination service. Therefore, it is essential to investigate honey bee monitoring in agricultural landscapes in order to answer empirically at two priority issues: (1) identifying key resources use in the agricultural landscape (composition, origin and dynamic); and (2) investigating the influence of the lack of food resource on the life history of honey bee.

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Supporting Information

Appendix S1: Key-words used in 'Web of Science'.

Honey bee → TS=(honeybee* OR honey bee* OR honey-bee* OR Apis)

Colony losses → TS=(decline* OR loss OR colony losses CCD OR Colony Collapse Disorder OR colony collapse disorder OR Colony collapse disorder OR kill* OR mortality)

Parasites and pathogens → TS=(pest OR pests OR parasit* OR pathogen* OR bacteria* OR virus* OR viral OR mite* OR microbe* OR fungus OR Nosem* OR Varroa OR Acarapis)

Genetic diversity and vitality → TS=(genetic* OR vitality OR phylogenetic* OR allozyme) AND (variability OR varying OR variation OR diversity OR biodiversity OR select*)

Habitat loss → TS=(habitat OR landscape) AND (loss* OR fragmentation OR degradation OR isolation OR heterogeneity OR connectivity OR structure OR configuration)

Flower availability → TS=(malnutrition OR food shortage OR food-shortage OR nutritional stress OR energetic stress OR flower availab* OR flower diversity OR resource availab* OR resource diversity OR pollen availab* OR pollen diversity OR nectar availab* OR nectar diversity)

Pesticides → TS=(pesticide* OR intoxication OR insecticide* OR herbicide* or fungicide*)

Agricultural landscapes → TS=((agricultur* OR farm*) AND (area* OR landscape* OR habitat OR habitats OR environment*)) OR (agroecosystem* OR agro-ecosystem*)

Diet composition → TS=((diet* OR resource* OR source* OR aliment* OR food OR origin OR composition OR collect* OR harvest* OR characteriz* OR quality OR content OR protein OR amino acid) AND (nectar OR honey OR pollen OR palynolog* OR melissopalynolog* OR pollen analys* OR flower OR flowers OR flora* OR botanic*))

Foraging → TS=(forag* OR select*) AND (resource* OR patch OR patches OR flower OR flowers OR range OR distance OR pattern)

Appendix S2: Papers with honey bee diet considered and their principal contents

References	Agrosystem	Site replication	Annual replication	Seasonal pattern		Botanical origine		Resource quality	Landscape influence
				pollen	nectar	pollen	nectar		
Requier et al. (submitted)	×	×	×	×	×	×	×	×	×
Coffey & Breen (1997)		×	×	×	×	×	×		
Köppler et al. (2007)	×	×	×			×			
Odoux et al. (2012)	×			×		×		×	
Dimou & Thrasyvoulou (2007)	×		×	×		×			
Forcone et al. (2009)		×	×				×	×	
Pernal & Curry (2001)	×					×		×	
Carpes et al. (2009)		×				×		×	
Forcone (2008)		×	×				×		
Forcone et al. (2005)		×	×				×		
Modro et al. (2007)		×				×		×	
Lazarova & Atanassova (2012)		×	×				×		
Salonen et al. (2009)		×	×				×		
Makhloufi et al. (2010)		×					×	×	
Aronne et al. (2012)		×				×	×		
Cook et al. (2003)	×							×	
Pernal & Curry (2002)	×							×	
Bonvehi & Jorda (1997)						×		×	
Somerville & Nicol (2006)						×		×	
Somerville & Nicol (2002)						×		×	
Telleria (1993)				×		×			
Sanchez & Lup (2011)		×					×		
Fagundez & Caccavari (2006)		×					×		
Forcone et al. (2011)						×		×	
Oliveira et al. (2010)		×					×		
Leonhardt & Blüthgen (2012)						×		×	
Hocherl et al. (2012)	×							×	
de Almeida-Anacleto et al. (2012)		×				×			
Herbert & Shimanuki (1978)						×		×	
Nicolson & Human (2013)	×							×	
Salonen & Julkunen-Tiitto (2012)							×	×	
Upadhyay & Bera (2012)			×				×		
Sereia et al. (2011)							×	×	
Moreti et al. (2011)			×			×			
Sabo et al. (2011)	×						×		
Feas et al. (2010a)							×	×	
Feas et al. (2010b)							×	×	
Aronne & De Micco (2010)		×					×		
Bastos et al. (2004)							×	×	
Pires et al. (2009)							×	×	
Canini et al. (2009)							×	×	
Naab et al. (2008)							×	×	
Sodre et al. (2007)		×					×		
Yao et al. (2006)						×	×		
Dag et al. (2006)							×	×	
Barth (2004)						×	×		
da Silveira et al. (2012)						×			
Boff et al. (2011)						×			
Dimou & Thrasyvoulou (2009)						×			
Ramirez-Arriaga et al. (2011)							×		
Flores & Sanchez (2010)							×		
Poderoso et al. (2012)							×		
Villanueva & Roubik (2004)						×			
Caccavari & Fagundez (2010)							×		
Adekanmbi & Ogundipe (2009)							×		
De Novais et al. (2009)						×			
Dongock et al. (2007)							×		
Baroni et al. (2002)							×		
Herrero et al. (2002)							×		
Aira et al. (1998)							×		
Diaz et al. (1998)						×			

Objectifs de thèse

Cette revue introductive permet d’établir une relative méconnaissance de l’écologie de l’abeille domestique dans son environnement agricole actuel, engendrant une incapacité d’évaluer le réel impact du stress environnemental sur le déclin de l’abeille (manque de ressources florales en particulier), mais également la conception de mesures de conservation. L’acquisition des ressources alimentaires est vitale pour tout organisme et par conséquent, la disponibilité et l’abondance des ressources dans l’environnement agissent comme des facteurs déterminants et limitants sur la survie, la croissance et la reproduction des organismes (Stearns, 1992). L’agriculture intensive agit sur la dynamique spatiale et temporelle des ressources. Par exemple à travers la présence abondante de ressources lors des floraisons des cultures de colza et de tournesol agissant comme des ressources de masse pour les abeilles domestiques. Ces floraisons massives sont attractives pour les apiculteurs, assurant un potentiel de production de miel (VanEngelsdorp and Meixner, 2010). Cependant, les durées de floraison courtes et discontinues engendrent des conséquences sur la dynamique temporelle des ressources alimentaires disponibles pour les abeilles.

L’objectif général de cette thèse est d’analyser l’influence de la dynamique spatio-temporelle des ressources sur (1) l’acquisition de ces ressources et (2) l’histoire de vie des colonies d’abeilles. Afin d’obtenir des résultats utilisables en apiculture, j’ai pu utiliser des ruches gérées comme en apiculture classique, en paysage céréalier intensif. L’hypothèse générale de mon travail présume la présence d’une période de restriction de disponibilité florale entre les floraisons massives du colza et du tournesol, engendrant une déplétion d’approvisionnement alimentaire pour les colonies d’abeilles domestiques. En période de restriction alimentaire, les organismes opèrent des compromis d’allocation des ressources entre survie, croissance et reproduction (Roff, 1992, Stearns, 1992). Tout choix effectué dans ces compromis d’allocation de la ressource aura des répercussions dans la dynamique adaptative des organismes et dans leur histoire de vie future (Cameron et al., 2013). Dans un deuxième temps, nous prédisons que cette restriction d’approvisionnement alimentaire, qui génère un compromis d’allocation des ressources au sein des colonies et des individus, aura des conséquences sur la survie hivernale des colonies. La pénurie de disponibilité florale entre la floraison du colza et du tournesol serait donc assimilable à une disette alimentaire, agissant négativement sur la fitness des colonies d’abeilles.

La première étape de cette étude a été d’analyser des dispositifs de suivi d’abeilles domestiques en condition réelle de gestion apicole et à l’échelle du paysage. Ces suivis nous ont permis de collecter un jeu de données empiriques très conséquent sur les traits d’histoire de vie des abeilles, à différentes échelles temporelles d’une part (durant toute la saison de développement des colonies, Avril à Octobre ; et pendant 5 années consécutives), et spatiale d’autre part (composition et structure paysagère). Les analyses effectuées l’ont été à deux échelles biologiques : celle de l’individu (histoire de vie et comportement des butineuses), et celle de la colonie. Deux questions principales ont été abordées. Tout d’abord, (1) quelle est la composition et la dynamique temporelle du régime alimentaire (reflétant l’acquisition des ressources) des abeilles en paysage céréalier intensif ? Nous avons étudié la part de différentes ressources florales mais également étudié la part relative des différents compartiments paysagers (à savoir, cultures versus milieux semi-naturels) dans la composition du régime alimentaire. La deuxième question aborde l'hypothèse d'une réaction adaptative des colonies et des individus face à cette déplétion d'approvisionnement alimentaire, par des mécanismes de régulation et compromis d'allocation des ressources. (2) Ces compromis d'allocation peuvent-ils engendrer des coûts reportés plus tard en saison (« carry-over effects »)?

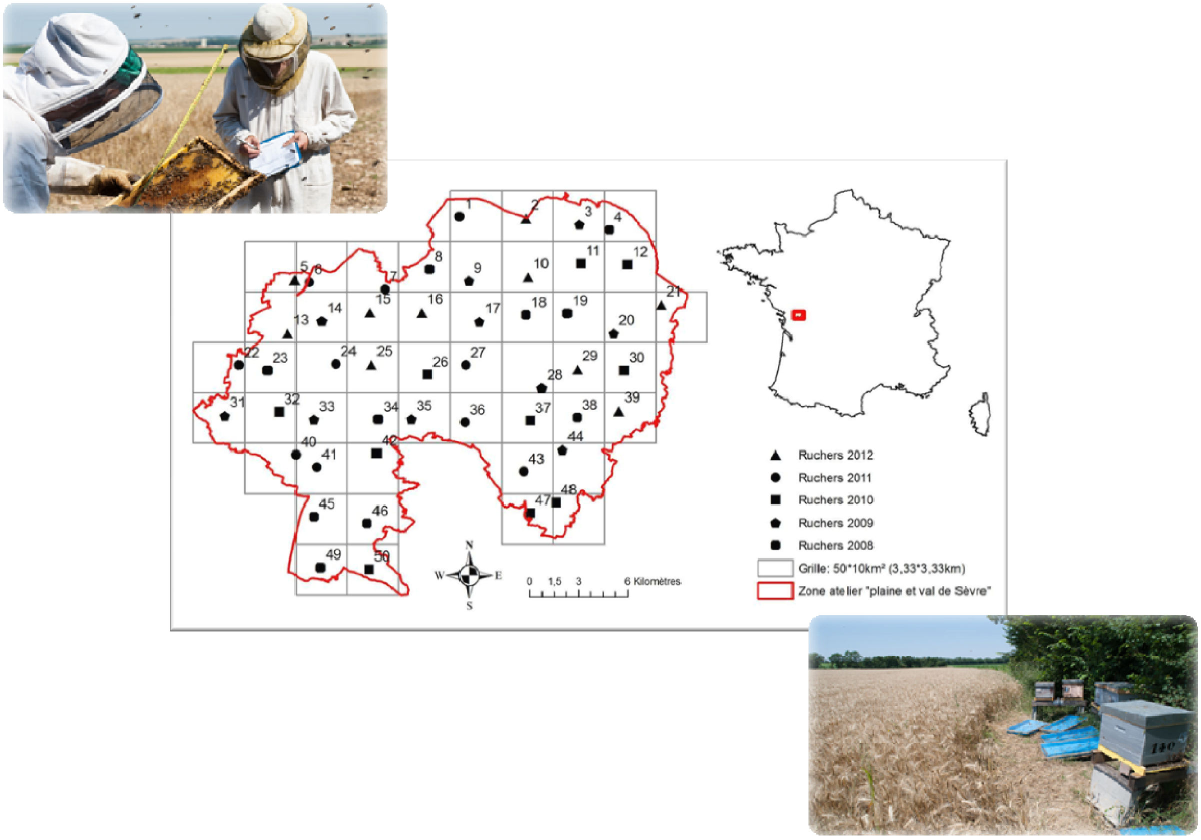
L'enjeu sous-jacent de cette thèse est de ré-évaluer l'influence du stress environnemental (en particulier, manque de ressources florales) dans le cadre des causes multifactorielles du déclin des colonies d'abeilles, mais également de constituer des connaissances de base sur l'écologie de l'abeille afin de pouvoir calibrer de façon judicieuse des mesures de conservation en faveur de l'abeille domestique, de l'apiculture et du service de pollinisation en paysage agricole intensif.

MATERIEL & METHODES

Monitoring des colonies

Monitoring de l'histoire de vie des colonies à l'échelle du paysage

Cet article développe et détaille le design spatial et temporel du monitoring ECOBEE mis en place au sein d'une vaste plaine agricole céréalière. Les méthodes et mesures d'estimation des différents traits d'histoire de vie des colonies et des variables paysagères sont également énoncées, afin de mettre en avant les possibles applications de ce vaste jeu de données empiriques dans la problématique actuelle du déclin de l'abeille domestique et de l'apiculture en paysage agricole intensif.



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Title: ECOBEE: a tool for long-term bee colony monitoring at landscape scale in West European intensive agrosystems.

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ECOBEE: a tool for long-term bee colony monitoring at landscape scale in West European intensive agrosystems

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Summary

In Central Western France as in many other areas, traditional apicultural practices have been replaced by more intensive practices in order to compensate for colony losses and current decreasing yields. One neglected aspect of apicultural practices concerns the choice of the locations by professionals to settle apiaries in intensive agrosystems with regard to landscape features, a choice which appears to be largely empirical. ECOBEE is a colony monitoring scheme specifically intended to provide beekeepers and researchers with basic ecological data on honey bees in intensive agrosystems, as well as colony population dynamics. ECOBEE was launched in 2008 in a long-term ecological research territory, with three specific aims: (i) to monitor seasonal and inter-annual population dynamic parameters of honey bee colonies in a heterogeneous farming system; (ii) to provide relevant and robust datasets to test specific hypotheses about bees within the frame of theoretical or applied research, such as the influence of landscape planning, agricultural inputs or human pressure (iii) to offer opportunities for assessing the effectiveness of agro-environmental schemes or the effects of changes in agricultural policies on the honey bee development. We herein present an overview of the ECOBEE platform, the type of datasets collected over the first four years of monitoring and their possible application and use. These include colony size, brood surfaces, food reserves and harvested pollen species composition. We found that colony dynamics were largely influenced by the phenology of the main mass-flowering crops foraged by bees, namely oilseed rape and sunflower. Furthermore, we have detected a sharp food shortage period, in late spring between oilseed rape and sunflower blooms, possibly temporarily constraining colony demographic sustainability. We further discuss the research perspectives offered by the ECOBEE monitoring package, especially with regard to spatial ecotoxicology.

Keywords: honey bee monitoring; floral resources; colony dynamic; agrosystem landscape; agricultural practices; France; intensive cereal farming; AES

Introduction

The Common Agricultural Policy (CAP) that was set up in Europe in 1962 has resulted in profound changes in rural farmland habitats. In particular, farming systems strongly intensified over the past 50 years, which led to a homogenization of agrosystems, now accounting for a high proportion of total land cover (e.g. 46% in France and 61% in the Poitou-Charentes French region (Marot and Maurand, 2012). The homogenization of agricultural lands – particularly in areas of mixed farming – has resulted in an over-mechanization of agricultural practices, along with an increase in field size, a reduction of the number of cropped species at the regional scale, and a more systematic use of agro-chemicals. Repeated warnings have been given about the negative environmental impacts associated with agricultural intensification, among others the erosion of biodiversity (Benton et al., 2003, Tscharntke et al., 2005). For instance, in large portions of agricultural landscapes, hedgerows and grasslands have been removed in order to simplify mechanization and farming practices.

In parallel, professional beekeeping activity in farmland habitats has been considerably modified over the past 20 years. Traditional apicultural practices have been replaced by more intensive practices in order to compensate for colony losses and decreasing honey yields (Maxim and van der Sluijs, 2010, vanEngelsdorp and Meixner, 2010, Robert-Vérité and Bova, 2012). Apicultural adaptations to changing environments include artificial syrup feeding, mass queen breeding, seasonal migration and professionalization (GEM, 2005, Saddier, 2008). Beekeeping sustainability in farming landscapes is currently threatened and beekeepers tend to seasonally move honey bee colonies away toward areas with less human activity and more diversified natural resources. The underlying causes responsible for these latter changes in beekeeping practices are not fully understood. In particular, the choice of appropriate apiary locations for honey production in intensive agrosystems, which to a large extent must rely on landscape features, remains largely empirical to date.

French agricultural farmlands have traditionally been areas of high honey production. Indeed, some regions have become famous for their honey from sainfoin, sarrasin, or lucerne (Louveaux, 1996). However annual crops such as oilseed rape and sunflower, the cultivation of which has increased threefold in France over the last 30 years (Marot and Maurand, 2012), have had profound consequences for honey bees and beekeeping as these mass flowering crops are very attractive to honey bees. In the recent past, these crops provided economically valuable honey flows in intensive cereal systems. In particular, sunflower honey accounted for up to 80% of total Centre-West France honey production in 1994 (Aubert, 2002 , Mollier et al., 2009). This new resource for honey bees has considerably modified the annual bee colony dynamics as well as the professional beekeeping practices and equipment. In particular, some atypical colony behaviours such as brood production stopping or a massive exit of honey bees which then hang below the hive entrance were described during the sunflower honey flow (Aubert, 2002).

However, since 1993, i.e. the approximate period when the honey bee decline began in France, the honey production gradually decreased by half in Central Western French region (Aubert, 2002 , Saddier, 2008). The precise reasons for the honey bee decline is hotly debated in France as in other countries among beekeepers, farmers and agricultural industries/companies (Allier et al., 2010). Different possible causes have been suggested, such as pesticides, parasites and diseases, lack of floral resources and biodiversity erosion in cropping landscapes (Maini et al., 2010). Nowadays, about 30% of the beehives have to be restocked every year in agricultural regions (FranceAgriMer, 2012). Special consideration must also be given to risk assessment of pesticide use in the agricultural environment, since pesticide use may affect both short and long term survival of bee foragers, contribute to the collapse of hives, and decrease flora abundance and richness. Pesticide risk is not randomly distributed in agricultural landscapes as it is directly related to the agricultural systems, i.e. crops, their succession, and the farming practices. In addition, since mass floral/mass-flowering species lead to a substantial mobilization of foragers to store honey and pollen, such crops may modify the annual cycle of the colony and generate hazards to the bee health (in short or long term). As shown by recent work on homing failure, risk assessment must take into account the foraging and spatial ecology of honey bees (Henry et al., 2012a, EFSA., 2013).

Currently, a critical question for beekeepers is to understand and predict where to set up their apiaries in intensive agro-ecosystems, i.e. which particular landscape would be optimal. Indeed, potential honey production around an apiary is difficult to predict, and there is a global need to understand honey bee ecology in intensive cereal systems better, in order to fulfil major expectations from beekeepers such as how to ensure year-round food resource availability for bees (Janssens et al., 2006). Beyond spatial disruptions (Briane, 1991, Sande et al., 2009), it appears that temporal disruptions in food resource availability also arise from the simplification of arable landscapes and the general decrease in weed species. The common agricultural policy of the European Union has encouraged agro-environmental schemes (AES) dedicated to promote floral resources for bees, such as flowering fallows or field margins. Their effectiveness is however highly variable depending on the local landscape context (Decourtye et al., 2010, Henry et al., 2012c). The quality and quantity of pollen resources is also a keystone issue for honey bee population dynamics. Trees and crop weeds provide most of their pollen supplies (Odoux et al., 2012). Since a deficient pollen intake reduces the ability of the colony to sustain brood production, and may lead to cannibalism of larvae (Schmickl and Crailsheim, 2001), we need to know more about floral resource availability and pollen quality, particularly in intensive agro-ecosystems where the use of chemical and the simplification of crop rotations has resulted in a general decrease in quantity and perhaps, quality, of food resources for bees.

To cope with the numerous concerns of professional beekeepers in modern agricultural environments, and to further provide researchers with basic, accurate, long-term ecological data on honey bees under current beekeeping practices, we have developed a colony monitoring scheme, called ECOBEE. ECOBEE was launched in 2008 in a long-term ecological research territory, with three specific objectives: (i) to monitor the seasonal and inter-annual dynamics of honey bee colonies in a heterogeneous farming system at the landscape level, (ii) to provide relevant and robust datasets to test specific hypotheses about bees within the frame of theoretical or applied research, such as the influence of landscape planning, agricultural inputs or human pressure (iii) to offer opportunities for assessing the effectiveness of agro-environmental schemes or the effects of changes of agricultural policies on honey bee development.

The ECOBEE monitoring scheme consists of the joint collection of honey bee ecological and environmental data (Table 1). Ultimately, the ECOBEE datasets may be analysed jointly to investigate temporal and spatial issues in the ecology of honey bees in an intensive agrosystem. This includes the assessment of land use and farming practices effects on colony health and dynamics, but also the temporal effects induced by variations in meteorological conditions and floral resource phenology, both at seasonal and inter-annual levels.

Herein, we provide an overview of the datasets acquired during the first four years of ECOBEE monitoring. Detailed descriptive statistics on seasonal colony dynamics, spatial variations in colony size, inter-annual variations in food reserves and harvested pollen species composition are provided, as a prime illustration of the temporal and spatial issues dealt with by ECOBEE.

1. Honey bee ecological variables	1.1. Colony life history	• Health monitoring, collapse events • Requeening events (swarming, division…) • Beekeeping tasks (artificial feeding, honey yields…)
	1.2. Colony dynamics	• Colony size • Brood area • Drone brood area • Food reserve mass
	1.3. Resource use	• Harvested pollen species composition • Honey-embedded pollen species composition
2. Environmental variables	2.1. Floral resource phenology monitoring 2.2. Land use monitoring 2.3. Climatic data	

Table 1 : List of the main recorded variables within the frame of the ECOBEE monitoring scheme. See text for detailed field methods.

Materials and methods

The study area

The study area “Zone Atelier Plaine & Val de Sèvre” is a Long-Term Ecological Research (LTER) located in the Poitou-Charentes Region, Western France (46°11’N, 0°28’W, 43m average altitude, Fig 1). The main interests of this zone lie in its large size and the historical data related to monitoring of land use by the CNRS-CEBC over the past 19 years (1994 onwards). The site is bordered by the town of Niort to the north, and Chizé forest massif to the south. Approximately 28,000 inhabitants live in the area, in c. 40 villages. The study area size is 45,000 ha, of calcareous plains which are subjected to a warm-temperate oceanic climate. With a mean annual precipitation of 840 mm and regular summer dryness, mixed oak forests would be the potentially natural vegetation (Bohn et al., 2000/2003). Rain is scarce in summer, however bees are not supposed to suffer from drought. Woodlands are mostly reduced to small and scattered remnant patches (mean patch size = 1.87 ha) and hedgerows are widespread in some areas especially in the cattle breeding sectors. Woods are composed of medium-sized trees, maple, ash, cherry or oak (*Acer, Fraxinus, Prunus cerasus* or *Quercus*), with a strong presence of elm (*Ulmus*). Wood edges are major food resources for the honey bees, in particular in spring (Odoux, 2012). Some of the main human derived landscape features of this area are a suburban zone in the North including many garden areas, a motorway on a North-South axis carrying heavy road traffic, and an electricity substation creating a concentration of high voltage power lines in some areas.

Environmental data used by ECOBEE mainly concerns land use, but other data sets of interest include hedges, hydrography or soils. Crops consist of cereals (>45%), spring-sown crops (maize and sunflower), oilseed rape (15%) and grasslands (about 18%). Fodder crops are permanent or temporary, based on legumes (clover, lucerne, sainfoin…), or grasses (ray-grass, fescue, orchard grass, foxtail millet…). The farming system encountered is mainly a mixed farming type. The Poitou-Charentes region has been subjected to agricultural intensification over the last fifty years, with traditional mixed farming systems being eventually replaced by cereal systems only, a process that still continues today. Currently 40% of the 650 present farms in the study area are cereal-only farms. Average farm size is 94 ha (Agreste, 2012) but some reach more than 200 ha.

Half of the study site has been designated as a NATURA 2000 SPA (FR5412007 – Plaine de Niort Sud-Est) under the Bird Directive. This status allows the implementation of Agri-environmental Schemes (AES), a tool deriving from the second pillar of the CAP. In France, AES are mainly 5-year contracts between volunteer farmers and the government. These contracts are intended to promote the implementation of environmentally friendly agricultural practices in return for an annual subsidy to offset the costs and possible income reductions involved (Berthet et al., 2012). For example, some of the contracts promoted on the study site aim to encourage the return of meadows and grasslands, and agricultural management practices favorable to insects and flowers, imposing in some cases strong constraints with regard to mowing dates in order to protect ground nesting birds. The area under AES in 2011 was nearly 10,000 ha. Plant species diversity and insect abundance has been enhanced, contributing to bird conservation (Bretagnolle et al. 2011a, b).

The study material

All honey bee colonies originated from a livestock managed by a local professional apicultural association (ADAPC) using *A. mellifera mellifera x caucasica* strains. At the time of launching the monitoring program, the queens were one year old. Colonies were checked to ensure they were disease-free. The beehives were Dadant-Blatt model (on 10 brood chamber frames) in pine woodwaxed microcrystalline. Colonies were set up and managed as a sedentary apiary according to local professional beekeeping practices. Supers were added as honey reserves grew. They were generally harvested twice a year, after the oilseed rape and the sunflower honey flows. Queen cells (CFPPA Venours College) from the same strain were introduced for requeening colonies. All colonies were fed with commercial syrup in May and June in order to compensate the apparent food shortage between oilseed rape and sunflower flowering periods (see discussion).

The spatial design

The 450 km^2 study area was divided into 50, 10km² square plots whose size (Fig.1) was set to encompass the mean foraging distance of honey bees (about 1.5 km in such landscapes (Steffan-Dewenter and Kuhn, 2003). Each year from 2008 onward, ten new experimental plots were randomly chosen from the 50 available plots. In each selected plot, a 5-hive apiary was set up, as close as possible to the centre (usually ± 500m), and was monitored during the whole apicultural season, spanning from April to September (see *Data collection*).

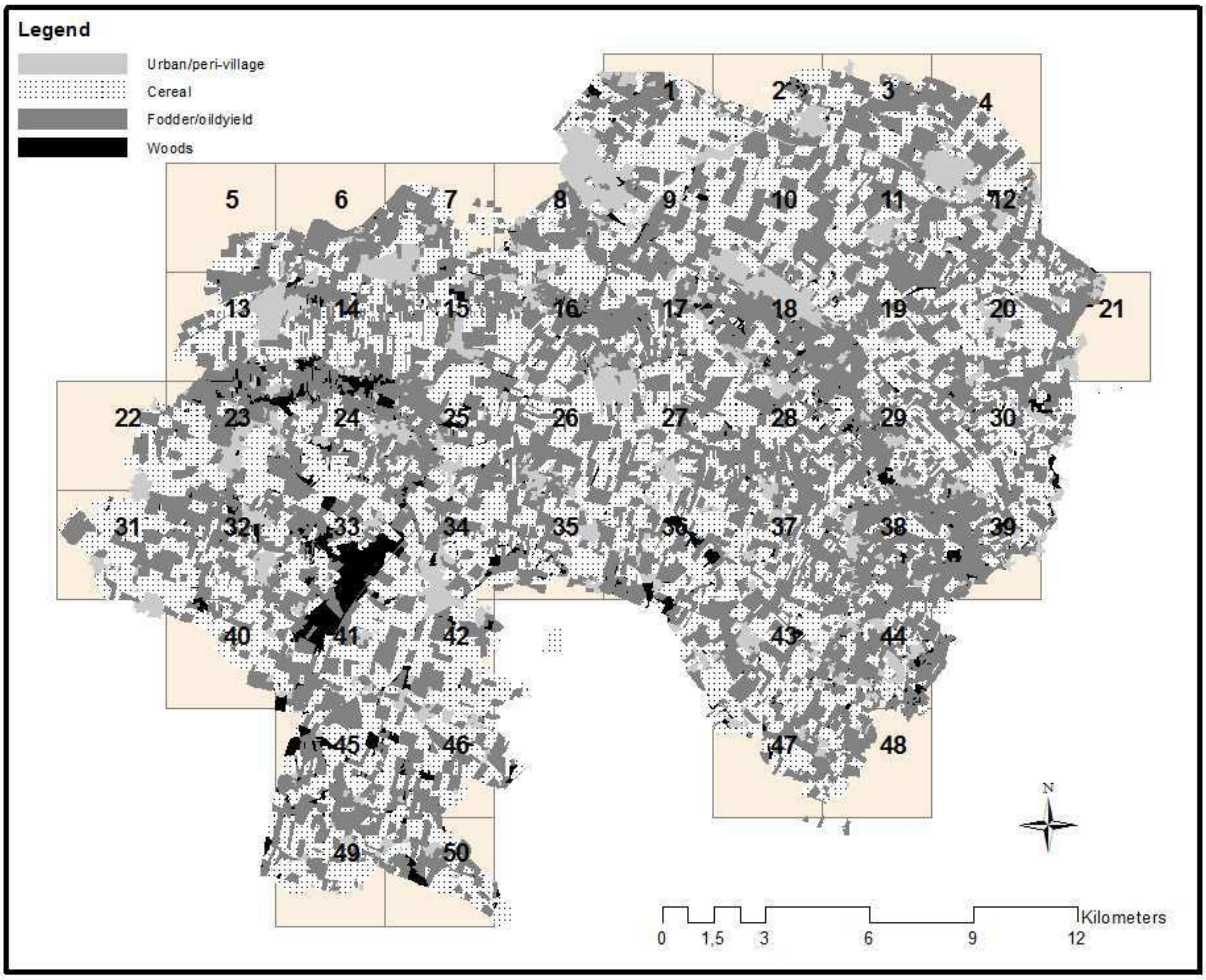

Figure 1 : Study area "Plaine & Val de Sèvre", in Centre West of France.

At the end of the apicultural season, the hives were gathered together in a wintering apiary. After overwintering, colonies started the monitoring program again with a different status in terms of population structure and queen age. Hives were then randomly re-assigned to the new experimental plots. New colonies intended to replace lost ones were equally allocated among plots. The hives were set up in plots in March or April, and remained on site until late September.

Data collection

Since we made regular (twice a month), and sometimes intrusive measurements within the hives that could potentially affect colony dynamics, we systematically kept two hives of the apiary as control (i.e., undisturbed) hives. Thus, only three of the five colonies in each experimental plot were monitored every two weeks during the apicultural season. The two *control* colonies were only checked at the beginning and end of the apicultural season. They were also used as *substitution* colonies because they could have been used to replace *experimental* colonies of the same plot that collapsed in the course of the monitoring season. Parameters measured in the course of the monitoring protocol are detailed below.

Honey bee ecological variables

Colony life history

Every single beekeeping manipulation and survey is stored in a database (software Microsoft Access) to keep track of each colony's history. This includes first and foremost the general health status of colonies, i.e. whether they have shown evidence of population weakening, demographic slowdown or diseases. Demographic events such as colony division, swarming or requeening were noted, as well as all the current practices including artificial feeding, honey super set up or harvest, etc.

Colony dynamics

Studied demographic parameters included: the adult bees population size, the brood (eggs, larvae, pupae) size, and the food reserves quantity. This allowed the derivation of four parameters that are used to describe the state of these demographic compartments: (i) colony size, i.e. the number of adult honey bees, (ii) the total brood area, indicating which effort is allocated by the colony to the production of new adult workers, (iii) the drone brood area, indicating which effort is allocated to sexual reproduction, (iv) the honey reserve mass. These colony parameters were assessed at each monitoring visit, either every two weeks for the monitored hives or at the beginning and at end of the season for the control hives, using the following methods:

- The colony size (number of adult individuals) was assessed by weighing each brood frame with and without bees, as well as the hive bottom and the honey supers. Colony size was then assessed considering an average of 10^{-4} kg/bee. The resulting values slightly underestimated real colony size because it didn't take into account the portion of foraging bees that had left the colony at the time of measurement.
- Brood surfaces, on both side of each brood frame, were assimilated to ellipses (Fresnaye, 1961, Mallet and Charles, 2001, Vallon et al., 2008) and therefore approximated using length L and width W measurements (cm) following the formula

S (cm^2)= ¼(L.W.π). Brood surfaces were then totaled across all the frames of each colony. Isolated empty cells within brood surfaces were ignored.

- Food reserve mass (stored honey and pollen) was assessed on each frame, both from the main chamber and the honey supers, as the difference between total frame mass and the mass of empty frames before introduction in the hive. Whenever necessary, brood mass was also removed from the resulting mass difference. Brood mass values were derived from brood surfaces, using the formula m (kg) = $m_{\max} \times S / S_{\max}$; were $S_{\max}$ is total frame surface (1632 cm^2) and $m_{\max}$ is the total brood mass expected for a frame surface entirely covered by brood (0.6385 kg, predicted from the linear regression between 6000 brood surface values and the corresponding full-empty frame mass differences).

Resource use

To assess resource use, palynological analyses were performed on pollen harvest samples and on honey samples.

- Harvested pollen species composition indicates which floral resources are foraged for pollen. To sample harvested pollen, each of the three monitored hive entrances were periodically fitted with a pollen-trap (24-h sampling every ten days, except when queens were in nuptial flight period). Pollen species composition was assessed at the apiary level after homogenisation of pollen samples among colonies within experimental plots. Palynological analyses for pollen identification followed Louveaux et al. (1978) with at least 300 pollen grains identified on both microscope slides taken in each sample (Tamic et al., 2012).
- The honey-embedded pollen was intended to give information on plants foraged for nectar. Assuming that incidental pollen grains were ingested by nectar foragers and subsequently stored with honey, the most abundant pollen species found in honey were likely to reveal the identity of plant species intensively foraged for nectar (Louveaux et al., 1978). Honey samples from each apiary were processed by an external laboratory.

Environmental variables

Floral resource phenology monitoring:

The availability of floral resource species was monitored through phenological surveys conducted in the close vicinity of the study area (20km east, INRA Poitou-Charentes, UE Entomologie laboratory). The phenological database, called *Apibotanica*, is available on-line (www.poitou-charentes.inra.fr/entomologie) and further described in Odoux et al. (2011). Blooming dates were monitored for more than 400 botanical species of interest, including flowering crops.

Land use monitoring:

Land cover has been recorded twice a year every year since 1994 on every single field (c.19000) of the study area, and stored on a GIS database (ArcGIS, version 10). About 50 land cover categories were recorded by observers driving on every single track of the study area (Bretagnolle et al., 2011) .

Weather data

Detailed meteorological data for the region was available from a national French meteorological station (Météo-France, 2012) located in Niort, on the northern edge of the study area. Relevant variables likely to influence honey bee foraging activity and colony development was available on an hourly basis, including temperature, precipitation, wind, solar radiation, daylight duration, etc.

Data analysis

Four years after launching the ECOBEE monitoring platform, we are now able to give an overview of the seasonal dynamics of a typical colony in the studied intensive agrosystem. The four main colony dynamic parameters were modeled as a function of time using Generalised Additive Mixed Models (GAMMs) with the R software (the R Foundation for Statistical Computing, version 2.11.1). GAMMs are modeling techniques allowing the fit of temporal splines while giving the possibility of taking into account repeated measurements on statistical units in a nested design (i.e. colonies nested within experimental plots and within years). Models were fitted to data using the maximum likelihood method. Due to the many zeros in the drone brood surface dataset, we found it more appropriate to model drone brood occurrence frequency (presence-absence) using a binomial family distribution.

In a second step, we focused on colony size and tested whether its temporal dynamics would be further influenced by landscape composition. To do so, we recomputed the colony size temporal GAMM after adding land use covariates that were liable to influence colony dynamics. Relevant land use covariates were the surfaces (ha) of oilseed rape, woody habitats, grasslands, legume fields (alfalfa) or cereal fields, measured within a 1.5-km array from apiaries, i.e. the usual foraging range of honey bees. Possible model improvements were assessed using the Akaike Information Criterion, considering each land use covariate taken separately. More detailed descriptive statistics on (i) honey reserves and (ii) pollen species richness were further provided to support the colony dynamics results and illustrate temporal and spatial variations, respectively, in colony supply.

Results

Typical colony temporal dynamics

The GAMMs showed a significant time effect on each of the four colony parameters, indicating that measured parameters do not vary randomly among colonies but follow a general temporal pattern (Fig. 2). Brood surface and drone brood occurrence frequency (temporal spline effect: $F_{8,1219}=102.5$, $p<0.001$ and $F_{1,1226}=246.6$, $p<0.001$, respectively) are at their maximum early in the season, when the hives are set up in the study area. They gradually decrease during the season, eventually down to nearly zero for the drone brood occurrence frequency. Colony size, i.e. adult population size peaks later in the season ($F_{7,979}=41.66$, $p<0.001$), after the oilseed rape blooming period, with an average of about 27,500 honey bees. Honey reserves ($F_{8,1219}=114.8$, $p<0.001$) show two distinct peaks, namely a low one during the oilseed rape blooming period, and a high one during the sunflower period.

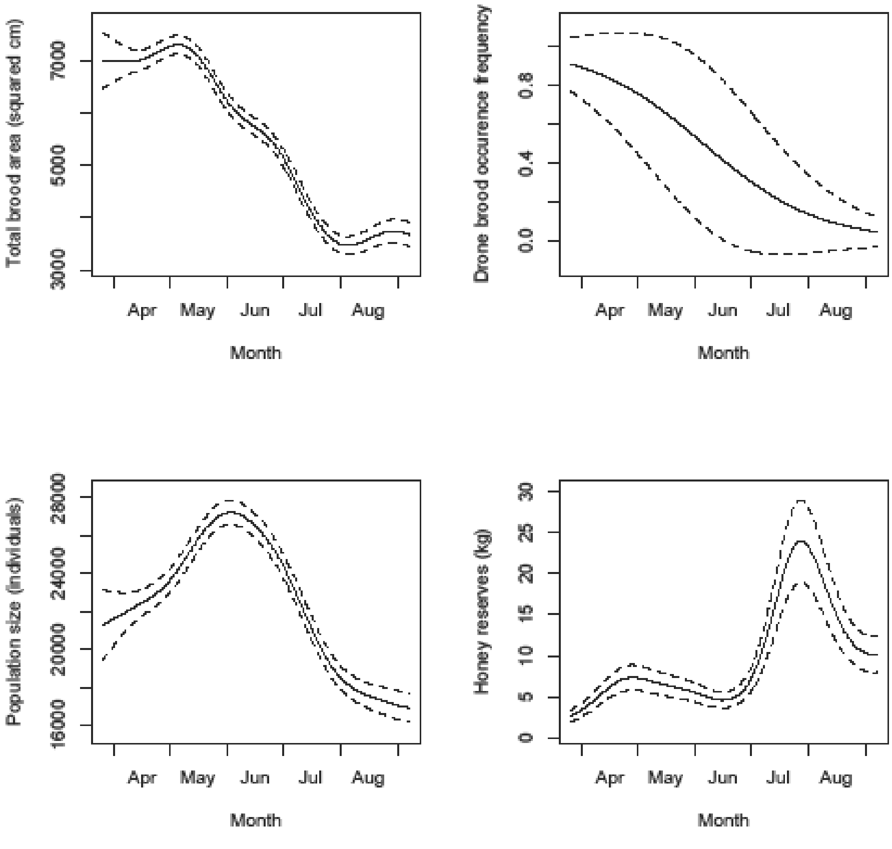

Figure 2 : Average seasonal dynamics of colony parameters predicted by the GAMMs (±1SE), including total brood area, drone brood occurrence frequency, colony size (adult population) and honey reserve mass. Data proceed from 208 colonies monitored over the 2008-11 beekeeping seasons.

Inter-annual variations in food reserve dynamics

Colony seasonal dynamics are likely to be influenced by meteorological and plant phenology, as revealed by the substantial inter-annual variations found in the ECOBEE datasets. Although food reserve dynamics followed the same general pattern over years (Fig. 3) with a steep increase during sunflower blooming period (mid-June-late July), important temporal lags existed from one year to the next. For instance, a lag of up to >20 days was found between years 2008 and 2011 in sunflower honey flow (Fig. 3). Inter-annual variations are probably mostly driven by weather conditions, as the earliest sunflower honey flow was obtained in 2011, i.e. the year with the most precocious spring.

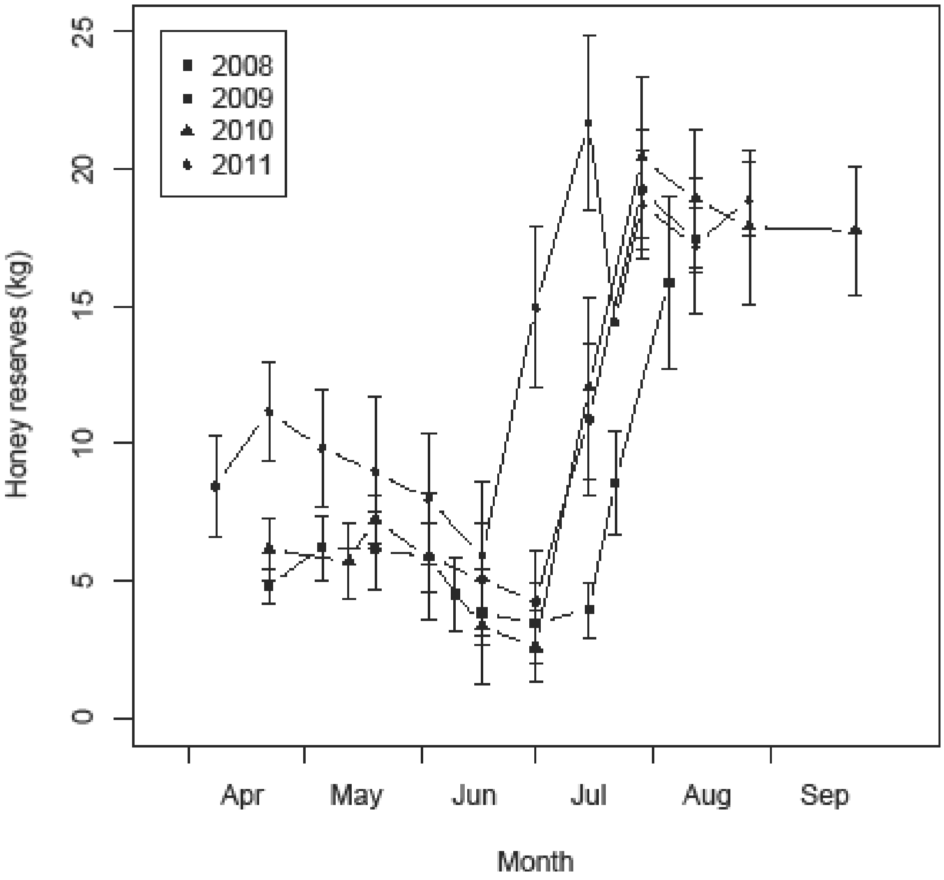

Figure 3 : Inter-annual variations in the dynamics of body chamber food reserves for 30 experimental colonies each year in 2008 to 2011.

Landscape effects and spatial variations in resource use

To better assess how landscape composition might influence the seasonal dynamics of colony size, we rescaled the temporal axis on each year's specific oilseed rape blooming end date, as estimated from phenological surveys, and tested the contribution of the main land use covariates, taken separately, to the improvement of the temporal model. Compared with the simple temporal GAMM, the "woody habitat" covariate allowed for a substantial improvement of model fit (AIC decrease = 6.9), whereas all the other candidate covariates did not (AIC increases = 1.8, 2.9, 3.8 and 4.1 for grassland, alfalfa, oilseed rape and cereal surfaces, respectively). The effect of woody habitats was highly significant (Likelihood ratio test statistic = 8.88, P=0.0029), and positively influenced colony size, and possibly sustainability, after oilseed rape blooming period (Fig 4).

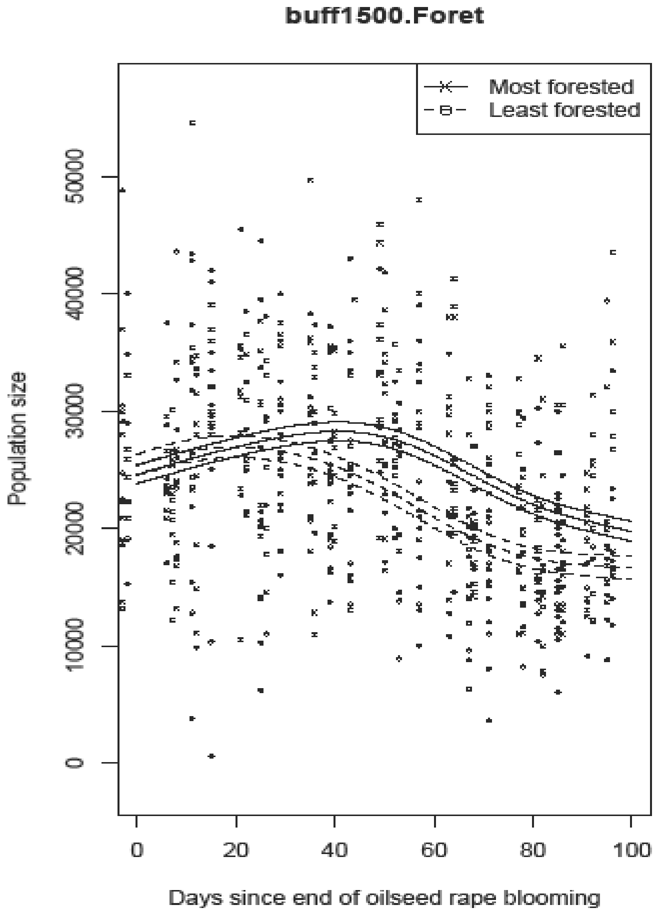

Figure 4 : Influence of woody habitats on the colony size dynamics after oilseed rape period. The temporal axis was rescaled on each year's specific end date of oilseed rape blooming. Curves show the expected colony size (±1SE) for the least and most forested environments, as defined by the median value of woody habitat surfaces measured within a 1.5-km radius from colonies (1.0 to 15.6 ha vs. 15.6 to 263 ha for the least and most forest areas, respectively).

Pollen diversity

Landscape heterogeneity is further expected to influence floral resource availability, and therefore honey bee foraging activity. This can be shown by comparing species composition in pollen samples among contrasted experimental plots. Available pollen data suggests that the diversity of collected pollen varies markedly among apiaries. For instance, in the absence of mass-flowering crops (e.g. week #21, between oilseed rape and sunflower flowering periods, Fig. 5), the most diversified pollen samples displayed twice as many species as the least diversified ones. Harvested pollen diversity gradients may be further compared with landscape heterogeneity gradients.

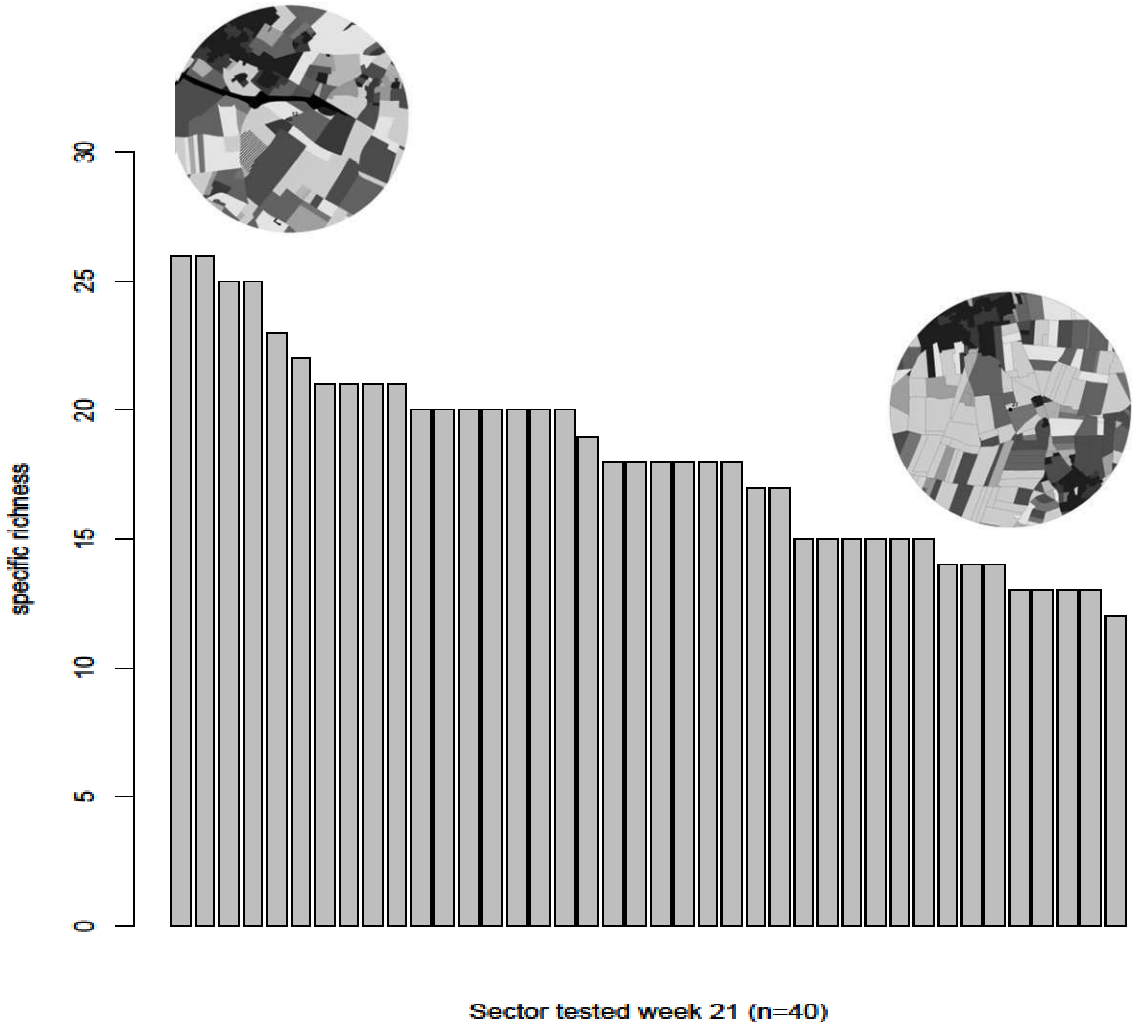

Figure 5 : Pollen species richness for the week #21 in pollen samples from 40 experimental plots (years 2008-2011). The two 1500m landscape buffers illustrate land use heterogeneity in the environment of the apiaries with the most and least diversified pollen samples.

Discussion

Up to now, the ECOBEE platform monitoring scheme has allowed identification of important patterns regarding the ecology of honey bees in our studied intensive agrosystem. First, colony temporal dynamics follow a strong temporal pattern, most likely scaled on the phenology of the two main mass flowering crops in the region, namely oilseed rape and sunflower. Second, the seasonal food storage dynamics appears to be largely influenced by mass-flowering crop phenology, itself driven by year-specific meteorological conditions. Third, floral resource use varies tremendously among colonies, probably reflecting the environmental conditions of each local landscape context.

We further suspect that colonies undergo a food shortage period between the two mass-flowering crops, encompassing the late May to early July period. Honey supers are removed by beekeepers early in this period – after oilseed rape blooming – while colony size reaches its seasonal peak. During this period, food reserves available to bees in the hive body chamber gradually decrease (in June; see Fig. 3), indicating that the colony as a whole might deplete honey at a faster rate than foragers actually store it. Yet, the diversity of wild floral resources is high during this period, as indicated by the phenological surveys (Odoux et al, 2012). We further suggest that woody habitats, intensively used by honey bees for pollen early in the beekeeping season (Requier et al., in prep.), buffer the population decrease after the oilseed rape period (Fig. 4), possibly improving the resilience of colonies and their overall size later on during the sunflower honeyflow. Further analyses remain to be done to document the food shortage hypothesis and assess the economic benefits of maximizing woody habitats in the close vicinity of apiaries early in the season.

ECOBEE is a monitoring scheme that allows a detailed characterization of honey bee temporal and spatial dynamics according to landscape, seasonal and yearly variations. Our quasi-experimental scheme is able to provide considerable amount of data on honey bee colonies ecology and behaviour in relation and response to environmental constraints. Each year, over 6,000 observations are collected from 50 hives, in parallel to landscape data (19,000 crop fields coupled with farming and environment features), which allow us to investigate relationships between bee life history traits, apidology and farmland landscapes. In addition, the spatial and temporal variability of landscape parameters in the study area offers the possibility of testing the effects of different cropping systems, including experimental ones (AES), on honey bee ecology and performances with regard to food reserve (honey) acquisition. We believe our scheme is a robust experimental tool for beekeeping observations and for ecological research of honey bee traits in intensive farming landscapes. ECOBEE is also a tool that may provide guidance for beekeepers searching for suitable environmental contexts to set up healthy apiaries.

The coupled land use - colony monitoring data on a large scale will provide a strong background to test for different environmental factors related with food resources as well as some stressors like diseases, predators or the use of agro-chemicals. With regard to the latter, we have initiated standardised data collection through large-scale surveys of farming practices, with the objective of evaluating possible relationships between the use of pesticides and colony sanitary state in a spatially explicit approach, using for example “Treatment Frequency Indicator” (TFI) (Allier, 2012). The ECOBEE survey also offers the opportunity to foster model-based assessment of pesticide effects at the colony level through demographic simulations (Henry et al., 2012b). Finally, our experiments are currently serving as a reference work for the conception of innovative farming systems favourable to pollinators (POLINOV project, coordinated by ACTA), in collaboration with INRA (INRA SPE Dept), CNRS, ADAPC, and French technical institutes (ITSAP, CETIOM, ARVALIS, Chambre d’Agriculture). This research therefore contributes to both the preservation of threatened apicultural activities and to the development of new and innovative agricultural policies (see Berthet et al. 2011).

Ackowledgments

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Monitoring des individus

Monitoring de l'histoire de vie des butineuses

Cet article présente une méthode d'adaptation de l'utilisation de l'outil RFID (Radio-Frequency IDentification) sur l'abeille domestique afin de retracer l'histoire de vie des butineuses selon leurs activités de vol, en conditions naturelles. Différents traits d'âge et comportementaux majeurs ont été estimés grâce à la collecte automatique d'un vaste jeu de données durant toute la durée de vie de nombreux individus, simultanément et sans interruption.

Paper in prep.

Title: Monitoring life history traits in free ranging honey bees using Radio-Frequency Identification (RFID)

Authors: Requier F., Aupinel P., Brun F., Bretagnolle V., Decourtye A.

Monitoring life history traits in free ranging honey bees using Radio-Frequency Identification (RFID)

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Key-words: Honey bee, *Apis mellifera* L., life history traits, age-polyethism, RFID tags, automatic monitoring, flight behavior, foraging

1. Introduction

The current widespread decline of honey bee populations is particularly alarming (Potts et al., 2010a), because the pollination service provided by bees is vital for biodiversity and especially wild plants (Tilman et al., 2001, Biesmeijer et al., 2006) as well as ensuring crop yields in agricultural landscapes (Rader et al., 2012, Garibaldi et al., 2013). In addition, beekeeping industry is decreasing (VanEngelsdorp et al., 2008, Ellis et al., 2010, Potts et al., 2010b). Despite comprehensive research efforts on this major honey bee colony losses, no single explanation has emerged (Cox-Foster et al., 2007, Anderson and East, 2008, Potts et al., 2010a), and the hypothesis of synergetic effects between multiple drivers is favored (Potts et al., 2010a). Among these, environmental drivers such as pesticides and decrease of flower availability have been recently reappraised. For instance, Henry et al. (2012) provided evidence of behavioral disruption in honey bee foragers due to a common pesticide (thiamethoxam, a neonicotinoid), which decreases the survival probability of colonies in agricultural landscapes. This study comforts the need to investigate the ecology of honey bees, such as the life history and the behavior of foragers in field-realistic conditions (Desneux et al., 2007). Individual monitoring *in situ* may indeed provide empirical evaluations of environmental stress acting on honey bees. The latter may include pesticides, but also the decrease of flower availability, which was recently suggested to be a potential important driver of honey bee decline (Naug, 2009, Decourtye et al., 2010, Decourtye et al., 2011a, Di Prisco et al., 2013).

However very few tools are currently available for monitoring honey bees at individual level in the wild. Honey bee is an eusocial insect, living in colonies of several tens of thousands individuals (Winston, 1994). Among these, worker bees are the only individuals that perform foraging task in order to supply the entire colony in resource, with about 2,000 workers foraging daily (Seeley and Visscher, 1985). These individuals are actually old adults, because of an age-polyethism in honey bee (Ben-Shahar, 2005, Whitfield et al., 2006, Seeley, 1982). Actually not all workers will become foragers (Omholt and Amdam, 2004, Amdam et al., 2004, Rueppell et al., 2007). In addition, the tracking of foragers is really a technological challenge, because of their very small body size (about 12 mm and 100 mg, Winston, 1994) and their wide foraging area (about 2km, Visscher and Seeley, 1982, Beekman and Ratnieks, 2000, Steffan-Dewenter and Kuhn, 2003). Only the harmonic radar technology has been used successfully for tracking the flight movements of honey bee foragers (Capaldi et al., 2000, Riley et al., 1996). However this monitoring method has limits: first, very few foragers have been tracked (about ten in average, Riley et al., 2005, Menzel et al., 2011, Menzel et al., 2005), and second the tracking area was quite restricted (about 900 m of radius in ideal conditions without any obstacle, Riley et al., 2005).

RFID (Radio-Frequency IDentification) technology has recently been adapted to bees by miniaturization (Streit et al., 2003). This tracing tool is able to monitor individuals, for a long time (up to the entire life of the individual), and in large numbers simultaneously. RFID has been used so far in order to investigate the homing flight ability of foragers (Henry et al., 2012, Pahl et al., 2011), or flight activity of foragers with artificial food feeding in controlled conditions (i.e. trip records between a feeder and the hive, Schneider et al., 2012, Decourtye et al., 2011b, Gill et al., 2012). In this study, we extend the use of RFID technology to investigate individual long-life monitoring in field conditions, in particular with regard to the various tasks workers may perform according to their age. Our method is based on recording flight activity patterns of individuals to infer their life history traits.

2. Material and methods

2.1. Experimental design

Three honey bee colonies from a livestock managed apiary of *A. mellifera mellifera × caucasica* strain in INRA Le Magneraud (46°09' N, 0°41′ W) which is located in Central-Western France, in the Poitou-Charentes Region, were placed in 2011 on the ECOBEE platform (Odoux et al., 2014). ECOBEE is located within the Long-Term Ecological Research *Zone Atelier Plaine & Val de Sèvre* (46°23'N, 0°41' W), about 30 km of INRA Le Magneraud. Each colony was distant from each other by at least 1.5 km, the mean foraging distance of honey bees (Steffan-Dewenter and Kuhn, 2003), and placed in a field border. Colonies were set up and managed as a sedentary apiary according to local beekeeping professional practices (Odoux et al., 2014), during the whole beekeeping season, spanning from late March to October. Queens were one year old and their state of health was checked for any visible disease symptoms. The beehives were the Dadant-Blatt model (with 10 brood chamber frames) in pine wood-waxed microcrystalline.

During the entire experiment, a total of 2100 newly emerged workers (one day-old) were collected from the same hive in the livestock apiary. Each worker was equipped with an unique RFID-tag (Fig. 1a, mic3® - TAG 64 bit RO, iID2000, 13.56 MHz system, 1.0 mm × 1.6 mm × 0.5 mm; Microsensys GmbH, Erfurt, Germany), weighting about 3 mg (i.e., 3 % of an adult bee body weight). The RFID weight is low compared to honey bee capacity in carrying up 70 mg of nectar and 10 mg of pollen (Winston, 1994). Tagged bees were set up in free living colonies at six different dates (i.e. the first day of each month between April to September), in cohorts of 75-150 individuals per colony which would not affect the demographical dynamic of the colonies consisting in about 22,000 adult individuals (Odoux et al., in press, see details in appendix S1). Tags were fitted on the thorax of bees using dental adhesive (TempoSIL®2, Coltène/Whaledent s.a.r.l., Le Mans, France) in order not to affect flight properties of bees (Fig. 1a). Tagged bees were introduced into the three field colonies (hereafter called site).

Each time a tagged bee was passing a reader placed at the beehive entrance (working distance of 3 mm; (Streit et al., 2003), it was identified and recorded with date and time (with a precision of one second) (Decourtye et al., 2011b). In order to investigate the in-and-out activities of honey bees, we placed two rows (A and B, see Fig. 1c) of fives RFID-readers (iID2000, 2k6 HEAD; Microsensys GmbH, Erfurt, Germany) at the entrance of bee-hives (Fig. 1b). Stairs were placed at the bottom of each row of RFID-readers in order to slow the speed of bee passage under the readers to ensure their detection (Fig. 1c). This new RFID-design thus allowed discrimination of “in-trip” vs. an “out-trip”, according to the sequence (*seq*) of consecutive recording (*hit*) between rows A (inner row) and B (outside row, Fig. 1c, see below for method). To ensure continuous monitoring during the entire experiment (8 months) for each equipped hive, we set up an electrical continuous supply using a set of two batteries (power: 12V, 92Ah, C20). Particular attention was paid to batteries replacement every 3 or 4 days.

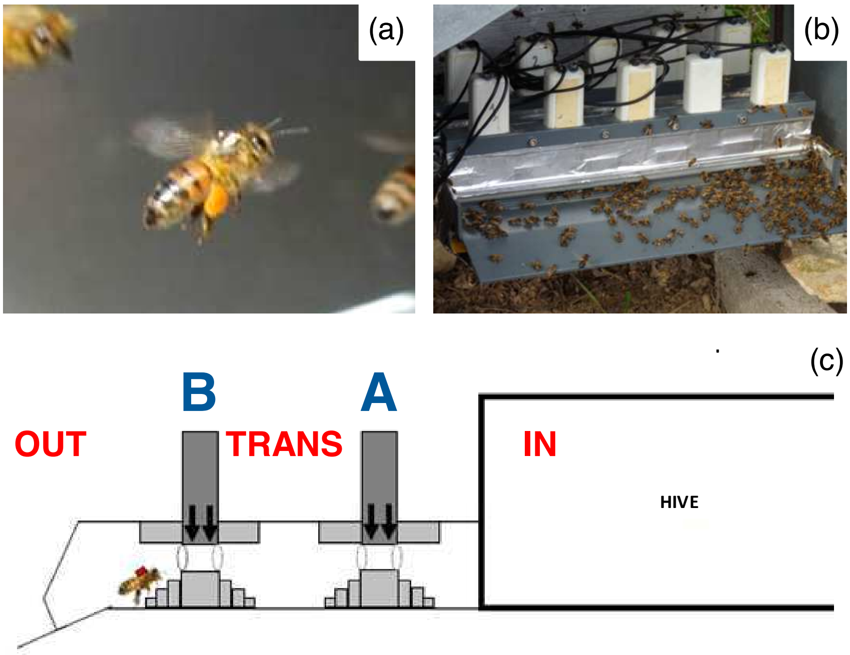

Figure 1 : Honey bee RFID monitoring equipment. (a) A pollen-forager honey bee equipped with RFID-tag, (b) A hive entrance equipped with two rows of five RFID-readers, (c) conceptual schema of RFID adaptation for detecting in-and-out activities of honey bees.

2.2. Data collection and formatting

All *hits* were recorded independently in each RFID-reader. The reader software (‘‘BeeReader’’, Tag Tracing Solution, Valence, France) recorded the identification code and the exact time of the event (*hit*), for each RFID-reader in ASCII (text) files. Several steps of data management were then necessary, and are detailed below (Fig. 2a). The first step was to format the data (see Fig. 2a). Though minimal (3 days of data lost out of 8 months), some electrical disruptions caused gaps in data collection. A first filter replaced those gaps by “NA”, to be distinguished from true zeroes. In addition, double or multiple recordings were often collected when a bee moved too slowly under the RFID-reader (i.e. the detection of the same bee in the same drive at the same time). These were filtered by an arbitrary threshold of 1 second.

The second step consisted in transforming the *hit* collected in sequences *seq* A and B (see Fig. 2b). We took into account the identity of the RFID-reader for each *hit*, while row A was composed of readers 1 to 5 (inner row) and row B composed of readers 6 to 10 (outside row). We obtained a succession *seq* of *hit* (A and B) for each tagged bee during its entire life span; for example, the *seq* structure of the bee *A00103C00020BF30* (see Fig. 2b):

AAA**ABBA**AAAA**ABBBA**ABABBA**ABA**AAAABABABBBBAAAABBAAAABBAAABBAABBBAAAAABBABBBAABAAAAABBBAABAABBAAAABABABBABBBBAAAABBAABBBAABAABBAAABBABAAAA

In this study, we focused only on the out activity of the bees (i.e., *seq* = BB), reflecting the investment of bees in flight-learning (including hygienic, guarding, and orientation flights, Seeley, 1982) and foraging tasks. To ensure that the BB *seq* were real out-trips, we selected initially all ABBA *seq*, indicating that the bee was in the hive (A followed by B), then moved to outside the hive (AB *seq*), made a *trip* (BB *seq*) and then returned to the hive (BA *seq*). Then we further selected anomalous sequences such as those with a single or multiple B (e.g. ABA, ABBBA). For ABA *seq*, we considered the absence of the second B as a result of the first filter in order to delete duplicates (see above). We therefore considered that the ABA *seq* corresponded to very short *trips* (<1 s), and added a second B, such as BB = 0 s. For sequences types ABBBA *seq*, we considered that a single *trip* was performed.

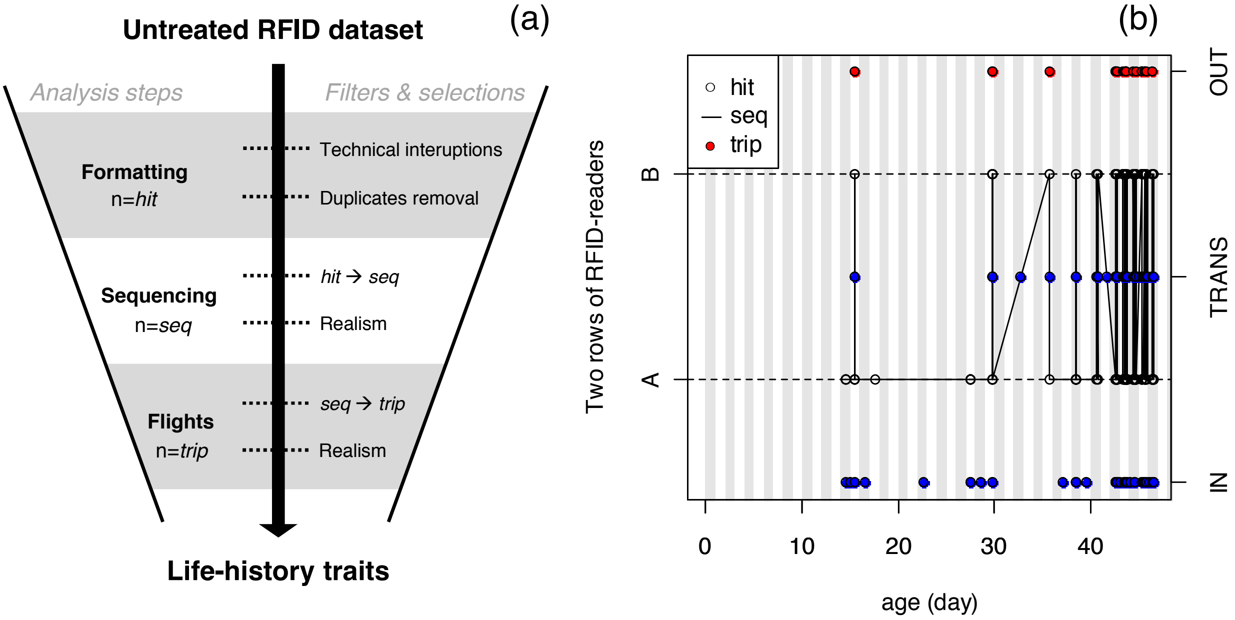

Figure 2 : RFID data structuration. (a) Analysis procedure with the three main steps and the six filters and selections performed before life history traits estimation, (b) An example of bee (*A00103C00020BF30*) monitoring with data structuration (n_{hit}=323, n_{seq}=131, n_{trip}=27).

The final step was to transform *seq* in *trip* (Fig. 2a). First, we considered the TRANS part of our experimental design (i.e. the part between the two RFID-reader rows A and B, see Fig. 2b), as inside the hive. Then we evaluated the *trip* parameters (i.e., the number, duration and hour of trips) only on the BB section of ABBA *seq*. A final filter was applied, selecting only the trips between 4 seconds and 2 hours. This minimum threshold (i.e. 4 s) is an estimate of time delays between the passages of the walking bee outer the row B of RFID-reader and the take-off. The maximum threshold (i.e. 2 h) is an arbitrary estimate of the maximum flight duration.

2.3. Life history traits characterization

We analyzed several traits, either in terms of behavior, or age related traits. The following five age traits were obtained:

- Age of First Exit (AFE) was defined as the BB>4s, and corresponded to the first date of *trip*. AFE informs on the length of honey bee allocation to in-nest tasks. For example, AFE was estimated at 15.3 days for bee *A00103C00020BF30* in Fig. 2b.
- Age of First Foraging (AFF) was estimated by a change in flight behavior between learning and foraging task (Capaldi et al., 2000). Because there was no clear cut difference between those, we combined several empiric indicators. In a first step, we invested the seasonal pattern of daily cumulative sum of the three parameters "number of trip per day" (*numberLM*), "sum of flight duration per day" (*timeLM*) and "sum of start times per day" (*hourLM*). We then identified a threshold of slope breakpoint in the seasonal pattern (see below in *Statistical analysis* section). This threshold was interpreted as the age of the change in behavioral flight (Fig. 3). In a second step, we added two other estimators that are quantitative thresholds, corresponding to the age where the bee performed for the first time more than 5 trips per day (*number*), and flight duration more than 15 minutes (time, Thom et al., 2000). These five estimators were then averaged to finally inform the value of AFF (*meanAFF*), and the variance among estimators provided an indication of uncertainty. For example, AFE were estimated to 23.9±1.1 for bee *A00103C0004029B0* in Fig. 3
- Lifespan (LSP) is provided by the last date of *hit* recording, related to the death of the bee. Most honey-bees die outside the hive, because the foraging is often the last task and outside environment is related to risk of predation and disorientation (Visscher and Dukas, 1997). In the opposite case, workers carry the dead individuals outside, as soon as possible in order to ensure the health of colony (about 12 mm and 100 mg, Winston, 1994). For example, LSP was estimated to 46.5 for bee *A00103C00020BF30* in Fig. 2b.
- Learning is provided by the age difference between AFF and AFE. Learning informs on the length of time allocation of honey bees to learn of flight and orientation flights.
- Foraging is provided by the age difference between LSP and AFF. Foraging informs on the length of time allocation of honey bees to foraging task.

In addition, we investigated three behavioral traits such as the number of trip per day, the mean duration of the trip per day (in second), and the mean time of the beginning of the trips per day.

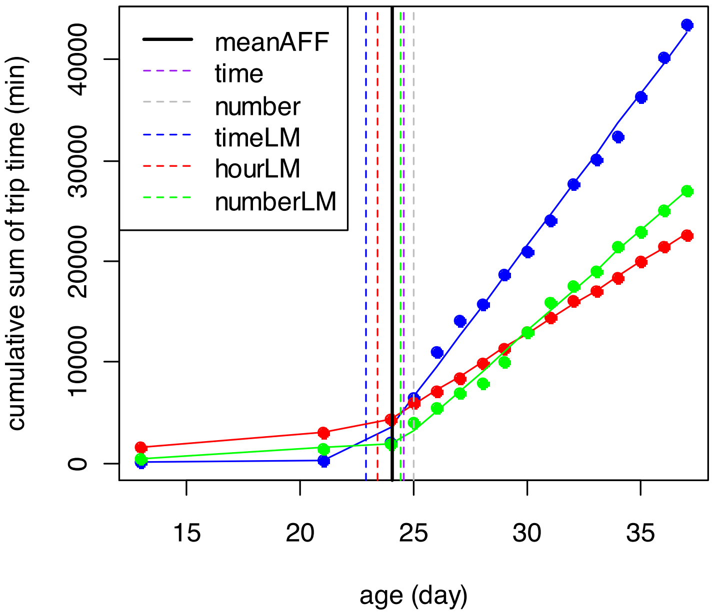

Figure 3 : Age estimated of First Foraging Flight (AFF) with the example of AFE estimation on one bee (*A00103C0004029B0*).

2.3. Statistical analyses

All statistical analyses were performed using the R environment for statistical computing, version 2.11.1 (R-Development-Core-Team, 2009). In order to estimate the age of first foraging (AFF), we performed the seasonal patterns of daily cumulative sum of the three behavioral parameters with Linear Models (LMs) using *lm* function in *stats* R-package and a Gaussian error structure, uniquely for bees which had more than five days of trips (Thom et al., 2000). In the opposite case (i.e. <5trips), we suggested that bee did not perform foraging task. We used the *breakpoints* function of the R-package *strucchange* in order to identify the age of the change in behavioral flight.

The influence of life history on task performances was tested using a Generalized Linear Mixed Models (GLMMs) with *lme* function in *nlme* R-package and a Gaussian error structure and the the origin of each honey bee (i.e. *cohort×site*) as an context random effect. We then used multiple comparison analysis using *glht* function in *multcomp* R-package.

In honey bee colonies, each worker bee is able to perform many tasks into the colony, including larval rearing and foraging. The age-polyethism theory predicts that these task allocations were performed according to a temporal succession from in-nest tasks to outside tasks (Seeley, 1982). However, the transition age between these two tasks categories determines the lifespan of the bees (Visscher and Dukas, 1997, Schmidhempel and Wolf, 1988, Rueppell et al., 2007, Becerra-Guzman et al., 2005). Therefore, we evaluated the correlation between life history traits allocation in honey bees, firstly between in-nest tasks and outside tasks, then between learning and foraging time, and finally between number of foraging trip and the duration of these trips. We used linear regression LMs and a Gaussian error structure in order to fit this trade-off (with bees which performed all tasks, n=1015).

3. Results

From the 2100 tagged bees, 230 (an average of 15.3±8.1 bees per cohort) did not provide any data (i.e., they never went out the hive), and an additional 29.09% provided only one *hit*. We collected a total of 402,471 *hits*, 256,462 *seq* and 21,104 *trip* on 1,870 bees during 193 days.

3.1. Life history of honey bee workers

A typical life history of an average honey bee worker was characterized by a mean lifespan of 28.1 days. The initial period consisting within in-nest tasks lasted 12.3±7.5 days (estimated by AFE, Fig. 4).

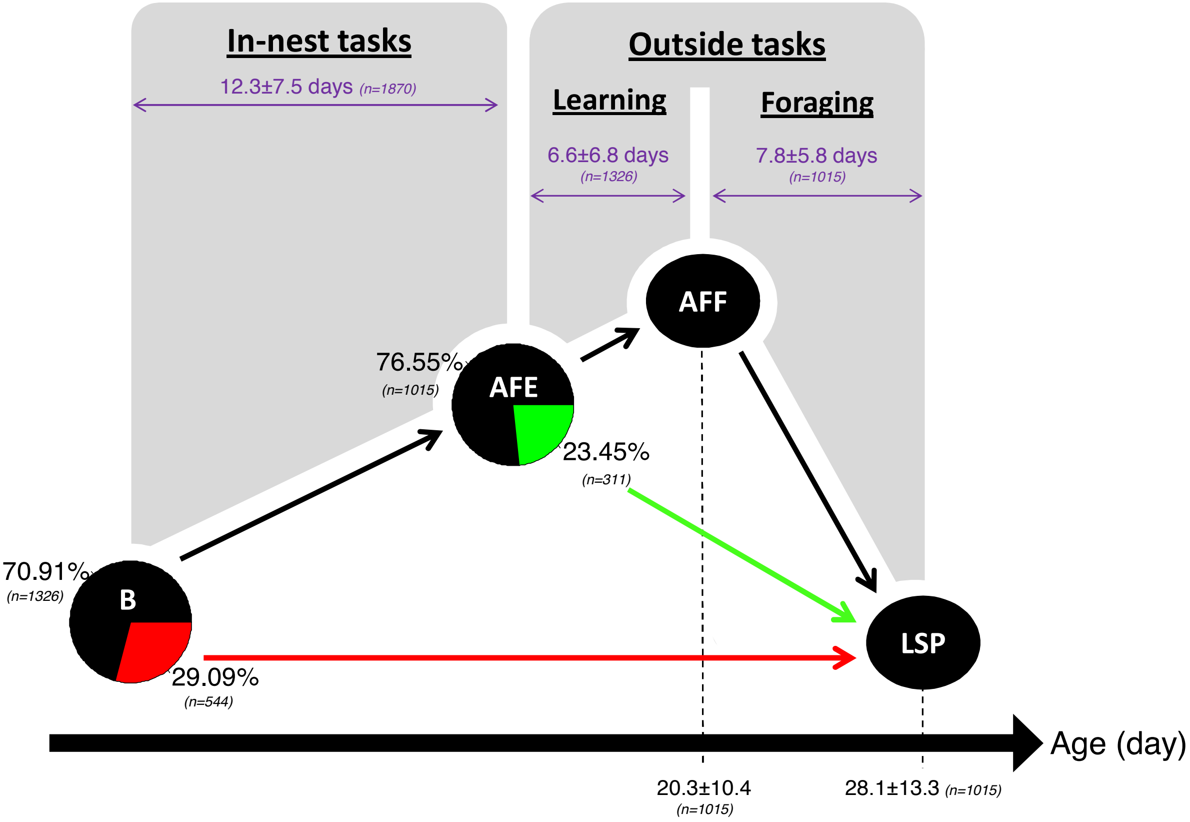

Figure 4 : Diagram of life history of honey bee workers, with the main ages and task durations. We presented the estimated value of each age of task transition and their respective task durations. We discriminated three groups of bees, individuals which performed foraging task in black (called 'forager'), bees which performed only learning outgoing flights in green (called "just learning'), and individuals which just performed in-nest tasks in red (called 'just in-nest').

Then, the first outside period, corresponding to learning of surrounding environment, lasted 6.6±6.8 days. The final outside period corresponding to foraging task properly, lasted 7.8±5.8 days, with the age of first foraging flight at 20.3±10.4 days old (Fig. 4). During foraging task, honey bees performed an average of 2.3±1.5 trips per day with flight duration about 40.6±23.1 minutes (Fig. 7). However, these average values showed a huge variation. For instance, all bees did not perform all tasks (i.e., in-nest tasks + learning + foraging): 29.09% of the monitored bees (n=1870) did not perform outside tasks (bee group in red, Fig. 4), and among outgoing bees (n=1326), 23.45% did not perform foraging trips (in green, Fig. 4). Overall, only 54.3% of the 1,870 monitored bees performed a complete life history with the temporal succession of in-nest tasks, learning and foraging tasks (in black, n=1015, Fig, 4).

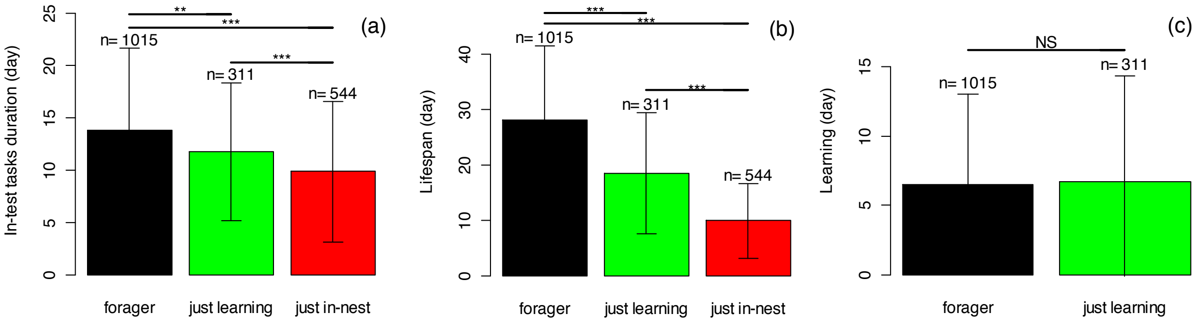

Figure 5 : Influence of life history on task duration, (a) in-nest tasks, n=1870, (b) lifespan, n=1870, (c) learning, n=1326.

These three groups of bees (i.e. forager in black, just learning task in green and just in-nest tasks in red) differed significantly in the duration of in-nest tasks (*GLMM*, $F_{1,1868}$=55.95, p<0.001, Fig. 5a) and lifespan ($F_{1,1868}$=633.26, p<0.001, Fig. 5b). Actually, these two traits were highly correlated ($F_{1,1868}$=1053, p<0.001). Conversely, the duration of learning task did not vary between forager bees and "just learning" bees ($F_{1,1868}$=0.90, p=0.34, Fig. 5c).

3.2. Flight behavior of honey bee workers

During the learning period, forager and just learning bees differed in their learning behavior: foragers performed more learning trips per day than "just learning" bees (*GLMM*, $F_{1,1314}$=25.5, p<0.001, Fig. 6a), and their trips were longer ($F_{1,1314}$=76.12, p<0.001, Fig. 6b). There was however no difference in the hours of learning activity between these two groups of bees ($F_{1,1314}$=2.15, p=0.14, Fig. 6c).

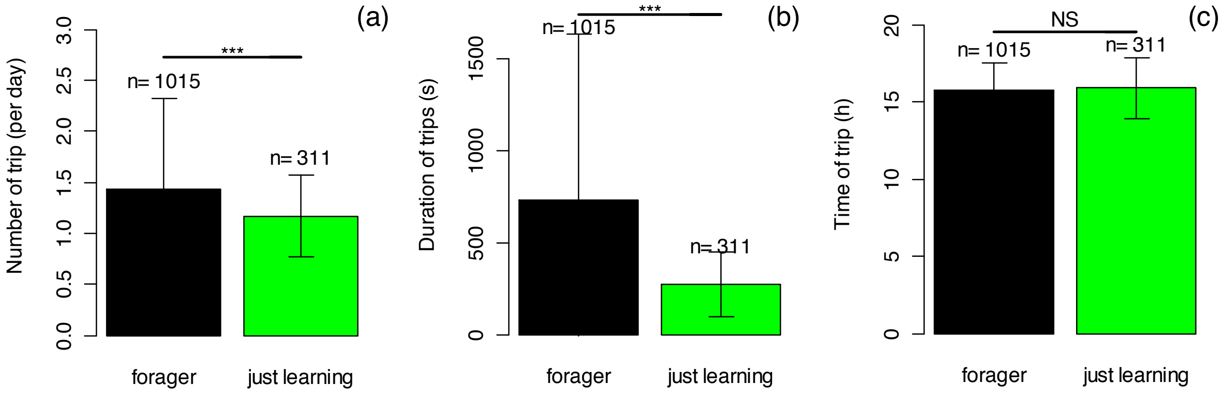

Figure 6 : Influence of life history on flight behavior, (a) number of trip per day, (b) duration of trips, (c) hour of the beginning of trips, n=1326.

Finally, honey bee foragers showed a strong behavioral variation between learning and foraging activities. Foragers increased their number of trips between learning and foraging activities (*GLMM*, $F_{1,1013}$=264.02, p<0.001, Fig. 7a), and performed longer trips in foraging than in learning ($F_{1,1013}$=1161.6, p<0.001, Fig. 7b). Surprisingly, we found a daily temporal segregation between foraging and learning activities, with learning flights being performed later in the day than foraging flights ($F_{1,1013}$=274.34, p<0.001, Fig. 7c).

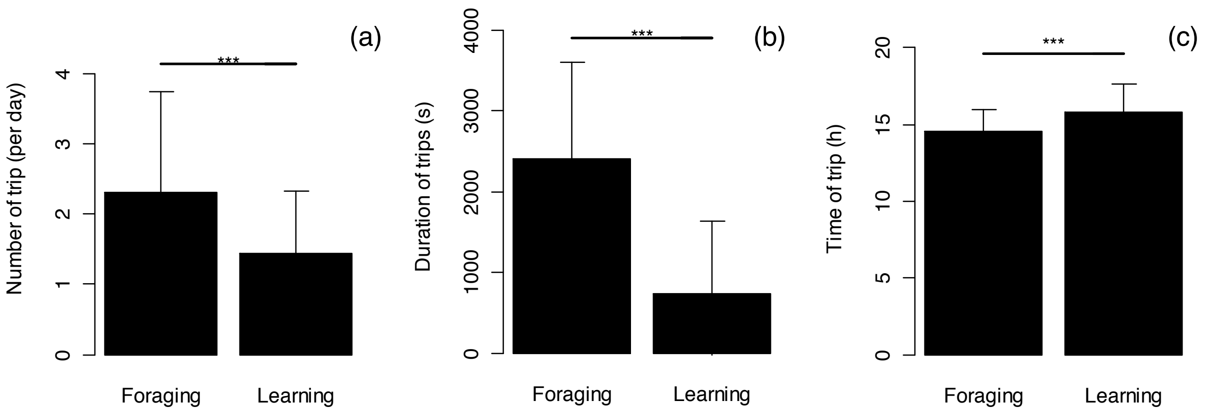

Figure 7 : Difference in flight behavior between learning and foraging task of forager bees, (a) number of trip per day, (b) duration of trips, (c) hour of the beginning of trips, n=1015.

3.3. Correlations between life history traits

Time in in-nest tasks and outside tasks were highly positively correlated (*GLMM*, $F_{1,1013}$=1075, p<0.001, Fig. 8a). A similar pattern emerged between flight-span learning time and foraging time ($F_{1,1013}$=1106, p<0.001, Fig. 8b). We found a negative correlation between the number of trips per day and the duration of the trips ($F_{1,1013}$=57.9, p<0.001, Fig. 8c), suggesting possibly a trade-off between length and number of trips. However, there was again huge between individual variations.

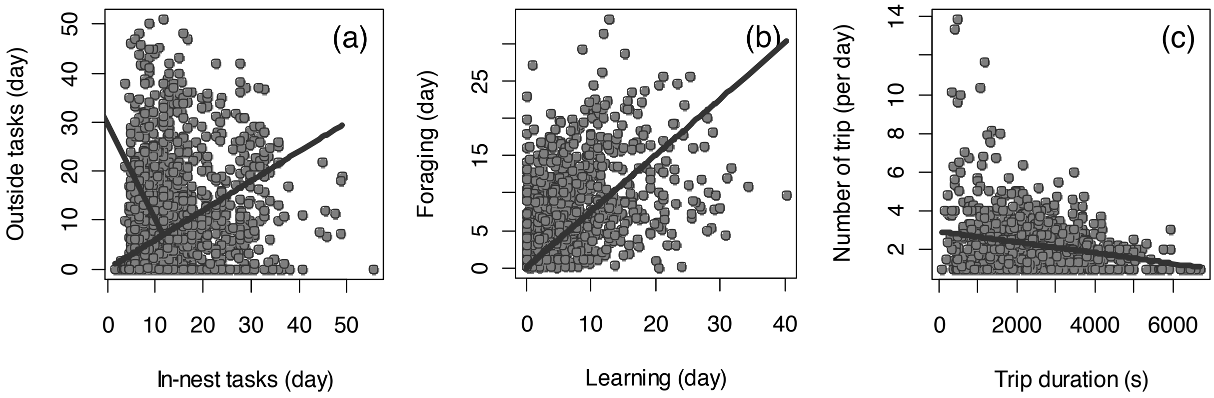

Figure 8 : Correlations between life history traits of forager bees, (a) between in-nest and outside tasks, (b) between learning and foraging tasks, (c) between behavioral allocation in foraging tasks, n=1015.

4. Discussion

The fairly recent adaptation of RFID technology to honey bees opens the potentials to study honey bee ecology in the wild. Indeed, this long-life monitoring provided life history and behavior data for thousands of worker bees in a single study in real conditions. This technique is also less invasive than The strong miniaturization of tag-transponders used insured the biological representativeness of the data collected, i.e. used tags are four times lighter than markers used by harmonic radar, these markers demonstrated as not affecting the flight behavior of bees (Capaldi et al., 2000).

Life history traits estimated by RFID-tool

Our estimated life history parameters appear to be fully consistent with data obtained from the literature. Average lifespan was found in our study to be 28.1±13.3 days, compared to 30 days (Schmidhempel and Wolf, 1988, Winston, 1994), 19.9-25.9 days in Becerra-Guzman et al. (2005), 33.0-38.4 days in Amdam et al. (2009). In addition, our results confirmed that the longevity is largely accounted for by the in-nest tasks, and in particular the brood-nursing allocation (Amdam et al., 2009). Indeed, the lifespan of honey bee depended on age of first exit from the hive, the shift age between in-hive tasks and out-hive tasks (Visscher and Dukas, 1997, Becerra-Guzman et al., 2005, Rueppell et al., 2007, Schmidhempel and Wolf, 1988). In our study, we found an allocation of 12.3±7.5 days to in-nest tasks: values from the literature range from 6 days in Capaldi et al. (2000), 8.9 days in Seeley (1982), and 25.7 days in Winston and Punnett (1982).

The foraging task is preceded by a period of flight learning and spatial orientation in order to record visual and olfactory landmarks around the colony (Gould and Towne, 1988), according to age-polyethism of honey bees (Ben-Shahar, 2005, Whitfield et al., 2006, Seeley, 1982). These learning flights are concentrated near the hive, about few hundred meters (i.e. median of 189m in Capaldi et al., 2000) while foraging flights are taking place at much greater distances (about 2km, Visscher and Seeley, 1982, Beekman and Ratnieks, 2000, Steffan-Dewenter and Kuhn, 2003). This difference in flight distances suggested possible differences in in number of trips and trip durations, with a transition between these two periods that could be detected with the RFID. Indeed our results are consistent with previous studies, i.e. orientation flights are shorter in duration than foraging flights (i.e. 1 to 10 min/trip and >10 min/trip, respectively, Thom et al., 2000).

We estimated the age of foraging at 20.3±10.4 days, an estimated value of the same order of magnitude as average range of previous studies such as: 12.8 days in Dukas (2008), 14 days in Capaldi et al. (2000), 20.6 days in Seeley (1982), and 25.6 days in Winston and Punnett (1982). Consensual literature talk about an average of 7 days to foraging task duration (7.7 days in Visscher and Dukas, 1997, and 6.8 days in Dukas, 2008) which is close to our estimation: 7.8 days. Finally, our average estimates of foraging behavior have been also similar to literature (i.e. 2.3±1.5 and 40.6±23.1 min for the number of trips per day and their durations, respectively). Indeed, Fewell and Winston (1996) and estimated the duration of a foraging flight between 38 and 50 min (for pollen), and Thom et al. (2000) accounted for an average of 3.5 foraging flights per day.

A wide plasticity of life history

We found a wide variance in the life history traits that may result from age plasticity known in worker bees (Corona et al., 2005). Adult workers perform two main tasks into the colony, the food supply of the entire colony population (outside task, i.e. foraging) and the brood-rearing (in-nest tasks including also cell cleaning, feeding and capping brood and queen attention, Winston, 1994, Seeley, 1982). Proximal factors may explain this plasticity of life history and behavior of bees, such as endogenous factors (e.g. Becerra-Guzman et al., 2005, Huang and Robinson, 1996, Schulz et al., 1998).

Over the eight experimental months (April to October), a bee colony shows strong temporal variation in the number of larvae, the number of workers and the quantity of reserves (Odoux et al., 2014). Previous studies have shown that when a colony endorse a shortage of older workers, about 10% of young workers (aged 7 to 10 days) become early foragers (Huang and Robinson, 1996, Schulz et al., 1998). Other studies have demonstrated that colony could regulate the foraging intensity in response to food starvation with an increase of workers allocation to foraging task (Schulz et al., 1998), and foraging behavior (Fewell and Winston, 1996).

Exogenous factors may also generate much variability in honey bee activity (e.g. Becerra-Guzman et al., 2005, Rueppell et al., 2007). Bad weather conditions prevent bees from flying (Crailsheim et al., 1999, Blaschon et al., 1999, Riessberger and Crailsheim, 1997). In addition the lifespan of honey bee is depending on the age of first exit from the hive (Toth et al., 2005, Feigenbaum and Naug, 2010), because survival probability of bees lowers with predation and risk of loss by disorientation (Visscher and Dukas, 1997). In addition, some scientists have shown that the foraging distance of bees increases with the spatial depletion of flower resource availability (Beekman and Ratnieks, 2000, Steffan-Dewenter and Kuhn, 2003), suggesting that flower availability could modify the foraging behavior.

Conclusion

Our results were very consistent with previous values from the literature, which confirms the validity of this new monitoring method for investigating the life history and behavior of bees in natural conditions. Compared to previous methods, RFID allows collecting a large amount of data, improving our knowledge on the ontogeny and the plasticity of flight activity in the honey bee. Indeed, contrary to past studies which monitored only few individuals, few life history traits and few days (about tens in average, see Dukas, 2008: 32 individuals, Visscher and Dukas, 1997: 33 bees, Schmidhempel and Wolf, 1988: range of 40 to 60 bees, Capaldi et al., 2000: 29 bees), RFID-method allowed monitoring thousands of bees automatically and continuously during their entire lifespan. This method may also be used as an empirical baseline for the modeling honey bee colony (i.e. multi-agent system and mechanistic model, see Becher et al., 2013 & 2014) by more accurate estimations of life history parameters and their plasticity. In addition, this adaptation method of RFID-tool could help to measure the real impact of current environmental stress on the honey bee in farmland habitats, such as pesticides and decrease of flower availability.

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Supporting Information

Appendix S1: The number of honey bees tagged and introduced, per colony (site) and per date (cohort), n=2100.

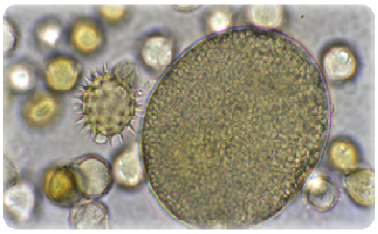
Introduction date	**colony A**	**colony B**	**colony C**
05/04/2011	150	150	Ø
06/05/2011	150	150	Ø
01/06/2011	75	75	Ø
30/06/2011	150	150	150
28/07/2011	150	150	150
25/08/2011	150	150	150

RESULTATS

Régime alimentaire

Régime alimentaire des abeilles domestiques en paysage agricole intensif

Cet article met en évidence l'influence de la dynamique des ressources florales en paysage agricole sur le régime alimentaire, tant sur sa dynamique que dans sa composition. En effet, la dynamique des ressources florales dominée par les trois cultures majeures, colza, tournesol et maïs, provoque l'apparition d'une forte déplétion d'approvisionnement alimentaire en pollen et nectar. Des ressources plus marginalement présentes en paysage agricole intensif telles que les zones boisées et les adventices jouent un rôle prépondérant dans le régime alimentaire des abeilles, par leur diversité florale mais également leur utilisation importante pendant la période de restriction florale, pouvant contrer l'intensité de la déplétion d'approvisionnement alimentaire.



Paper in revision in Ecological Applications

Title: Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds in spring.

Authors: Requier F., Odoux J. F., Tamic T., Moreau N., Henry M., Decourtye A., Bretagnolle V.

Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds in spring

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Summary

In intensive farmland habitats, pollination of wild flowers as well as crops may be threatened by the widespread decline of pollinators, both wild and managed. With the honey bee, the causes of decline appear multifactorial, including habitat loss (for nesting and foraging), diseases, pathogens, and pesticides. Environmental stress has recently been reconsidered with regard to the exposure to agrochemicals as well as a lack of available food resources, both acting on the honey bee diet. However, our knowledge of honey bee diet in intensive agricultural landscapes is currently rather poor.

In this study we investigated simultaneously nectar and pollen diet in an agricultural landscape, at a large spatial scale and over five consecutive years. We studied the seasonal contribution of mass-flowering crops (rapeseed and sunflower) versus other floral resources, in relation to pollen quality and landscape composition.

From April to October, pollen and nectar biomass collected by honey bees followed a bimodal seasonal trend, marked by a two-month food supply depletion period between the two oilseed crops mass-flowerings (may for rapeseed and july for sunflower). The nectar was mainly collected on crops while pollen came from a wide diversity of herbaceous and woody plant species, from semi-natural habitats or weeds in crops. Weeds in particular happened to play a critical role in honey bee diet, as a dominant pollen resource between the mass flowering crops (up to 40%). The pollen diet composition was partly determined by the nutritional value of the collected pollen and by the local landscape composition.

Our study highlights (i) a food supply depletion period of both pollen and nectar resources during late spring, contemporaneously with the demographic peak of honey bee populations, and (ii) a high botanical richness of pollen diet, mostly proceeding from trees and weeds. Our results support the Agri-Environmental Schemes intended to promote honey bees and beekeeping sustainability through the enhancement of flower availability in agricultural landscapes.

Key-words: agricultural landscape, Agri-Environmental Schemes, *Apis mellifera* L., floral resources, food selection strategy, honey reserves, palynological identification, pollen collects, pollen quality

Introduction

Over the past 50 years, European agricultural landscapes have been profoundly modified to meet the growing food requirements of humanity (Godfray et al., 2010). Intensification of agricultural systems has resulted in a major loss of habitats and biodiversity in rural landscapes (Tilman et al., 2001), with strong declines in bird, insect and plant populations (Sotherton, 1998). Bees in particular have undergone a substantial decline in agricultural landscapes in Europe and North America (Winfree et al., 2009). Through pollination, insect pollinators and especially bees maintain wild plant species and also ensure yields, to a varying extent depending on crops (Klein et al., 2007, Rader et al., 2009). Social bees, especially the honey bee *Apis mellifera* L., are usually regarded as important pollinator agents in farmland landscapes owing to the very high numbers of foragers reared by a single nest (Rader et al., 2009). Both wild and honey bees are however currently declining in agricultural landscapes, and their decline seems best explained by a multifactorial stress between three groups of interacting drivers: genetic diversity and vitality, pests and pathogens, and environmental stressors (Potts et al., 2010, Vanbergen et al., 2013).

Environmental stress has recently been reappraised, especially with regard to the consequences of exposure to agrochemicals (e.g. Gill et al., 2012, Henry et al., 2012, Osborne, 2012) as well as the lack of available food resources, i.e. flowers (Naug, 2009), both acting on honey bee foraging ecology. Paradoxically however, our current knowledge about honey bee diet in intensive cereal systems is poor, since most studies investigating floral resources have focused mainly on foraging strategy (e.g. Beekman and Ratnieks, 2000, Steffan-Dewenter and Kuhn, 2003). Larvae, adult workers and queens all depend exclusively on floral resources (except for honeydew) from which they obtain their entire energy. Honey bees are generalist flower foragers that use both pollen and nectar (Haydak, 1970). Nectar is the main source of energy for adult workers and may be stored in the form of honey. Pollen is another essential component of bee nutrition as it is consumed by both adults and larvae (Keller et al., 2005), and is the only source of proteins and lipids (Haydak, 1970). Despite the widely recognized importance of floral resources, the honey bee diet composition has been remarkably poorly documented so far in intensive farmland habitats (but see Sabo et al., 2011 for nectar diet, Koppler et al., 2007 and Odoux et al., 2012 for pollen diet). To the best of our knowledge, no study has ever considered both pollen and nectar diet in farmland landscapes.

Here we investigated simultaneously the two components of the honey bee diet (pollen and nectar) in intensive farmland habitats, using empirical data collected at a large spatial scale over five consecutive years. Food resources brought back to hives were surveyed throughout the beekeeping season (March to October), and in varying farmland landscape configurations. Our agrosystem includes two oilseed crops, rapeseed *Brassica napus* and sunflower *Helianthus annuus*. Yields of these two oilseed crops are known to be improved by honey bee pollination, though the extent of this increase varies among studies (e.g. Oz et al., 2009, Bommarco et al., 2012) and is partly accounted for by other insects as well (Rader et al., 2009). In return, oilseed crops are highly rewarding for honey bees, because they constitute mass-flowering crops and provide resources in abundance (Rollin et al., 2013), which explains why beekeeping activity is so developed in intensive cereal farming systems (VanEngelsdorp and Meixner 2010). However, mass-flowering occurs over a restricted period of time (about 20-30 days) and with a two months gap in between rapeseed and sunflower (April and July, respectively). Our aims were therefore to describe the seasonal variations in honey bee diet in terms of pollen and nectar collected biomass, especially with regard to the relative contribution of crops versus other plant species. Given the two-phased mass flowering pattern, with distinct blooming periods for rapeseed and sunflower during the beekeeping season, we predicted a bimodal pattern of collected pollen and nectar biomass. To further assess the relative contribution of mass-flowering crops and other floral resource to the honey bee diet, we determined the botanical origins of pollen and nectar. We predicted that honey bees would make a greater use of wild floral resources, such as species from semi-natural habitats, in the absence of mass-flowering crops. Then we investigated the possible links between the nutritional value of pollen and their occurrence in honey bee diet, which would reflect a honey bee selection strategy for flower resources based on nutritional criteria. Finally we assessed to which extent the dissimilarity of dietary composition among apiaries varies with distance, as a surrogate approach to detect an effect of landscape context on dietary composition.

Materials and methods

Study area and experimental design

Our study area, the Long-Term Ecological Research (LTER) *Zone Atelier Plaine & Val de Sèvre* is located in central western France (46°23’N, 0°41’ W; see appendix S1). Land use of every single field (around 18000) in the study site (450 km2) is recorded twice a year since 1995 and stored onto a Geographical Information System (ARCVIEW 9.2). The agricultural landscape of the study area is characterized by a high proportion of arable land (>75% of land use), of which a large part is dedicated to cereal production (45% of cereals) in addition to other crops (10% sunflower, 9% rapeseed, 8% maize).

Field work was conducted from 2008 to 2012. Considering an average foraging distance of 1.7 km radius in such landscapes (Steffan-Dewenter and Kuhn, 2003), the study site was divided into 50 squares of 3.3 km a side (10 km2, see appendix S1 and Odoux et al., 2014). Every year, ten of these squares were randomly chosen (with no replacement) and in each of the squares, a 5-hive apiary was set up as close as possible to the square’s centre, and was monitored from late March to October. On average, each apiary had access to 50.1±30.3 ha (mean±SD) of rapeseed and 73.5±36.0 ha of sunflower within a 1.5 km distance radius (n=50). All honey bee colonies originated from a livestock managed by a local professional apicultural association (ADA PC), from an *A. mellifera mellifera* $_\times$ *caucasica* strain. Colonies were set up and managed as a sedentary apiary according to local beekeeping professional practices. Phenological periods were obtained with *Apibotanica* dataset (www.poitou-charentes.inra.fr/entomologie/) for all plants including oilseed crops.

Pollen and nectar sampling

The assessment of the contribution of mass-flowering crops to total pollen and nectar diet was based on the identification of pollen grains found in pollen loads and nectar brought back to the hive by foragers. In order to collect pollen loads, the entrance of each of the 5 hives per square were fitted with a pollen-trap for 24-h every 10±2 days from late March to October. Pollen traps were set only after ensuring that the queen was laying eggs and when weather conditions allowed foraging, i.e. when outside temperature was above 12°C. Pollen traps consist of a grid with three lines of 5.3 mm diameter holes through which foragers are forced to crawl and may lose their pollen loads. Such pollen traps retain about 10±5% of all the collected pollen brought back to the hive (Lavie and Fresnaye, 1963). Assuming it reflected pollen composition of daily pollen collect and that the percent retained is stable, the weight of the collect provided an estimate of daily collected pollen biomass. Pollen trap contents were pooled among the five hives of apiaries within each sampling date, resulting in a total of 780 pollen samples. Samples were freed from exogenous impurities, oven-dried for 48h at 45°C and weighed to the nearest 0.01g (Sartorius balance).

Contrarily to pollen sampling, nectar sampling requires to open the hives. To avoid invasive effects, we therefore carried out nectar biomass measurements at a slightly lower frequency (every 14±2 days, from late March to October) and only three out of five hives were opened per apiary (Odoux et al., 2014). For each hive, frames were weighed without honey bees, the brood area measured, then converted to mass, and finally subtracted from the total frame mass in order to estimate the total biomass of food reserves included in brood-chamber and super-chamber, cumulating therefore nectar and honey (see appendix S1 and Odoux et al., 2014). In the following, we call this mass “nectar”, though it comprises both nectar and honey. A total of 1460 biomass measurements were obtained. Honey reserves in the super-chamber were extracted twice a year, conformingly to local beekeeping practices, just after the rapeseed and sunflower honey flows (May and August, respectively). Honey was sampled for each square during beekeeping harvest. Only these samples (n=67) were used for floral identification, following the protocol detailed below.

Flower species identification in pollen and nectar

From the 780 pollen samples, a subset of 450 samples was selected in order to get at least one sample per square and month (see appendix S2). For each of the 450 pollen samples (later labeled as a session), four grams (on average 14±13% of the total collected per session) were randomly withdrawn after homogenisation, then diluted in water and mounted onto microscope slides. To improve accuracy, pollen identification was duplicated for each sample. Slides were stained with Fuschin and examined at ×400 magnification with an Olympus BH-2 microscope fitted with a numeric camera (Software 2I system-Paris Pégase Pro V4). Pollen was identified to species whenever possible (see appendix S3 for a complete list of species) or else to genus (e.g., for the closely related *Prunus avium*, *P. spinosa* and *P. cerasus*). For each slide, a counting "transect" was performed until at least 300 pollens were counted and identified (Tamic et al., 2011), representing c.1.6% of the 4-g pollen sample. The weak point of this quick method is to underestimate rare species (Louveaux et al., 1978) or overestimate those with large diameter (Dasilveira, 1991), but provide a satisfying image of the actual bee diet. In order to stay closer to the diet issue, we converted the species frequency of occurrence (averaged over the two samples) into percentage in weight out of the sample, considering the pollen grain n as a sphere with a species-specific diameter d and assuming weight is independent of species identity. For each floral species i, the relative contribution over the apiary sample for a given session j was obtained as:

$$Biomass_{i.j} = \frac{(n_i \times d_i)_j}{\sum_i (n_i \times d_i)_j} \times Biomass_j$$

In addition, in order to avoid overweighting rare species, all analyses were duplicated excluding species recorded in less than 1% of the session.

Determining the botanical origin of nectar is less straightforward and notoriously more difficult than for pollen (Louveaux et al., 1978, Anklam, 1998, Von Der Ohe et al., 2004). Usual methods rely on melissopalinology, i.e. the identification of incidental pollen grains ingested by nectar foragers and subsequently regurgitated and stored in honey reserves (see Louveaux et al., 1978, Sabo et al., 2011, for similar procedures). This approach may help identifying the main plant species foraged for nectar, but is not adequate for quantitative assessment of the less common species. Incidental pollen grains unrelated with nectar foraging may also artificially raise the species richness estimates of nectar botanical origin. For those reasons, we only present broad categories of contribution to biomass: dominant species (>45% of biomass), common species (15 to 45%), and rare species (<15%; see also Louveaux et al., 1978). Pollen were identified from 500 grams samples that were randomly withdrawn for each honey sample from the super-chambers, and obtained by homogenising equal amounts of honey from the five hives within each monitored apiary. The botanical origin of honey was assessed only for the two periods of honey extraction by beekeepers (n=67; see appendix S2).

Nutritional characteristics of pollens

We quantified the nutritional value of pollen (protein and mineral contents) for the floral species which accounted for more than 1% of the diet biomass on an annual basis, i.e. 15 species (see appendix S3). To do so, we first isolated monospecific pollen subsamples of those target species by manually extracting specific pollen loads from pollen samples where those species were classified as dominant (>50% in biomass, see appendix S1). The purity of monospecific subsamples was controlled by microscope observations and validated if the target species accounted for >95% of pollen grains. Among all these monospecific subsamples, we randomly chose three subsamples per non-crop species and up to seven subsamples per crop species in order to account for possible differences in quality between different crop varieties which have been bred for other characteristics than their attractiveness to bees. For each of the 56 monospecific subsamples eventually selected, we extracted and freeze-dried 5g of pollen loads. The mineral content of the dry matter was assessed after a 6h calcination at 550°C, the crude protein content was assessed from the determination of nitrogen content, using Kjeldahl method (N×6.25; ISO 5983 norm) and a Vapodest 45 Gerhardt automat, and the gross energy was obtained using an IKA C 7000 calorimeter system.

Statistical analyses

Pollen collects pooled among the five hives per square were expressed as biomass dry weight (log-transformed; n=780) and used as a proxy of the foraging activity of the bees from a given square at a given date. Nectar biomass was obtained by considering only the weight of the frames in hives brood-chambers (log-transformed; n=1460), i.e. excluding honey located in the super-chamber, be it collected or not by beekeepers. These weights could include nectar and honey, and possibly some pollen bread though this is negligible since pollen is stored in quite small quantities. Seasonal patterns in pollen and nectar biomass were modelled as a spline function of time using Generalised Additive Mixed Models (GAMMs, see appendix S4). Time was rescaled on Julian dates from the first of January and corrected for inter-annual deviations of climatic conditions (see appendix S4). As seasonal patterns in both collected pollen and nectar biomass were clearly non-linear (see Results below), we divided the season into temporal classes, separated by a peak of collected biomass (see method in appendix S4).

We then investigated the floral resource taxonomic identity in honey bee diet (pollen and nectar) using four broad resource types based on life forms and management, namely cropped species, herbaceous-plant species, woody-plant species, and finally horticultural-plant species (see appendix S3). We used the Chi-squared test to compare, among temporal classes, the relative contribution of these four resource types to the collected dry biomass of pollen. Likewise, we tested the ten most frequently used floral species sorted in abundance on a yearly basis.

Floral resource diversity in pollen (n=450 samples) and nectar (n=67 samples) was estimated using the total observed species richness (number of distinct pollen species), the expected species richness returned by the Chao 1 estimator as well as the Shannon index of species equitability (see appendix S4). Equitability ranges from 0 (only a single species sampled) to 1 (all species being equally sampled). The seasonal variations of species richness and equitability of foraged plants were tested using a

Generalized Linear Mixed Models (GLMMs) and a posteriori multiple pairwise comparisons.

The relationship between pollen nutritional value (protein and mineral contents) and dietary preferences (contribution to collected biomass) was tested using Spearman’s test for all samples with at least one of the 15 most frequently collected species (reducing sample size to n=448). Correlation coefficients were then plotted against month in order to depict possible seasonal variations in honey bee preference patterns.

In order to compare pollen collect composition among apiaries, we computed the Jaccard dissimilarity index (see appendix S4), ranging from 0 (complete similarity between two samples, i.e. squares) to 1 (complete dissimilarity). The pairwise dietary dissimilarity values between apiaries were modelled as a function of pairwise Euclidian distance between apiaries. Under the assumption that the local landscape context influences honey bee diet, we expected neighbouring apiaries to display more similar diets than apiaries located further away from each other. This hypothesis was tested using landscape variable descriptors (listed in Appendix S5) obtained from GIS and focusing on a 1500m radius around each apiaries (see Steffan-Dewenter and Kuhn, 2003 for the choice of radius). We used model selection by AIC from GLMMs (see appendix S4) in order to infer on the relative contribution of landscape context (distance between pairwise squares), date (month) and identity of pairwise squares on the dissimilarity of diet composition (see appendix S8). We used GAMMs to fit the influence of the best candidate parameters returned by the AIC-based model selection (see method in appendix S4).

All statistical analyses were performed using the R environment for statistical computing (R-Development-Core-Team, version 2.11.1).

Results

In our intensive cereal system, honey bees were found to collect a highly diversified range of floral species, with a total of 228 identified species in pollen samples (n=450) and an expected total species richness estimated to 271±17.6 by the Chao estimator (appendix S3) from April to September. The sampling completeness had therefore reached an acceptable level of 84%. Despite acknowledged limitations due to sampling for honey (see methods above), we found a tentative estimate of 82 floral species (n=67; total expected richness = 101±11.8, sampling completeness = 81%).

Seasonal patterns in pollen and nectar collected biomass

Once corrected for inter-annual variations (see appendix S6), we found strong within year seasonal variations in the pollen and nectar collected biomass. During the studied period, the pollen collects presented a bimodal biomass pattern, with two collecting peaks (May 10 to June 6 and July 14 to August 16, Fig. 1a) preceded and followed by lower collecting periods. Seasonal variations in pollen biomass were highly significant (*GAMM*, $F_{6,773}=7.333$, $p<0.001$). Unexpectedly, the first peak of pollen biomass occurred 1-month after the peak of rapeseed blooming. Conversely, the second peak, in July, coincided with the blooming of sunflowers/maize.

Likewise, the nectar biomass in brood-chamber of hives (i.e., excluding honey collected by beekeepers in super-chambers) followed a bimodal pattern ($F_{4,1455}=375.9$, $p<0.001$) with a poorly marked peak in spring (from April 12 to May 10) and a sharper one in summer (from July 19 to August 21). These two periods coincided with the blooming periods of the two dominant oilseed crops, i.e. successively rapeseed and sunflower (Fig.1b). Therefore, pollen and nectar collects peaked simultaneously only during the sunflower blooming period.

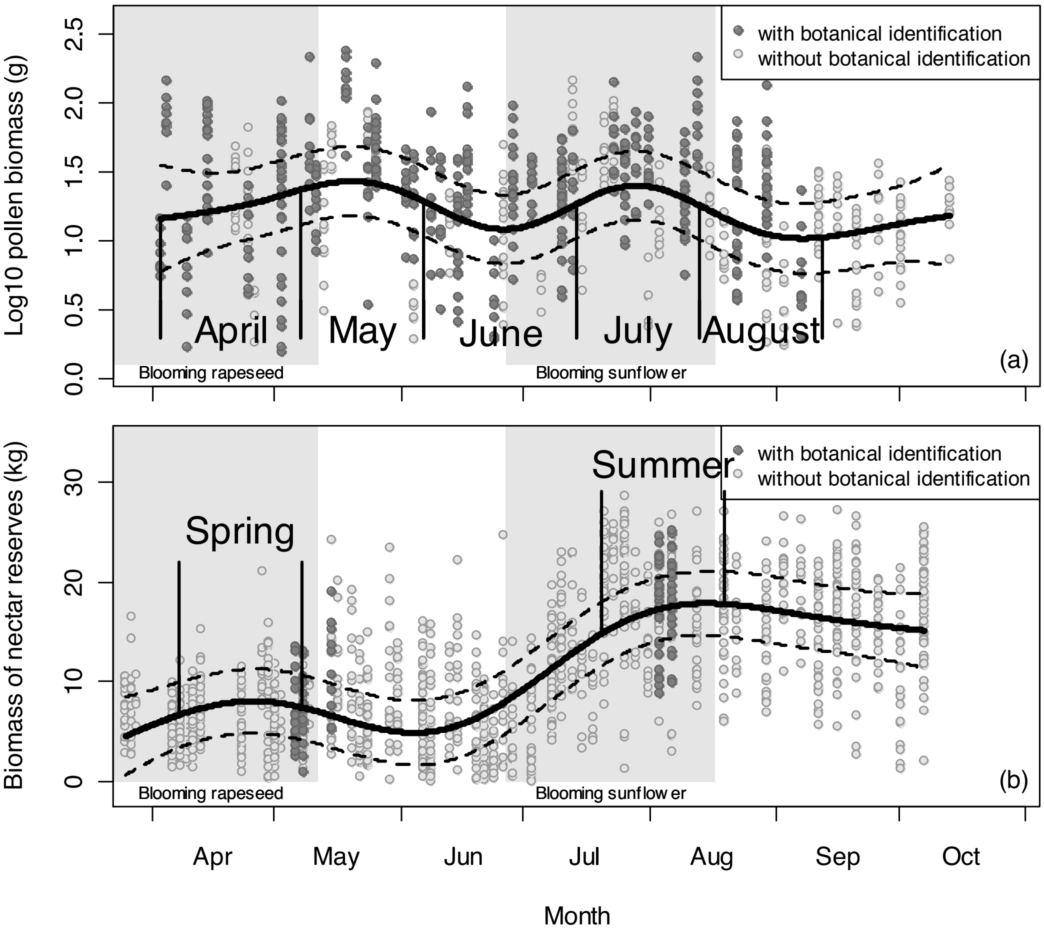

Figure 1: Seasonal patterns of (a) pollen collects (log10 transformed) with the five temporal classes (n=780 samples) and (b) nectar reserves into brood chamber with two temporal classes (n=1640). The thick line is the fitted model by the *GAMM* (see methods) with dashed lines showing confidence interval (CI 95%). Each point represents an observed value. Month periods are also indicated.

Floral taxonomic diversity in pollen and nectar collects

On average, honey bees from a given apiary (i.e. 5 hives), collected 15±4.5 pollen species on a daily basis (Fig.2a) with a rather low equitability value of

0.51±0.14 (Fig.2b), suggesting unequal collects among plant species. Deleting species with less than 1% occurrence per session however returned different values, a much lower species richness (6±2.2) and a higher equitability (0.68±0.17). Species richness and diversity were highest in May while April showed the lowest species richness (Figs 2a and 2b). Overall, honey bees foraged on much fewer plant species for their nectar requirements than for their pollen requirements (82 vs. 228 species, respectively, see appendix S3), a difference actually amplified when deleting species with less than 1% occurrence per session (27 and 147 species, respectively). This may however be partly explained by the lower nectar diet sampling effort (two sampling sessions against five for pollen diet). In total, 90.2% of species collected for nectar were also collected for pollen, but 8 species were specifically collected for nectar. On a daily basis, the number of collected species as well as their equitability were lower for nectar than for pollen (*GLMM*, $F_{1,464}=42.83$, $p<0.001$, Fig.2c and $F_{1,464}=207.75$, $p<0.001$, Fig.2d, respectively), indicating that despite different sampling efforts between pollen and nectar collecting, honey bees did forage on fewer species for nectar than for pollen. Restricting the data set to species for which occurrence exceeded 1% did not alter the difference for species richness ($F_{1,464}=221.30$, $p<0.001$) while there was no difference anymore for equitability ($F_{1,423}=0.03$, $p>0.05$)..

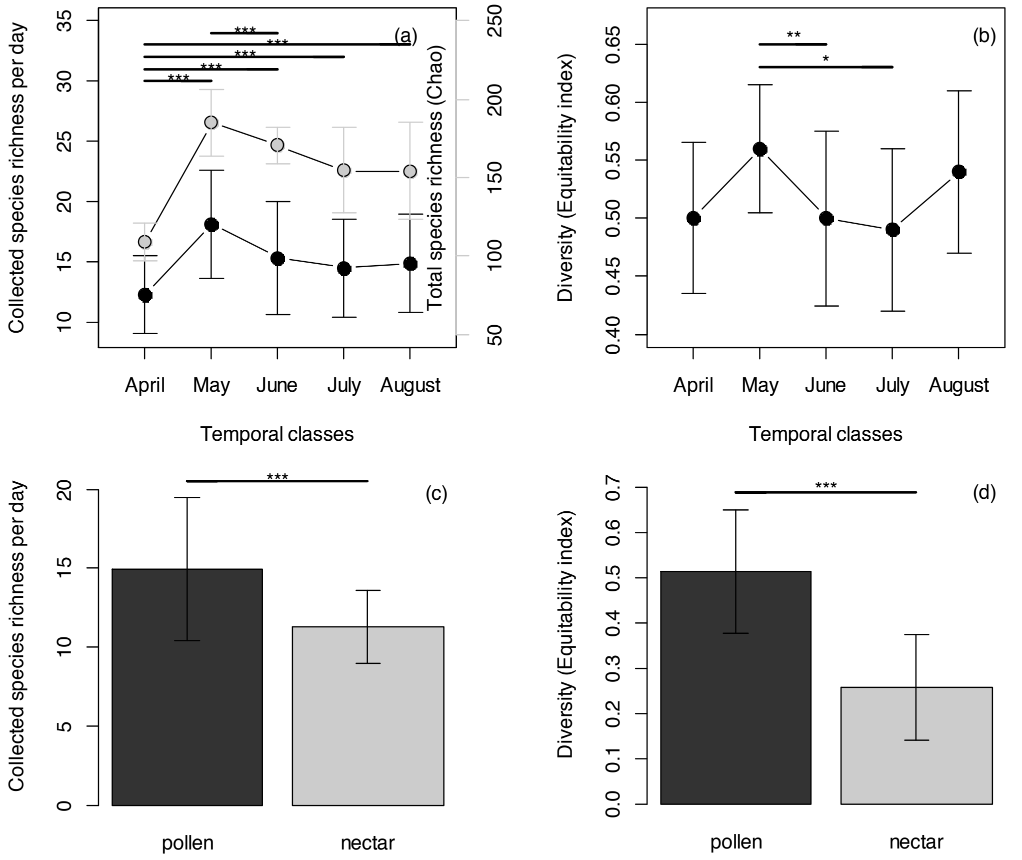

Figure 2: Average (±1SE) of (a) the collected species richness per pollen sample (daily basis) in black and the total species richness estimated using Chao estimator in gray, (b) the floral species diversity per sample (Equitability index), for each temporal classes for pollen harvests, n=450, (c) the species richness and (d) the species diversity differences between pollen and nectar, n=67, *p<0.05, **p<0.01, ***p<0.001

Crop *versus* wild flowers in pollen and nectar collects

Overall, on an annual basis, cropped plant species were the second most dominant source of pollen and accounted for 30.19% of total pollen biomass (Fig.3a). Compared to all the other three resource types combined along the season, crops were less foraged than other resource types ($\chi^2=14.80$, df=1, $p<0.001$). Early in the season, crops (represented by rapeseed) were less used for pollen (11%) than other floral resources. In contrast, honey bees heavily relied on woody and herbaceous plant species (including weeds) from semi-natural habitats to meet their pollen requirements ($\chi^2=8.43$, df=1, $p<0.01$), totaling more than 60% of annual pollen diet. Woody-plant species, including *Crataegus sp.*, *Prunus sp.* and *Acer sp.* (Fig.3b) accounted for the bulk of the pollen diet in April. The poppy Papaver rhoeas was the most collected herbaceous plant species and was the second most dominant species in the annual pollen diet after maize and before sunflower. Poppy was mostly collected in June, i.e. during the depletion period of pollen biomass between crop blooms (Fig.3b). Horticultural plant species, typically found in gardens, were only marginally used (Fig.3a). Contrary to pollen, nectar diet mainly proceeded from the dominant cropped plant species ($\chi^2=68.88$, df=1, $p<0.001$, Fig.3c). During spring, nectar biomass was largely dominated by rapeseed, while sunflower dominated during summer Fig.3d).

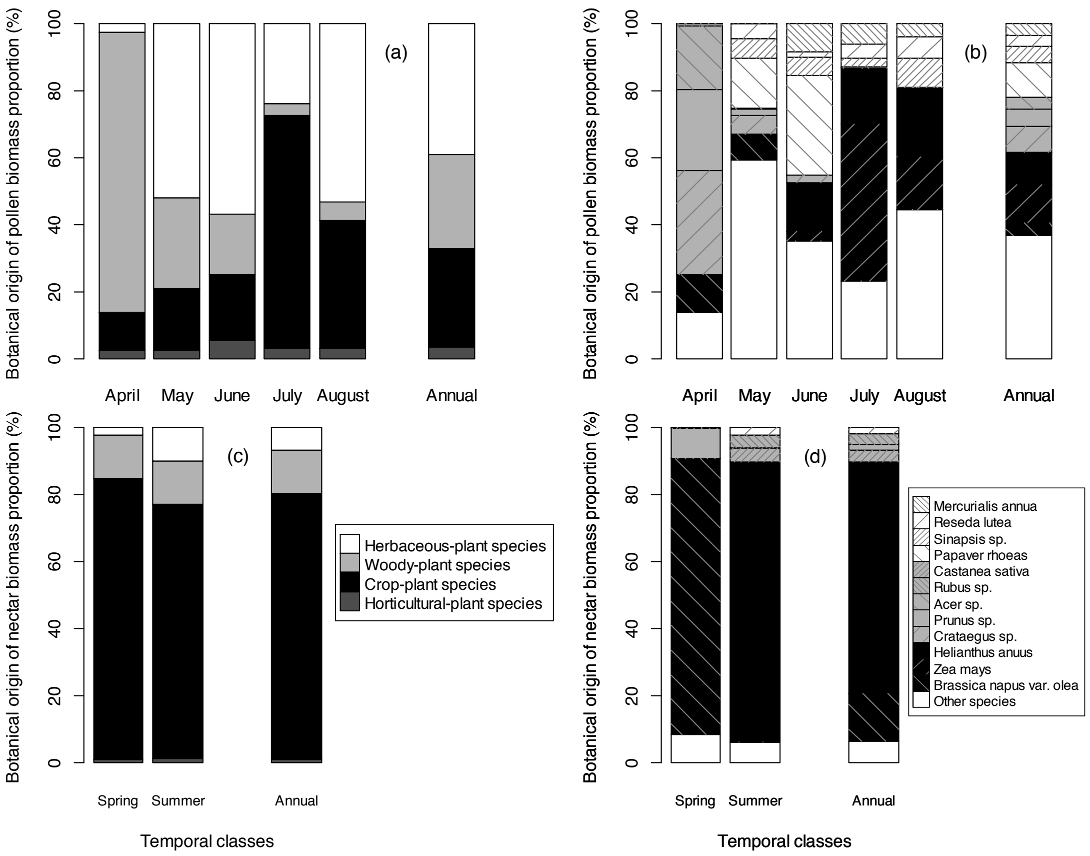

Figure 3: Botanical origin and taxonomic identity of collected pollen (a, b) and nectar (c, d) resources, expressed in biomass proportions (n = 450 and 67 samples, respectively). See text for details on relative biomass assessment.

Nutritional value of dominant foraged pollen species

Using only the 15 most frequently collected species, we found that the relationship between the specific biomass collected and parameters depicting pollen quality (protein and mineral values) decreased with season ($F_{1,14}$=71.1, p<0.01 and $F_{1,14}$=30.0, p<0.05, respectively). Early in the season (April), honey bees foraged on pollens of high nutritive values, since protein- and mineral-rich pollens contributed most to collects (Fig.4). One noticeable exception was rapeseed pollen, being little collected although rich in proteins, minerals and energy (see appendix S7). In contrast, at the end of the season, honey bees did not collect pollens according to their nutritional value since there was no, or even a negative relationship between biomass proportion of pollen species and their nutritional values. In particular, maize and sunflower pollens where heavily collected despite their low protein and mineral contents.

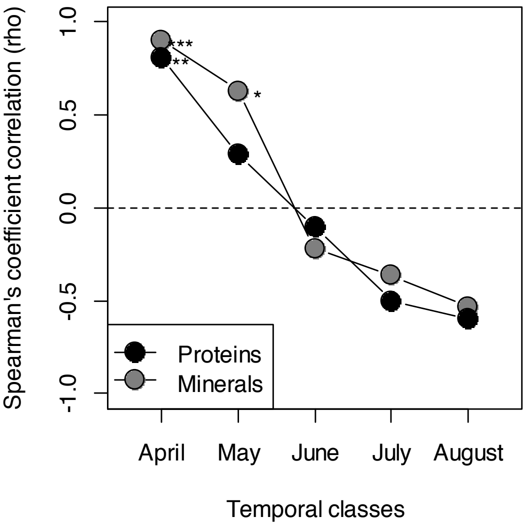

Figure 4: Spearman's correlation coefficient of regression models between the protein and mineral values of pollen, and their harvest (weight in biomass proportion, log10 transformed), according to season (month); n=56; *p<0.05, **p<0.01, ***p<0.001.

Influence of the landscape context

The diet dissimilarity value of pollen collect composition between apiaries (at same time periods and years) was on average 0.47±0.11. This intermediate value indicates that from one square to another, half of species were different, therefore indicating local dietary specializations. This was further supported by a low average species richness collected per apiary and per year (62.8 species) relatively to the total pool of species collected overall (228 species). In other words, each apiary exploited on average only 29.3% of the total collected floral community at the study site scale (range: 20.6-42.1%, n=50).

The pairwise square dissimilarity of pollen compositions was best accounted for by landscape context (pairwise distance between apiaries) and by date (month, see appendix S5). The pairwise dissimilarity in pollen composition varied significantly with time (*GAMM*, $F_{3,1057}$=12.01, p<0.001, Fig.5a) with pollen diet dissimilarity being maximal in June, i.e. during the depletion period of pollen biomass between the two crop blooms. Pairwise dissimilarity in pollen composition increased also with geographic distance ($F_{1,1059}$=42.08, p<0.001) and with the landscape composition dissimilarity ($F_{1,1058}$=7.723, p<0.01, Fig.5b), thus suggesting a landscape effect on the composition of collected pollen.

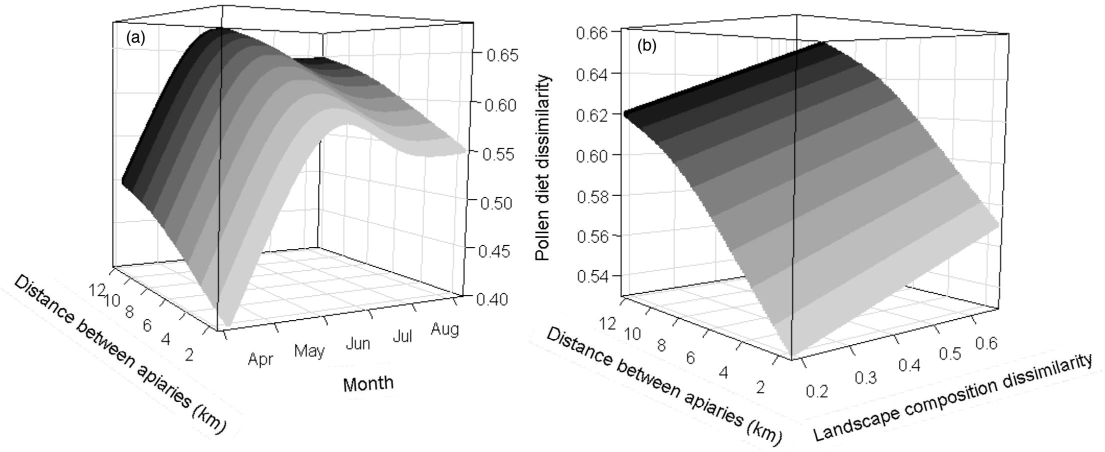

Figure 5: Diet dissimilarity of pollen composition between pairwise of squares (a) as a function of time (month corrected for phenological lag) and pairwise distance between apiaries, and (b) as a function of landscape composition dissimilarity and pairwise distance (from a GAMM model, n=1061). The gray gradient represents graphical depth.

Discussion

It has only recently become obvious that diet of honey bees may be an essential driver of their health (Brodschneider and Crailsheim, 2010, Foley et al., 2012), and that recent landscape changes may contribute to explain the current decline of honey bees through nutritional stress (Naug, 2009). Honey bee diet in cereal farmland systems is driven by oil seed crops, which is not surprising given their predominance in such landscapes: indeed, collected pollen biomass follows a bimodal seasonal pattern resulting from oilseed crop blooming periods and their intense collecting by bees. In addition, our study demonstrates that honey bees forage on a very wide floral community, mainly composed of weeds and trees. The annual crops actually provide a secondary contribution to pollen collect though a dominant contribution to nectar collect. Finally, honey bees experience a two-month food supply depletion period between crop flowering blooms. We provide below an interpretation of these results and evaluate their implication both for bee health and for sustainable honey production.

Why seasonal patterns of nectar and pollen collects are dissimilar and do not strictly follow crop blooms?

We showed a strong temporal bimodal pattern in pollen and nectar biomass foraged by honey bees in intensive farmland landscapes due to strong seasonality in flowering crop blooms. These bimodal patterns in both pollen and nectar present a long period of apparent food supply depletion between the two peak periods (see below and Odoux et al., 2012), that either result from a super-abundance of flower resource during oilseed crops blooming, or from a food-shortage period during the absence of these oilseed crops.

The coincidence between crop blooms and honey bee diet actually fitted well for nectar, but not so for pollen whose first collecting peak occurred one month after rapeseed blooming. Therefore, while the nectar income appears mostly driven by the availability of the two dominant oilseed crops in intensive farmland habitats (Varis, 2000), pollen diet seems to be driven by crops only in summer. At the first pollen peak, honey bees massively foraged on floral species from semi-natural habitats rather than rapeseed for pollen needs. Rapeseed crop accounts for c.10% of the crop surface, similar to sunflower and maize, therefore suggesting that rapeseed was counter selected compared to sunflower for pollen diet. Rapeseed has good nutritional value in terms of proteins, minerals and energy, which is comparable to other species from semi-natural habitats that are used by honey bees in the same flowering period. However, other quality parameters may explain the apparent rapeseed avoidance by bees such as a lower pollen diameter (see appendix S7) which could increase the collect task, or a higher level of chemical treatments, in particular insecticides (Johnson et al., 2010). Moreover, Odoux et al. (2014) have shown that bee colonies are mainly at an immature stage during this period of mass-flowering (see Appendix S8 based on our own data). Nursing bees are the ones that mostly consume pollen to develop their glands which produce food for larvae (Haydak, 1970). Therefore we suggest that the relative avoidance of rapeseed for pollen by honey bees may result from a preference for high nutritional value and valuable pollen in order to feed the large number of bees at immature stages. Indeed we found that, within the studied period, the preferential use of pollen with high nutritional value (protein and mineral) decreased during the season, as with the number of immature bees (see appendix S8). However, in the absence of accurate data on floral resource availability (not collected in this study), one cannot conclude about potential honey bee selectivity with regard to pollen quality (see also Pernal and Currie, 2002).

The major contribution of weeds and trees to pollen collects

The observed bimodal pattern of honey bee diet, i.e. including a two-month food supply depletion period, may appear as an environmental stress for honey bee ecology as well as for beekeeping activity. This food supply depletion period could affect the survivorship of colonies because it appears during the peak of honey bee population size at the colony (larvae and adult; see appendix S8). Indeed, the quantity of available food affects the survivorship of honey bee larvae (Brodschneider and Crailsheim, 2010), the social interaction in the colony (i.e. cannibalism see Schmickl and Crailsheim, 2001) and the behavioural development in workers (Schulz et al., 1998). Such variations in food quantity have led beekeepers to provide supplements of food substitutes to prevent nutritional deficiency and colony failure, particularly at this period (pers. obs.).

We also found that honey bees used a very wide diversity of plants for their pollen needs throughout the season, even during the crop flowering blooms. Previous studies carried out in farmlands (Koppler et al., 2007, Odoux et al., 2012) already established that honey bees forage on a very large number of floral species. Honey bees need polyfloral pollen rather than monofloral (Schmidt, 1984) and furthermore the pollen resource diversity enhances resistance to fungal diseases, tolerance to pesticides and immunity in honey bees to parasites, diseases and pathogens (e.g. Alaux et al., 2010, Foley et al., 2012, Di Pasquale et al., 2013). The diversity of pollen collects allows the honey bee to balance the inability to synthesize most amino acids (Haydak, 1970), as well as other essential nutritional components (protein, lipids, fatty acids) involved in physiological development and immune defence (Huang, 2010, Morais et al., 2011). Our results thus suggest that honey bees need a wide variety of resources for their pollen diets in order to ensure the health of larvae, adults and the entire colony. This may explain why the bees have foraged on more species than expected during rapeseed blooming, especially since larvae are numerous. Interestingly, we found that pollen diet composition was most variable between apiaries during food supply depletion period, and was also best explained by landscape context variables, which were, to a large extent, specific to each apiary.

Implication for bee-pollinator conservation and sustainable honey production in agricultural landscapes

The enhancement of honey bee foraging habitat quality in farmlands can take a number of forms, although the most often used management techniques consists in sowing and maintaining diverse native or non-native flower-rich mixes in set-aside areas such as fallows, field margins and conservation buffer strips (Haaland et al., 2011, Wratten et al., 2012). These Agri-Environmental Schemes (AES) are carried out to sustain bee populations, and promote beekeeping activities. However, they have been developed so far without (or very little) knowledge of honey bee diet in intensive cereal systems. In such landscapes that comprise only rapeseed and sunflower as mass-flowering crops, a food supply depletion period in late spring is apparent. To overcome the need to feed the colonies artificially during this period of food supply depletion (a common current and new practice by local beekeepers), it is necessary to increase either the diversity of crops or the abundance and diversity of floral resources, either in crops (weeds), grasslands, or semi-natural elements (hedgerows, field margins). The first solution includes the reintroduction of entomophilous crops in cropping systems, such as Fabaceae (*Medicago sp.*, *Onobrychis sp.*, *Trifolium sp.*), which are extensively visited by bees (Rollin et al., 2013). These crops are furthermore preferred in the AES chosen by farmers, since their management is well known, their seeds are generally cheap, and they can be easily included within the crop rotation, providing farmers with forage crops (Decourtye et al., 2011). However, the costs of introducing these crops may be very high in sectors without cattle breeding (Van Buskirk and Willi, 2004).

Alternatively, since honey bees use all the available resources in the landscape to fulfill their pollen needs, increasing semi-natural habitat landscape cover and their associated flora may strongly contribute to honey bee diet. Honey bees continuously need a wide diversity in plants which semi-natural habitats are able to provide (i.e., hedgerows, forest edges, meadows and roadside strips). The conservation of semi-natural habitats and plant diversity are also consistent with the safeguarding of native bees (wild bees and bumblebees) and can enhance overall biodiversity and ecosystem services (Wratten et al., 2012, Nicholls and Altieri, 2013).

A third alternative are weeds, an essential resource for pollen diet because of their continuous flowering phenology (especially during the late spring period), and their high species richness which contributes directly to the pollen diversity need. Weeds represent a substantial part of the honey bee annual diet, in particular poppy. Weed conservation could be achieved with less intensive agricultural practices, including the reduction of the use of herbicides (and fertilizers) that are known to drastically restrict weed species richness and abundance (Marshall et al., 2003), as planned in some European countries (Barzman and Dachbrodt-Saaydeh, 2011). These promising solutions are consistent with the conservation of other beneficial species involved in other ecosystem services in agricultural landscapes such as pest predator insects (Bianchi et al., 2006), and the conservation of rare plant species and associated pollinators (Gibson et al., 2006).

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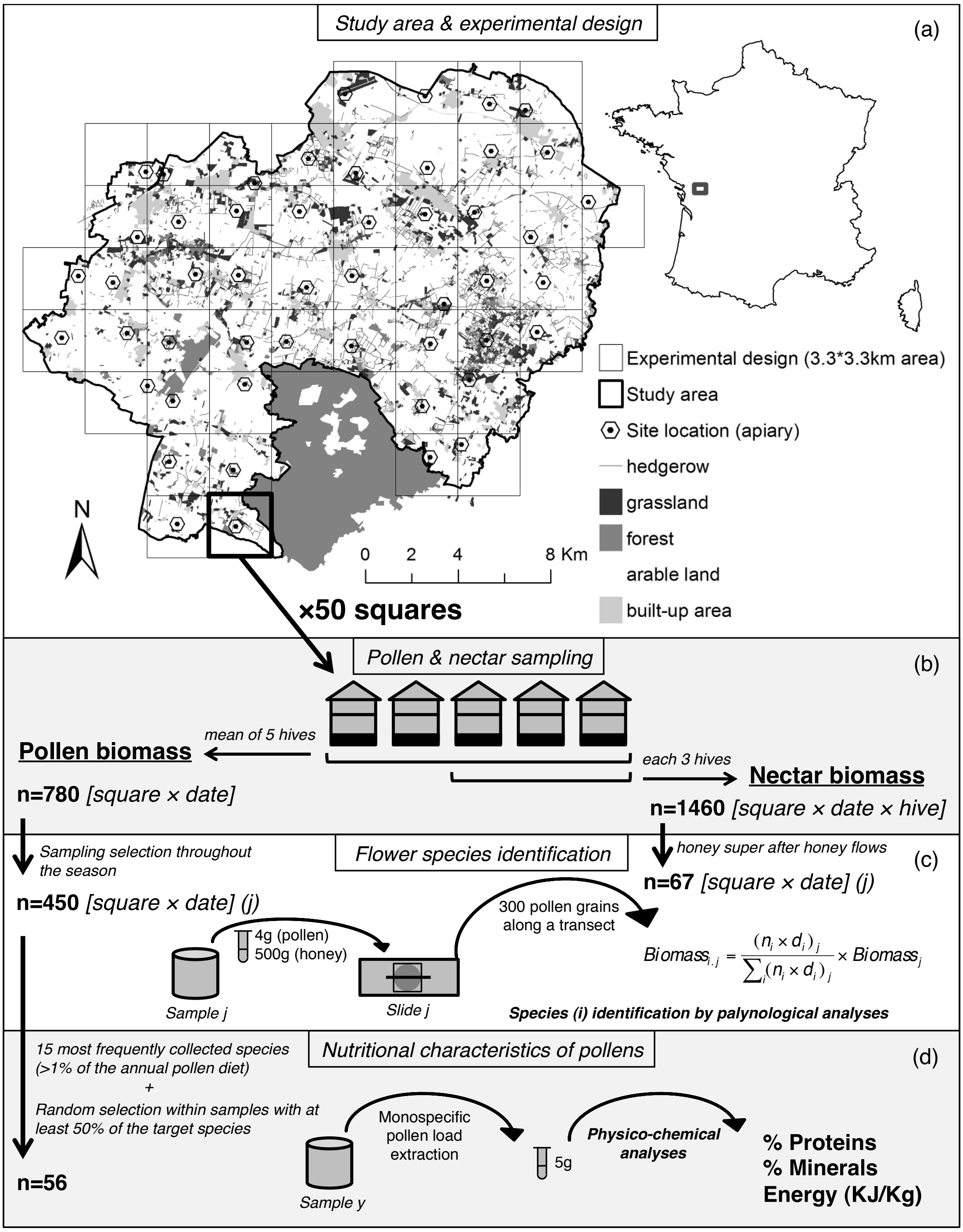
Supporting Information

Appendix S1: Study area, study design and sample sizes used in this study. (a) map of the study area, the LTER "Zone Atelier Plaine et Val de Sèvre", France, showing location of the 50 squares and the distribution of the main habitat types, grassland consists of permanent grasslands and arable land includes lucerne, (b) method of pollen and nectar sampling for each square, (c) palynological technics for flower species identification, and (d) physico-chemical sampling for nutritional characterisation of pollens.



Appendix S2: The number data samples for pollen and nectar.

Over the five years and cumulating the 50 squares (or apiaries), 780 samples of pollen (totalling over 22 kg) were collected from 250 bee colonies, of which a sub sample of 450 was selected for botanical identification (based on a minimum value of 80 samples per year, evenly distributed in the season). An additional 67 samples obtained at the end of each honey flow (totalling approximately 4500 kg of honey) were extracted for botanical identification.

		Pollen						Nectar		
		Annual	April	May	June	July	August	Annual	Spring	Summer
2008	Weighed	90	0	10	30	20	30	9	0	9
	Identified	80	0	10	30	10	30	9	0	9
2009	Weighed	160	20	30	40	30	40	20	10	10
	Identified	130	20	30	40	30	10	20	10	10
2010	Weighed	150	30	20	40	30	30	18	8	10
	Identified	80	20	10	30	10	10	18	8	10
2011	Weighed	190	30	30	30	40	60	20	10	10
	Identified	80	20	10	20	20	10	20	10	10
2012	Weighed	190	40	20	50	30	50	20	10	10
	Identified	80	30	10	20	10	10	0	0	0
Total	**Weighed**	**780**	**120**	**110**	**190**	**150**	**210**	**87**	**38**	**49**
	Identified	**450**	**90**	**70**	**140**	**80**	**70**	**67**	**28**	**39**

Appendix S3: List of plant species collected for (i) pollen and (ii) nectar, species affiliation within resource types and biomass proportion (%), ranked the most to the least annually collected by resource type.

(i) Pollen

Resource Types	*Species*	Annual	April	May	June	July	August
Crop-plant species	*Zea mays*	11	0	0	3	47	16
	Helianthus anuus	9	0	0	14	17	21
	Brassica napus var. olea	4	11	7	0	0	0
	Onobrychis viciifolia	1	<1%	3	0	0	0
	Pisum sativum	1	0	3	<1%	0	0
	Sorghum bicolor	1	0	0	0	4	1
	Faba sativa	1	<1%	2	<1%	0	0
	Setaria sp.	<1%	0	1	1	<1%	0
	Fagopyrum esculentum	<1%	0	0	<1%	1	<1%
	Phacelia tanacetifolia	<1%	<1%	<1%	<1%	<1%	<1%
	Trifolium hybridum	<1%	0	<1%	<1%	<1%	<1%
	Lupinus sp.	<1%	0	<1%	0	0	0
	Brassica sp.	<1%	0	<1%	<1%	<1%	<1%
	Trifolium incarnatum	<1%	0	<1%	<1%	0	0
	Vitis vinifera	<1%	<1%	<1%	<1%	0	0
	Cannabis sativa	<1%	0	0	0	<1%	<1%
	Oomycetes	<1%	0	0	<1%	<1%	0
	Medicago sativa	<1%	0	<1%	<1%	<1%	<1%
	Sorghum sp.	<1%	0	0	0	0	<1%
	Borago officinalis	<1%	<1%	<1%	<1%	0	<1%
	Medicago sp.	<1%	0	0	0	<1%	0
Herbaceous-plant species	*Papaver rhoeas*	10	<1%	15	30	<1%	<1%
	Sinapsis sp.	5	<1%	6	6	3	9
	Mercurialis annua	3	0	<1%	8	6	4
	Reseda lutea	3	<1%	4	1	4	7
	Trifolium sp.	3	<1%	8	2	1	2
	Cichorium sp.	2	0	0	1	1	10
	Trifolium pratense	2	0	7	0	0	0
	Daucus sp.	2	<1%	<1%	<1%	<1%	10
	Mercurialis sp.	1	0	<1%	<1%	<1%	7
	Ammi majus	1	0	<1%	<1%	5	<1%
	Poaceae	1	<1%	3	1	<1%	<1%
	Ornithogalum sp.	1	<1%	2	1	0	0
	Brassicaceae	1	<1%	2	<1%	<1%	1
	Vicia sp.	1	0	2	<1%	0	0
	Plantago sp.	<1%	<1%	<1%	1	1	<1%
	Taraxacum sp.	<1%	1	<1%	<1%	0	0
	Verbascum sp.	<1%	<1%	1	<1%	<1%	<1%
	Solanum nigrum	<1%	0	0	1	<1%	<1%
	Bryonia dioica	<1%	0	<1%	1	<1%	<1%
	Asparagus sp.	<1%	0	<1%	<1%	<1%	0
	Filipendula sp.	<1%	0	0	<1%	<1%	<1%
	Viola sp.	<1%	<1%	<1%	<1%	<1%	<1%
	Centaurea cyanus	<1%	<1%	<1%	<1%	<1%	<1%
	Chenopodium sp.	<1%	0	0	<1%	<1%	<1%
	Legousia speculum-veneris	<1%	0	<1%	<1%	0	0
	Centaurea sp.	<1%	0	<1%	<1%	<1%	<1%
	Centaurea jacea	<1%	0	<1%	<1%	<1%	<1%
	Convolvulus arvensis	<1%	0	<1%	<1%	<1%	<1%
	Campanula sp.	<1%	0	<1%	<1%	<1%	0
	Cirsium sp.	<1%	<1%	<1%	<1%	<1%	<1%
	Crepis sp.	<1%	<1%	<1%	<1%	<1%	<1%
	Calystegia sepium	<1%	0	0	<1%	<1%	<1%
	Torilis sp.	<1%	0	<1%	<1%	<1%	<1%
	Ambrosia artemisiifolia	<1%	0	<1%	0	0	<1%
	Veronica sp.	<1%	<1%	<1%	<1%	<1%	<1%
	Heracleum spondylum	<1%	0	<1%	<1%	0	<1%
	Silene sp.	<1%	<1%	<1%	<1%	0	<1%
	Arctium lappa	<1%	0	0	<1%	<1%	<1%
	Epilobium hirsutum	<1%	0	0	0	<1%	0
	Eupatorium cannabinum	<1%	0	0	0	<1%	<1%
	Hypericum sp.	<1%	0	0	<1%	<1%	<1%
	Lotus corniculatus	<1%	<1%	<1%	<1%	<1%	<1%
	Sanguisorba sp.	<1%	<1%	<1%	<1%	0	0
	Alliaria sp.	<1%	<1%	<1%	0	0	0
	Chaerophyllum sp.	<1%	<1%	<1%	<1%	0	0
	Malva sylvestris	<1%	0	0	0	<1%	<1%
	Melilotus sp.	<1%	0	<1%	<1%	<1%	<1%
	Polygonum persicaria	<1%	0	0	0	0	<1%
	Ranunculaceae	<1%	<1%	<1%	<1%	0	0
	Rumex sp.	<1%	0	<1%	<1%	<1%	0
	Achillea sp.	<1%	0	<1%	<1%	<1%	<1%
	Arctium sp.	<1%	0	0	<1%	<1%	<1%
	Barbarea sp.	<1%	<1%	<1%	0	0	0
	Carex sp.	<1%	<1%	<1%	0	0	0

Appendix S3 continued.

Resource Types	***Species***	**Annual**	**April**	**May**	**June**	**July**	**August**
Herbaceous-plant species	*Datura stramonium*	<1%	0	0	<1%	<1%	<1%
	Eupatorium sp.	<1%	0	<1%	0	<1%	<1%
	Geranium sp.	<1%	<1%	<1%	<1%	0	<1%
	Helianthemum sp.	<1%	<1%	<1%	<1%	<1%	0
	Hippocrepis sp.	<1%	<1%	<1%	<1%	0	<1%
	Lamiaceae	<1%	<1%	<1%	<1%	<1%	0
	Lotus sp.	<1%	<1%	0	<1%	0	<1%
	Lytrum salicaria	<1%	0	0	<1%	<1%	0
	Ranunculus ficaria	<1%	<1%	0	0	0	<1%
	Sanguisorba minor	<1%	0	<1%	<1%	0	0
	Verbena officinalis	<1%	0	0	<1%	<1%	<1%
	Anchusa sp.	<1%	0	<1%	<1%	0	0
	Anthriscus sp.	<1%	<1%	<1%	0	0	0
	Apiaceae	<1%	<1%	<1%	0	<1%	0
	Artemisia vulgaris	<1%	0	0	0	<1%	0
	Asteraceae	<1%	0	0	<1%	0	0
	Bellis sp.	<1%	<1%	<1%	<1%	<1%	0
	Buglossoides arvensis	<1%	0	<1%	0	0	0
	Capsella sp.	<1%	<1%	0	<1%	<1%	<1%
	Carthamus tinctorius	<1%	0	0	0	0	<1%
	Centaurea scabiosa	<1%	0	0	0	<1%	0
	Cerastium sp.	<1%	0	<1%	<1%	0	0
	Convolvulus sp.	<1%	0	0	0	0	<1%
	Coronilla sp.	<1%	0	<1%	<1%	<1%	0
	Daucus carota	<1%	0	<1%	0	<1%	0
	Dipsacus fullonum	<1%	0	0	0	<1%	0
	Echium vulgare	<1%	0	0	0	<1%	0
	Epilobium sp.	<1%	0	0	<1%	<1%	<1%
	Erodium sp.	<1%	0	<1%	0	0	<1%
	Fabaceae	<1%	0	<1%	0	0	0
	Falcaria sp.	<1%	0	0	<1%	<1%	<1%
	Filipendula vulgaris	<1%	0	0	<1%	0	<1%
	Fumaria sp.	<1%	0	<1%	0	0	0
	Galium sp.	<1%	0	0	<1%	<1%	0
	Lathyrus sp.	<1%	0	<1%	<1%	<1%	0
	Leontodon sp.	<1%	<1%	0	0	0	0
	Leucanthemum sp.	<1%	0	<1%	<1%	<1%	<1%
	Linaria sp.	<1%	0	0	<1%	<1%	0
	Malvaceae	<1%	0	0	<1%	0	<1%
	Nasturtium sp.	<1%	0	0	<1%	0	0
	Origanum sp.	<1%	0	0	<1%	<1%	<1%
	Persicaria maculosa	<1%	0	0	0	0	<1%
	Potentilla sp.	<1%	<1%	<1%	<1%	0	0
	Senecio sp.	<1%	0	0	<1%	0	<1%
	Solanum dulcamara	<1%	0	<1%	<1%	<1%	<1%
	Torilis nodosa	<1%	0	0	0	<1%	0
	Typha latifolia	<1%	0	0	<1%	0	0
	Urtica dioica	<1%	0	<1%	<1%	<1%	0
	Valeriana sp.	<1%	0	<1%	0	0	<1%
	Veronica persica	<1%	0	0	<1%	<1%	0
	Vicia cracca	<1%	0	<1%	0	0	0
Woody-plant species	*Crataegus sp.*	8	31	6	2	0	0
	Prunus sp.	5	24	2	0	0	0
	Acer sp.	4	19	<1%	0	0	0
	Rubus sp.	3	<1%	<1%	9	4	<1%
	Cornus sanguinea	3	0	11	<1%	0	0
	Quercus sp.	1	3	2	<1%	0	0
	Castanea sativa	1	0	0	4	<1%	0
	Hedera helix	1	0	0	0	0	5
	Salix sp.	1	4	<1%	0	0	0
	Sorbus sp.	1	1	1	0	0	0
	Rhamnus sp.	1	<1%	2	<1%	<1%	0
	Lonicera sp.	<1%	<1%	<1%	1	<1%	<1%
	Ligustrum vulgare	<1%	0	<1%	1	<1%	<1%
	Rosa canina	<1%	0	1	<1%	0	0
	Sambucus nigra	<1%	<1%	1	<1%	0	0
	Sambucus sp.	<1%	0	<1%	<1%	0	0
	Rosaceae	<1%	<1%	<1%	<1%	0	<1%
	Clematis vitalba	<1%	0	<1%	<1%	<1%	<1%
	Fagus sylvatica	<1%	<1%	<1%	0	0	0
	Caprifoliaceae	<1%	0	<1%	0	0	0
	Clematis sp.	<1%	0	0	<1%	<1%	<1%
	Crataegus monogyna	<1%	<1%	0	0	0	0
	Robinia pseudo-acacia	<1%	0	<1%	0	0	0
	Pinus sp.	<1%	<1%	<1%	0	<1%	0

Appendix S3 continued.

Resource Types	***Species***	**Annual**	**April**	**May**	**June**	**July**	**August**
Woody-plant species	*Viburnum lantana*	<1%	<1%	0	0	0	0
	Betula sp.	<1%	<1%	0	0	0	0
	Cornus mas	<1%	0	<1%	0	0	0
	Cornus sp.	<1%	0	0	0	0	<1%
	Corylus avellana	<1%	<1%	0	0	0	0
	Frangula dodonei	<1%	0	<1%	0	0	0
	Fraxinus excelsior	<1%	<1%	0	0	0	0
	Ilex aquifolium	<1%	0	0	<1%	0	0
	Quercus ilex	<1%	<1%	0	<1%	0	0
	Sambucus ebulus	<1%	0	0	<1%	0	0
Horticultural-plant species	*Magnolia sp.*	1	0	<1%	2	1	<1%
	Rosa sp.	1	<1%	1	1	<1%	<1%
	Elaeagnus sp.	1	0	0	<1%	1	2
	Parthenocissus sp.	<1%	0	<1%	1	1	<1%
	Buddleja sp.	<1%	0	0	1	1	<1%
	Amelanchier sp.	<1%	1	0	0	0	0
	Juglans regia	<1%	<1%	<1%	<1%	0	0
	Tilia sp.	<1%	0	<1%	<1%	0	0
	Malus sp.	<1%	1	0	0	0	0
	Aesculus hippocastanum	<1%	<1%	<1%	0	0	<1%
	Oenothera biennis	<1%	0	<1%	<1%	<1%	<1%
	Gleditsia sp.	<1%	0	0	<1%	0	0
	Cornus sericea	<1%	<1%	0	0	0	0
	Laurus nobilis	<1%	<1%	0	0	0	0
	Caryophyllaceae	<1%	0	<1%	0	0	<1%
	Cercis siliquastrum	<1%	<1%	<1%	0	0	0
	Eremophila sp.	<1%	0	0	<1%	<1%	<1%
	Eschscholzia californica	<1%	<1%	<1%	<1%	<1%	<1%
	Eucalyptus sp.	<1%	0	<1%	<1%	0	0
	Lagerstroemia sp.	<1%	0	0	0	<1%	<1%
	Liliaceae	<1%	<1%	<1%	<1%	0	0
	Platanus x hispanica	<1%	<1%	0	0	0	0
	Berberis sp.	<1%	<1%	<1%	0	0	0
	Buxus sempervirens	<1%	<1%	0	0	0	0
	Chelidonium majus	<1%	<1%	<1%	0	0	<1%
	Eschscholzia sp.	<1%	0	0	<1%	<1%	0
	Fraxinus ornus	<1%	<1%	<1%	0	0	0
	Olea sp.	<1%	0	<1%	<1%	0	0
	Passiflora sp.	<1%	0	0	<1%	0	<1%
	Prunus lauracerasus	<1%	0	<1%	0	0	0
	Pyracantha sp.	<1%	<1%	<1%	0	0	0
	Pyrus sp.	<1%	<1%	0	0	0	<1%
	Rhus sp.	<1%	0	<1%	<1%	0	0
	Rubus idaeus	<1%	0	0	<1%	0	0
	Sophora japonica	<1%	0	0	0	<1%	<1%
	Ailanthus altissima	<1%	0	<1%	<1%	0	0
	Albizia julibrissin	<1%	0	0	<1%	0	0
	Azara sp.	<1%	0	0	<1%	0	0
	Calendula sp.	<1%	0	0	0	0	<1%
	Camellia sp	<1%	0	0	0	0	<1%
	Ceanothus sp.	<1%	0	0	<1%	0	0
	Chamaerops sp.	<1%	0	<1%	0	0	0
	Chelidonium sp.	<1%	<1%	0	<1%	0	<1%
	Cotinus sp	<1%	0	<1%	<1%	0	<1%
	Cupressaceae	<1%	<1%	<1%	<1%	0	0
	Elaeagnus x ebbingei	<1%	0	<1%	0	0	0
	Erica sp.	<1%	<1%	<1%	<1%	0	0
	Fragaria sp.	<1%	<1%	0	0	0	0
	Gazania sp.	<1%	0	0	0	0	<1%
	Hepatica sp.	<1%	<1%	<1%	<1%	0	0
	Impatiens glandulifera	<1%	0	0	<1%	0	0
	Impatiens sp.	<1%	<1%	0	0	<1%	<1%
	Iris sp.	<1%	0	<1%	<1%	0	0
	Laburnum sp.	<1%	<1%	0	0	0	0
	Macleaya sp.	<1%	0	0	<1%	0	<1%
	Nicotiana sp.	<1%	0	<1%	0	0	0
	Nuphar lutea	<1%	0	<1%	<1%	0	0
	Papaveraceae	<1%	<1%	<1%	<1%	0	0
	Platanus sp.	<1%	<1%	0	0	0	0
	Rhamnaceae	<1%	0	0	<1%	0	0
	Sedum sp.	<1%	0	<1%	0	0	0
	Solidago sp.	<1%	0	0	<1%	<1%	<1%
	Spiraea sp.	<1%	0	0	<1%	0	0
	Symphoricarpos albus	<1%	0	<1%	<1%	0	0
	Syringa sp.	<1%	<1%	0	0	0	0
	Viburnum sp.	<1%	<1%	<1%	<1%	0	0
	Weigelia sp.	<1%	0	<1%	0	0	0
	Wisteria sp.	<1%	0	0	<1%	0	0

Appendix S3 continued.

(ii) Nectar

Resource types	*Species*	Annual	Spring	Summer
Crop-plant species	*Helianthus anuus*	15-45%	0	>45%
	Brassica napus var. olea	15-45%	>45%	<15%
	Phacelia tanacetifolia	<15%	<15%	<15%
	Zea mays	<15%	0	<15%
	Medicago sativa	<15%	0	<15%
	Onobrychis viciifolia	<15%	0	<15%
	Vitis vinifera	<15%	0	<15%
	Linum	<15%	<15%	0
	Fagopyrum esculentum	<15%	0	<15%
Herbaceous-plant species	*Reseda lutea*	<15%	<15%	<15%
	Mercurialis sp.	<15%	0	<15%
	Trifolium sp.	<15%	<15%	<15%
	Filipendula sp.	<15%	<15%	<15%
	Daucus sp.	<15%	0	<15%
	Poaceae	<15%	<15%	<15%
	Plantago sp.	<15%	<15%	<15%
	Papaver rhoeas	<15%	<15%	<15%
	Apiaceae	<15%	<15%	<15%
	Brassicaceae	<15%	<15%	<15%
	Trifolium pratense	<15%	<15%	<15%
	Solanum nigrum	<15%	0	<15%
	Lotus sp.	<15%	<15%	<15%
	Vicia sp.	<15%	<15%	<15%
	Cichorium sp.	<15%	0	<15%
	Chenopodium sp.	<15%	0	<15%
	Carduus sp.	<15%	<15%	<15%
	Centaurea sp.	<15%	<15%	<15%
	Hypericum sp.	<15%	0	<15%
	Primulaceae	<15%	<15%	0
	Taraxacum sp.	<15%	<15%	0
	Asteraceae	<15%	<15%	0
	Ranunculaceae	<15%	<15%	0
	Echium vulgare	<15%	<15%	<15%
	Arctium sp.	<15%	0	<15%
	Polygonum aviculare	<15%	0	<15%
	Centaurea cyanus	<15%	<15%	<15%
	Malvaceae	<15%	0	<15%
	Viola sp.	<15%	0	<15%
	Fabaceae	<15%	<15%	0
	Yeast	<15%	<15%	<15%
	Hippocrepis sp.	<15%	<15%	0
	Heracleum spondylum	<15%	<15%	<15%
	Sinapsis sp.	<15%	<15%	0
	Carex sp.	<15%	<15%	0
	Campanula sp.	<15%	0	<15%
	Galega sp.	<15%	0	<15%
	Artemisia vulgaris	<15%	0	<15%
Woody-plant species	*Castanea sativa*	<15%	<15%	<15%
	Prunus sp.	<15%	<15%	<15%
	Rubus sp.	<15%	<15%	<15%
	Quercus sp.	<15%	<15%	<15%
	Rhamnus sp.	<15%	<15%	<15%
	Salix sp.	<15%	<15%	<15%
	Acer sp.	<15%	<15%	0
	Cornus sanguinea	<15%	<15%	<15%
	Betula sp.	<15%	<15%	0
	Euonymus europaeus	<15%	<15%	0
	Pinus sp.	<15%	<15%	0
	Clematis sp.	<15%	<15%	<15%
	Corylus avellana	<15%	<15%	0
	Crataegus sp.	<15%	<15%	0
	Robinia pseudo-acacia	<15%	<15%	0
	Ligustrum vulgare	<15%	0	<15%
	Viburnum lantana	<15%	<15%	0
	Fagus sylvatica	<15%	<15%	0
	Lonicera sp.	<15%	<15%	0
	Rosaceae	<15%	0	<15%
Horticultural-plant species	*Buddleja sp.*	<15%	0	<15%
	Aesculus hippocastanum	<15%	<15%	<15%
	Parthenocissus sp.	<15%	0	<15%
	Wisteria sp.	<15%	0	<15%
	Liliaceae	<15%	0	<15%
	Caryophyllaceae	<15%	<15%	0
	Gleditsia sp.	<15%	<15%	<15%
	Tilia sp.	<15%	<15%	<15%
	Cupressaceae	<15%	<15%	0
	Cercis siliquastrum	<15%	<15%	0
	Fragaria sp.	<15%	<15%	0
	Buxus sempervirens	<15%	<15%	0
	Chamaerops sp.	<15%	<15%	0
	Thymus vulgaris	<15%	0	<15%

Appendix S4: Detail of statistical analyses.

All statistical analyses were performed using the R environment for statistical computing (R-Development-Core-Team, 2009).

- Seasonal patterns in pollen and nectar biomass

Seasonal patterns in pollen and nectar biomass were modelled as a function of time using Generalised Additive Mixed Models (GAMMs) with *gamm* function in *mgcv* R-package and a Gaussian error structure (Lin and Zhang, 1999). GAMMs are modeling technics allowing a temporal splines fit while accounting for repeated measurements within a nested design (i.e. dates are nested within squares within years).

We divided the seasonal patterns in both harvested pollen and nectar biomass into temporal classes, separated by a peak of harvested biomass using the *breakpoints* function of the R-package *strucchange*. We defined the mass-flowering temporal classes as the 30-days period encompassing the peak date. We used the same method for the nectar harvests whose seasonal pattern was also non-linear.

- Relative contribution of crops and other resource types

It is not possible to assign one habitat for each plant because of the presence of species in several habitats (e.g. *Reseda lutea* is both a weed in crops and is present in grasslands). We therefore first investigated floral resource in honey bee diet (pollen and nectar) using four broad taxonomic categories unrelated to the habitat, i.e. crop species, spontaneous herbaceous species, spontaneous ligneous species, and finally horticultural species. We used the Chi-squared test to compare the proportion in biomass for these four categories according to period, in order to test for non-random seasonal variation in the relative contribution of resource types, using the *chisq.test* function of the R-package *stats*.

- Floral resource diversity in pollen and nectar

Floral resource diversity in pollen and nectar was estimated using total species richness (S, number of distinct pollen species) as well as Equitability index ($E=[-\Sigma\ pi.\log2(pi)]/\log S$; where pi is the probability of occurrence of species i and S is the total species richness, Shannon, 1997).

Total species richness of foraged species by bees for pollen estimated by Chao method, since the number of species is rather high (see below) and the number of samples is finite (Chao, 1984). Chao 1 estimator is the sum of the observed number of species plus the quotient $a^2/2b$, where a is the number of species represented by one (singletons) and b the number of species represented by two (doubletons) individuals in the sample. Calculations were conducted using the *specaccum* function in the *vegan* R-package (Gotelli and Colwell, 2001).

The effect of month on equitability and species richness was tested using a Generalized Linear Mixed Models (GLMMs) with *lme* function in *nlme* R-package and a Gaussian error structure and the sampling square nested into year as an annual and spatial random effect. We then used multiple comparison analysis using *glht* function in *multcomp* R- package.

- <u>Influence of landscape context</u>

As a dissimilarity index we used the Jaccard distance with *vegdist* function in the *vegan* R-package between all pairs of squares to estimate the dissimilarity of diet composition between them within each year and temporal class (Faith et al., 1987, Chust et al., 2006).

In order to keep the influence of landscape context robust enough, we restricted it to pairs of squares located <12.5km apart from each other, corresponding to 1/3 of the study area width.

We used model selection by Akaike information criterion (AIC) from GLMMs with a Gaussian error structure and temporal classes nested into year as a seasonal and annual random effect

We used GAMMs with a Gaussian error structure and temporal classes nested into year as a seasonal and annual random effect to fit the influence of best parameters selected by AIC on the pollen diet dissimilarity.

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Appendix S5: Detail of landscape analyses

- Results of AIC Analysis for model selection (GAMM), L *i* = maximum likelihood for model *i*, Δ *i* (AIC) = [AIC*i* – min(AIC)], K *i* = number of estimated parameters for model *i*, *wi* (AIC) = the rounded Akaike weights, n=1061.

Model$_i$	K$_i$	L$_i$	AIC$_i$	Δ$_i$(AIC)	w$_i$(AIC)
Distance + Month	7	1	-1858,1	0	0,90
Distance * Month	11	0,12	-1853,8	4,30	0,10
Month	6	<0.01	-1808,8	49,30	<0.01
Distance	3	<0.01	-1714,4	143,68	<0.01
Distance + Month + Pair-square	136	<0.01	-1688,2	169,90	<0.01
Month + Pair-square	136	<0.01	-1688,2	169,90	<0.01
Null	2	<0.01	-1671,1	186,94	<0.01
Distance * Pair-square	132	<0.01	-1515,3	342,82	<0.01
Distance + Pair-square	132	<0.01	-1515,3	342,82	<0.01
Pair-square	132	<0.01	-1515,3	342,82	<0.01
Distance * Month * Pair-square	622	<0.01	283,3	2141,41	<0.01
Month * Pair-square	622	<0.01	283,3	2141,41	<0.01

- Landscape composition variables used in landscape composition dissimilarity analyses

We extracted the land-use around each apiaries in a radius of 1500m, corresponding to the foraging area estimated of honey bees in agricultural landscapes (Steffen-Dewenter et al., 2003). We cumulated the surface for each land-use and group these into 10 landscape variables:

- Forests and hedgerows
- Roadsides
- Grasslands
- Rapeseed crops
- Sunflower crops
- Leguminous crops
- Polliniferous cereal crops
- Other cereal crops
- Other crops
- Built-up areas

References

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Appendix S6: Annual phenological deviation estimated by cumulative temperature sum from a national French meteorological station (http://climat.meteofrance.com/) located in Niort, on the northern edge of the study area.

Substantial inter-annual variations in seasonal dynamics of colonies (e.g. honey reserves) have already been identified (Odoux et al., 2014), which are likely to have been caused by climatic conditions and plant phenology. The cumulative sum of temperatures above 0°C was used as a proxy of year conditions, as this parameter is well known to directly influence plant flowering phenology (Price and Waser, 1998, Cleland et al., 2007).

We first cumulated sum of temperatures above 0°C yearly for the period 1986-2012, and averaged year values to derive a baseline value. Then, each year value between 2008 and 2012 was substracted to the baseline value to account for the potential effect of annual climatic conditions. We calculated the temperature×days dynamic for each year and for the average. The day difference of a given year to the average (i.e., the residual) provided a proxy of year deviation compared to mean (see Fig.1 below), and we used this proxy as an annual phenological index in order to rescale the seasonal patterns of pollen and nectar harvests.

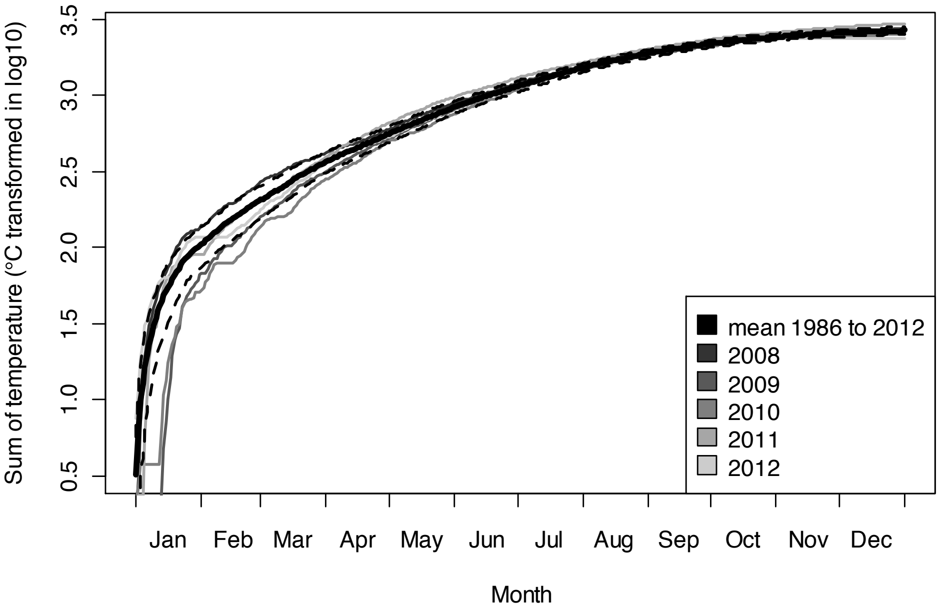

Figure: Temporal dynamic of the cumulated sum of temperatures above 0°C (log10 transformed) for each year between 2008 and 2012 and for the average period of 1986-2012.

References

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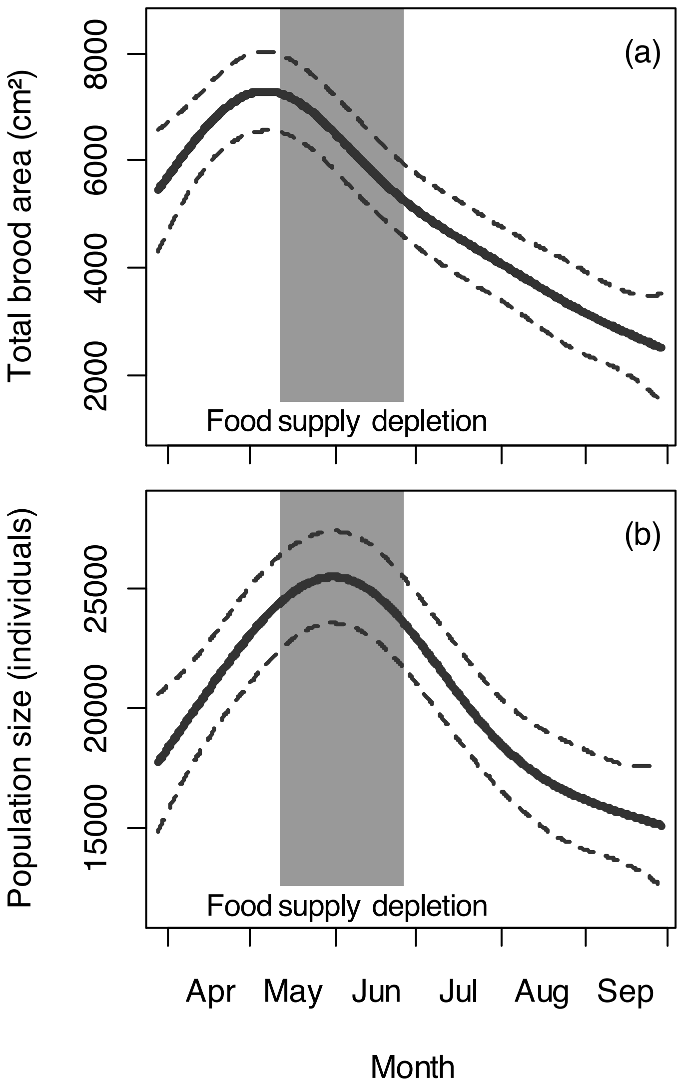
Price, M. V. & Waser, N. M. (1998) Effects of experimental warming on plant reproductive phenology in a subalpine meadow. *Ecology,* **79,** 1261-1271.

Appendix S3: Nutritional value of analysed pollen.

Species	n	Proteins (%)	Minerals (%)	Energy (KJ/Kg)	Pollen diameters (μm)
Papaver rhoeas	6	24.48 ± 1.75	2.17 ± 0.41	19820 ± 366.89	24.5
Sinapsis sp.	3	23.3 ± 0.1	2.7 ± 0.1	21398.67 ± 359.51	30.5
Mercurialis annua	3	16.2 ± 1.65	2.1 ± 0.26	20050 ± 428.68	23.5
Reseda lutea	3	27.5 ± 0.95	2.23 ± 0.15	19696 ± 378.72	22.5
Trifolium sp.	3	29.2 ± 0.87	3.1 ± 0.1	19700 ± 763.68	24.0
Trifolium pratense	3	28.17 ± 0.67	2.93 ± 0.12	20015 ± 289.91	36.0
Daucus sp.	3	19.17 ± 1.25	1.8 ± 0.2	19767 ± 18.38	17.5
Centaurea cyanus	1	24.1 ± NA	2.1 ± NA	NA ± NA	35.0
Zea mays	7	15.59 ± 0.74	1.6 ± 0.22	19154 ± 267.41	80.0
Helianthus anuus	6	16.33 ± 0.98	1.37 ± 0.05	20061 ± 412.78	39.5
Brassica napus var. olea	5	26.74 ± 1.54	2.54 ± 0.15	21644.33 ± 116.13	24.5
Crataegus sp.	3	28.23 ± 0.35	2.8 ± 0.1	20287.5 ± 68.59	38.0
Acer sp.	3	27.8 ± 0.78	2.77 ± 0.06	20796.5 ± 23.33	31.0
Rubus sp.	3	25.1 ± 0.53	2.5 ± 0.1	19247.5 ± 156.27	21.5
Cornus sanguinea	3	17.9 ± 0.2	3 ± 0.1	19638.5 ± 2.12	53.0

Appendix S8: Seasonal patterns of (a) total brood area (*GAMM*, $F_{4,1455}$=200.9,p<0.001) and (b) colony size (adult population, *GAMM*, $F_{4,1455}$=78.16,p<0.001) of honey bee colonies from the same study area (redrawn from Odoux et al. (in press) with one year update (2008 to 2012).



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Histoire de vie des colonies

Conséquence de la déplétion d'approvisionnement alimentaire printanière sur l'histoire de vie des colonies

Cet article étudie les conséquences de la déplétion d'approvionnement alimentaire énoncée dans l'article précédent, sur les mécanismes adaptatifs et évolutifs des colonies. Les coûts du compromis d'allocation des ressources pendant la période de déplétion d'approvionnement alimentaire printanière ont des répercussions irréversibles sur l'histoire de vie future des colonies, aboutissant à un affaiblissement des colonies qui réduit la survie hivernale des colonies d'abeilles domestiques.

Paper in prep.

Title: Carry-over effects of spring pollen supply depletion on honey bee colony winter survival in farmland habitats

Authors: Requier F., Odoux J. F., Henry M., Bretagnolle V.

Carry-over effects of spring pollen supply depletion on honey bee colony winter survival in farmland habitats

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Key-words: *Apis mellifera* L., life history evolution, pollen scarcity, nutritional stress, food allocation decision, colony dynamic, trade-off, carry-over effect, colony survival, *Varroa destructor*, agricultural landscape.

Introduction

The current decline of bee-pollinators is particularly alarming (Potts et al., 2010) given the pollination service they provide in agricultural landscapes, either in terms of conservation of wild flowers or insurance of crop yields (Biesmeijer et al., 2006, Rader et al., 2012, Garibaldi et al., 2013). In those farming landscapes, land-use changes have been identified as the major driver of the wild bee decline, with the loss of foraging and nesting resources (Winfree et al., 2009, Ricketts et al., 2008, Le Féon et al., 2010). The honeybee case is however more complex, better described as a syndrome of depopulation involving excess loss during over-wintering, which is caused by a multifactorial set of drivers including parasites and pathogens, lack of genetic diversity, and environmental stressors (Ellis et al., 2010, Williams et al., 2010, Kleinman and Suryanarayanan, 2012, Cox-Foster et al., 2007, Anderson and East, 2008). Two main interacting factors have been identified in particular: the ectoparasitic mite *Varroa destructor* (Kraus and Page, 1995, Moritz et al., 2007, Jaffe et al., 2009), and pesticide use, with their lethal or sub-lethal effects (Alston et al., 2007). Pesticides affect honey bee foraging, which led to some insecticides being eventually prohibited (Henry et al., 2012). Among pesticides, herbicides may also affect pollinators indirectly by decreasing floral resource availability (Gabriel and Tscharntke, 2007, Holzschuh et al., 2007): however, though suspected, no empirical study has yet investigated the consequences of decrease in flower availability on honey bee life history traits (Naug, 2009, Decourtye et al., 2010).

Honey bees depend exclusively on floral resources for feeding. Nectar is a carbohydrate source used by bees as an energy fuel, but is also transformed into honey for food reserve, allowing over-wintering colony survival (Winston, 1994). Pollen is the main source of protein, used to feed mainly the young adults, as well as queen and larvae (Haydak, 1970). Conversely to nectar, pollen is not stored (Brodschneider and Crailsheim, 2010, Keller et al., 2005b), and therefore is used in 'tense-flow' between colony needs and flower resources availability. Hence pollen is likely to affect life history traits and allocation trade-offs (see Stearns, 1989, for general review) in honey bees. Indeed pollen supply was shown experimentally to drive honey bee life history parameters (e.g. Brodschneider and Crailsheim, 2010, Keller et al., 2005a), affecting for instance in real time the number of larvae reared (brood) within the colony (Fewell and Winston, 1992, Pankiw, 2007). Pollen is collected by foraging bees to feed the brood, while pollen harvest is regulated by a feedback loop according to brood size and amount of stored pollen in the colony (Fewell and Winston, 1992, Camazine, 1993, Sagili and Pankiw, 2007). When a pollen scarcity occurs, the honey bee colony regulates its foraging intensity by increasing foraging workload (Schulz et al., 1998), through preferential allocation to pollen foraging over nectar (Fewell and Winston, 1992). However, if floral resources are limiting, the colony may further decrease its pollen consumption by reducing queen egg-laying (Schmickl et al., 2003), or even reducing larvae number through worker cannibalism (Schmickl and Crailsheim, 2001), before totally stopping activity (Keller et al., 2005a).

Since brood size constrains the incoming adult foraging population size (about 21 days later, eggs become adults), it is expected that a pollen scarcity should affect the population dynamic of honey bee colonies; though suspected (e.g. Keller et al., 2005a, Decourtye et al., 2010), this was never tested. Carry-over effects are defined as processes occurring in one season and influencing the fitness of individuals in the following season (e.g. Harrison et al., 2011). Generation time in adult honey-bees is about 2 months, carry-over effects would thus occur, in this species, within a breeding season. Our aims in this study are specifically to empirically test the hypothesis of existing carry-over effects of pollen scarcity to demography and life history trade-offs in honey bees in a real beekeeping management situation (i.e., with harvesting of honey, syrup supply and anti-*Varroa* treatment). In intensive farming systems (our situation), there is typically a two months period of pollen supply depletion occurring at the peak of larvae population growth in honey bee colonies (Requier et al., in revision). This depletion period occurs between rapeseed *Brassica napus* and sunflower *Helianthus anuus* mass-flowering periods (April and July respectively), a period without any other mass flowering crops nowadays in those cropping systems. Since our study site is large and spatially heterogeneous (in landscape configuration and flower resources), a resulting gradient of pollen supply depletion during this specific two months period (Odoux et al., 2014, Requier et al., in revision) was available. Using this gradient allowed to quantify its effects on bee life history traits and allocation trade-offs. Three major life history traits were considered: brood size (an indicator of reproduction effort), adult population size (an indicator of worker survival) and honey reserve (an indicator of colony survival such as effort allocated to over-wintering preparation). In addition, we analyze three important life history trade-offs: reproduction allocation trade-off (between female worker and male dispersers), behavioral adult allocation trade-off (between foraging and brood rearing tasks), and a space use trade-off within the context of constrained available apiary volume (space allocation between brood or honey reserve in the brood-chamber). First, we analyze real time (unlagged) response of life history traits and their trade-offs specifically during the pollen supply depletion period. We predict a correlated decrease of brood surface with intensity of pollen supply depletion, which should be more marked for drone brood. Second, we analyze with temporal correlograms the presence of potential carry-over effects on population parameters in relation to intensity of pollen supply depletion. Real time brood decrease should result in lagged carry-over effect on adult population 21 days later. Then, the latter should decrease honey reserve potential through reduced numbers of foragers. Third, through lagged effects of past selected life history traits on organisms evolutionary dynamics (Cameron et al., 2013), we predict that maladapted life history colony traits before the pollen supply depletion may have cascading effects later though amplification during pollen depletion period. In particular, we predict that wrong allocation within the brood versus honey reserve trade-off early in spring (April) may precipitate detrimental effects during pollen depletion period. Fourth, we investigate the colony survival under the interactive effects of these multiple carry-over effects and the influence of *Varroa destructor* pressure. We predict that parasite and pathogen pressures acts synergistically on lagged effects of pollen depletion, and decrease overall survival of honey bee colonies, especially winter survival.

Materials and methods

Study area and monitoring design

Our study area, the Long-Term Ecological Research *Zone Atelier Plaine & Val de Sèvre*, is located in central western France (46°23'N, 0°41'W), in the Poitou-Charentes Region. Land use of every single field (around 18000) in the study site (450 km2) has been recorded twice a year since 1995 and stored on a geographical information system (ARCVIEW 9.2, ESRI, Redlands, CA, USA). The agricultural landscape of the study area is characterized by a high proportion of arable land (>75% of land use), of which a large part is dedicated to cereal production (45% of cereals) in addition to oilseed crops (10% sunflower, 9% rapeseed) and maize (8%). Field work was conducted from 2008 to 2012. The study site has been divided into 50 equal $10km^2$ squares (see ESM1, Odoux et al., 2014, Requier et al., in revision) , so that the centers of neighboring squares were about 3km apart, i.e. twice the average foraging distance of honeybees for pollen harvest in such landscapes . Every year, ten of these squares were randomly chosen. In each of the squares, a 5-hives apiary was set up as close as possible to the square's centroid and was monitored during the complete beekeeping season (n=50 apiaries), spanning from late March to October. All honey bee colonies (n=250) proceeded from a livestock managed by a local professional beekeeping association (ADAPC), from an *A. mellifera mellifera × caucasica* strain. Queens were one year old at the time of launching and their health was checked disease-free for any visible symptoms. The beehives were the Dadant-Blatt model (with 10 brood-chamber frames) in pine woodwaxed microcrystalline. Colonies were set up and managed as a sedentary apiary according to local beekeeping professional practices (Odoux et al., 2014).

Estimating pollen supply depletion

Pollen harvest is depending on the larvae population size (Dreller et al., 1999) and flower resource availability in the foraging area, i.e. at about 1.5-2km around the colony (Visscher and Seeley, 1982, Steffan-Dewenter and Kuhn, 2003). Between rapeseed and sunflower mass-flowering periods, a two months pollen depletion period occurs. Given the vital functions ensured by pollen in physiological and morphological development of bees

(Brodschneider and Crailsheim, 2010, Keller et al., 2005b) and since it cannot be stored in hives, we used an index of pollen harvest as a proxy for estimating the colony environmental nutritional stress. Harvested pollen was collected at the entrances of each of the 5 hives per square, which were fitted with a pollen-trap for 24-h every ten days (on average; see ESM2). Harvested pollen depletion was estimated for each apiary (square): three parameters were available, pollen harvest biomass at start (Julian day c. 132) and end (Julian day c. 177) of the depletion period, and the slope of harvested pollen biomass with date between days 132 and 177. These three measures were correlated to each other (linear regression, *LM*, $F_{1,48}$=96.36, p<0.001, see ESM2), so we used the slope as our proxy.

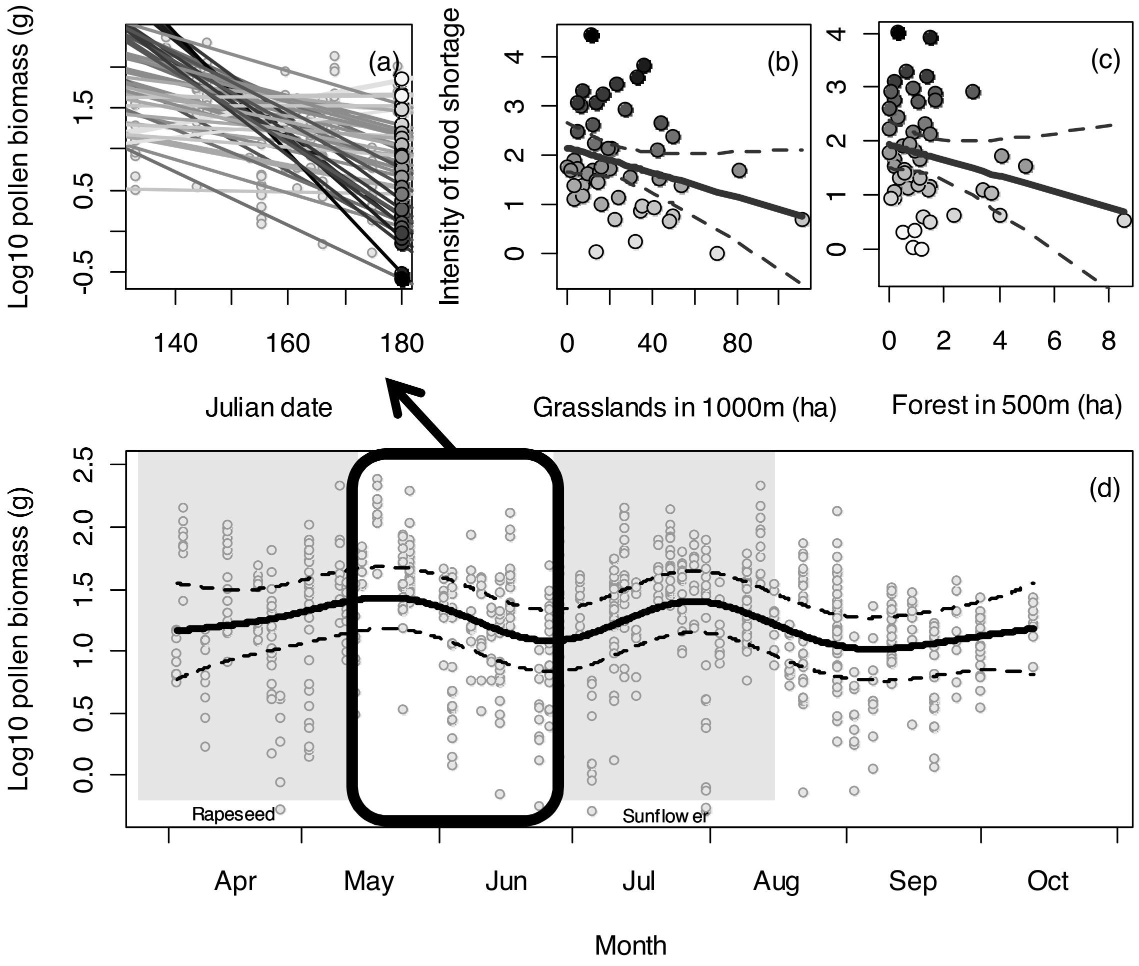

Figure 1 : Pollen supply depletion characterization, (a) the two pollen depletion estimators, depletion slope and last biomass estimated, (b) the linear regression between the pollen supply depletion intensity estimated by depletion slope and grassland surface (permanent grasslands) into 1,000m around the bee-hive, (c) the linear regression between the pollen supply depletion intensity estimated by last pollen biomass and forest surface (hedgerows and forest border) into 500m around the bee-hive, (d) the seasonal pattern of pollen harvests of honey bee colonies from the same study area (*GAMM*, $F_{6,773}$=7.333, p<0.001, n=780, redrawn from Requier et al., in revision)

Monitoring honey bee colony life history traits

For each 50 apiaries located in different landscape contexts, three of the five hives were sampled every two weeks during the beekeeping season (i.e. March to October). The two other colonies were used as control colonies, and were checked at the beginning and end of the beekeeping season. They were also used as substitution colonies if any of the monitored colonies of the same apiary collapsed during the season. In sum, due to this colonies turn-over, 250 colonies distributed in 50 apiaries were monitoring with c.15 sampling dates per year (the number of sampling dates varied according to year, see ESM3 for the complete data sampling). Overall, 1,636 measures (sampling date × apiary × colony) were available on the following life history traits: the brood area (sum of eggs, larvae, and pupae), the adult population size, and the honey reserve mass. These colony parameters were assessed at each date sampling (n=15).

In summary, brood size was estimated by brood area, obtained through measuring length and width of each brood from each frame (see ESM4 for details). We separated the drone brood since brood cannibalism may be sex biased (Wharton et al., 2008), and therefore brood size included worker brood surface and drone brood surface. Adult population size (number of adult individuals) was assessed by weighting each brood frame with *versus* without bees, as well as the hive bottom and the honey super-chambers. The resulting values slightly underestimated real adult population size because it did not take into account the portion of foraging bees that had left the colony at the time of measurement (see ESM4 for additional details). Honey reserve mass includes the supply of honey, nectar, and pollen, though the latter is negligible compared to nectar and honey weights because pollen is stored in very small quantities (Brodschneider and Crailsheim, 2010, Keller et al., 2005b). For each hive, frames were weighted without honey bees both from the main brood-chamber and the honey super-chambers, as the difference between total frame mass and the mass of empty frames before introduction in the hive (see ESM4). Similar to local beekeeping techniques, honey reserves in super-chambers were extracted twice a year, just after the rapeseed and sunflower honey flows (May and August, respectively). Colony survivorship was obtained for 241 colonies, as a binomial variable (dead *vs*. alive) over the 1,636 colony observations. A colony was considered “dead” when the hive was empty or if the colony was without queen in late winter. Finally, *Varroa* pressure was estimated at the beginning of the season by placing a greased board at the bottom of one of the five hives (i.e., one measure per apiary), and counting every two weeks the number of collected *Varroa* individuals (which were dead). A total of 878 measures were available (in 2012, no measures were available). Following local beekeeping techniques, an anti-*Varroa* treatment was applied systematically after sunflower honeyflow in September.

Quantifying honey bee life history trade-offs

Because relations could be non-linear, we always fitted Generalised Additive Models (GAMs, with *gam* function the *mgcv* R-package) with a Gaussian error structure, unless otherwise stated. For each measure of brood area, we distinguished brood dedicated to drones from those to female workers. We fitted with a GAM the relationship between worker brood area and drone brood area and then extracted the residual values (see ESM5), as a surrogate of sexual brood allocation. A positive residual value represents a preferential allocation to drone brood, a negative value to worker brood. The trade-off in behavioral adult allocation work was estimated according to the postulated hypothesis that the worker brood area (after transformation of surface into individuals, i.e. 1larvae= 0.3183 cm^2 surface of 1 worker cell) could predict the future worker population size, one generation (21 days) later. We plotted the relationship between the observed adult population (see method above) and this estimated value with GAMs. Residual values, when positive, indicate an over-presence of adult workers into bee-hive, and could be interpreted as a preferential allocation to in-hive tasks, such as brood rearing (see ESM5). Conversely, a negative value represents an under-presence of adult workers into bee-hive and could be interpreted as a preferential allocation to out-hive tasks such as foraging (this assumes however that mortality rates are equal between the two casts; we return to this point in the discussion). Finally, since a hive (managed honey bee colony) is constrained in volume, there is trade-off in space allocation. Beekeepers force honey bees to produce brood only in the brood-chamber, in order to harvest honey in super-chambers.

Therefore, bees have to compromise their space use within the brood-chamber between brood and honey, this later honey being indispensable for colony survival once super-chamber honey has been extracted by beekeepers. We fitted the relationship between honey reserve (i.e., biomass in brood-chamber only) and total brood area (worker + drone) with a GAM. A positive residual value represents a preferential allocation to honey reserve, versus a negative value to brood (see ESM5). Lastly, *Varroa* monitoring being rather empirical and subject to many bias, we first summed the number of dead Varroa for each apiary over the year, and then used for each count a standardized value (the ratio between the count of dead Varroa for that session out of the total annual count). Because the reproductive stage of *Varroa* is the most hurtful for honey bee, we focused on the temporal trend in *Varroa* pressure on worker brood. For this, we fitted the relationship between *Varroa* mortality counts and worker brood area with GAM. *Varroa* pressure was estimated using the residual values, with positive value representing strong *Varroa* pressure (see ESM6).

Statistical analyses

Substantial inter-annual variations in seasonal dynamics of colonies have already been identified (e.g. honey reserves, Odoux et al., 2014), which are likely to be caused by climatic conditions and plant phenology. We corrected for year deviation to the average 1986-2012 in temperature × days (see ESM 2 and Requier et al., in revision) to rescale the seasonal patterns of pollen and nectar harvests for each year. Seasonal patterns of all the six measured parameters were modeled as a function of time also using GAMMs and a Gaussian error structure (Lin and Zhang, 1999). GAMMs are modeling techniques allowing the fit of temporal splines while giving the possibility of taking into account repeated measurements on statistical units in a nested design (i.e. colonies nested within squares and within years). Time was expressed as the Julian date (corrected by annual deviation of climatic condition, see above) from the first of January of each year. Phenological periods were calculated with Apibotanica dataset (www.poitou-charentes.inra.fr/entomologie: Odoux et al., 2011) for rapeseed and sunflower.

Pollen supply depletion was quantified using two estimators, the slope of pollen harvest against time (LMs using *lm* function in *stats* R-package), and the last pollen biomass collected at the end of the pollen depletion period. Since the depletion period lasts more than 50 days, 4 to 5 pollen biomass samples for each apiary were available, allowing reasonable linear regression fitting. The last pollen biomass collected at the end of the pollen depletion period was obtained by using the LM prediction for the last day of depletion. These two pollen depletion estimators (i.e. slope and biomass) were centered-reduced and then reversed for easier interpretation, ranging from 0 to 4, where 0 corresponding to an absence of pollen depletion and 4 to a very high pollen supply depletion (see Fig. 1). Slope was used in order to test the influence of pollen depletion on life history traits before and during depletion period, while biomass was used in order to test for a carry-over effect after depletion period.

We used model selection by Akaike Information Criterion (AIC) from LMs with Gaussian error structure to fit the influence of the landscape parameters on these two pollen depletion indicators (see ESM2). Two landscape parameters in particular were focused, grassland (permanent grasslands) and forest (including hedgerows and forest borders) following previous analyses (Requier et al., in revision). These landscape variables were summed in successive buffers from 500m to 3,000m (i.e. twice the average foraging distance of honeybees for pollen harvest in such landscapes: Steffan-Dewenter and Kuhn, 2003).

Carry-over effects of pollen supply depletion on life history traits were tested using temporal correlations. A very high variability was apparent for the six parameters modeled (see below in Fig. 2). We first grouped the 1,636 colony observations into the 15 sampling dates (see appendix S3), cutting the temporal series into 15-days bins, and each hive observation were grouped and averaged at the apiary level for a given session. For each 15 sampling date we used *LM* regression between the apiary average of residuals from GAMMs (using for seasonal pattern estimation) and the pollen depletion intensity measured as the seasonal trend in pollen harvest per apiary (see Fig. 1). Thus, 50 values were available for each 15 *LM*s. The slopes of *LM* were plotted against sampling date, to infer of carry-over effect, and the p-value and R-square quantified the significance of the effect. Then, GAMs with Gaussian structure error were fitted in order to plot the results. In order to test a possible carry-over effect of pollen supply depletion on colony survival, we distinguished between summer survival (March to October) and winter survival (October year *y* to March year *y+1*).

All statistical analyses were performed using the R environment for statistical computing (R-Development-Core-Team, 2009) version 2.11.1.

Results

Pollen depletion (which occurs between rapeseed and sunflower blooming: Requier et al., in revision), was related to landscape composition, especially the surface of grassland available within 1km around the hive (*LM*, slope = -0.0126, $F_{1,48}$=4.035, p=0.05, Fig. 1b), and to a lower extant the surface of forest available within 500m around the hive (slope = -0.147, $F_{1,48}$=2.655, p=0.1098, Fig. 1c). These two results highlight a trend of decrease the pollen depletion intensity according to the increase of perennial habitats (i.e. grassland and forest) at a local scale (i.e. 1,000m and 500m, respectively).

Seasonal patterns in colony life history traits and allocation trade-offs

Every life history traits showed a seasonal pattern and a significant time effect, rejecting random or uniform temporal variation (see Figs. 2a-c). Worker brood area and the trade-off in brood allocation (*GAMM*, $F_{4,1631}$=189.1, p<0.001 and $F_{5,1630}$=38.12, p<0.001, respectively) reached their maximum early in spring (early May), at the end of rapeseed mass-flowering blooming thus before pollen depletion (Fig 2a & 2d). Then they gradually decreased along the season, but showed a second and sharper peak in early August, with specific allocation toward drone brood. Adult population size and behavioural cast trade-off peaked about one month after the brood area peak ($F_{4,1631}$=77.22, p<0.001 and $F_{2,1150}$=11.88, p<0.001, respectively), i.e. precisely within the pollen supply depletion period (Fig 2b & 2e). In particular, positive residual values were found in June (during pollen food-shortage), suggesting a preferential allocation of adults to in-hive tasks like brood rearing to the expense of foraging activities. Finally, honey reserves and space allocation trade-off showed a similar bimodal pattern ($F_{5,1630}$=238.0, p<0.001, $F_{4,1631}$=156.6, p<0.001, respectively), with a small peak during the oilseed rape blooming period, and a sharper one during the sunflower period (Fig 2c & 2f). The positive values of the residuals suggest preferential allocation to reserve (rather than to brood) in hive brood-chamber, both occurring during the two oilseeds mass-flowering blooming.

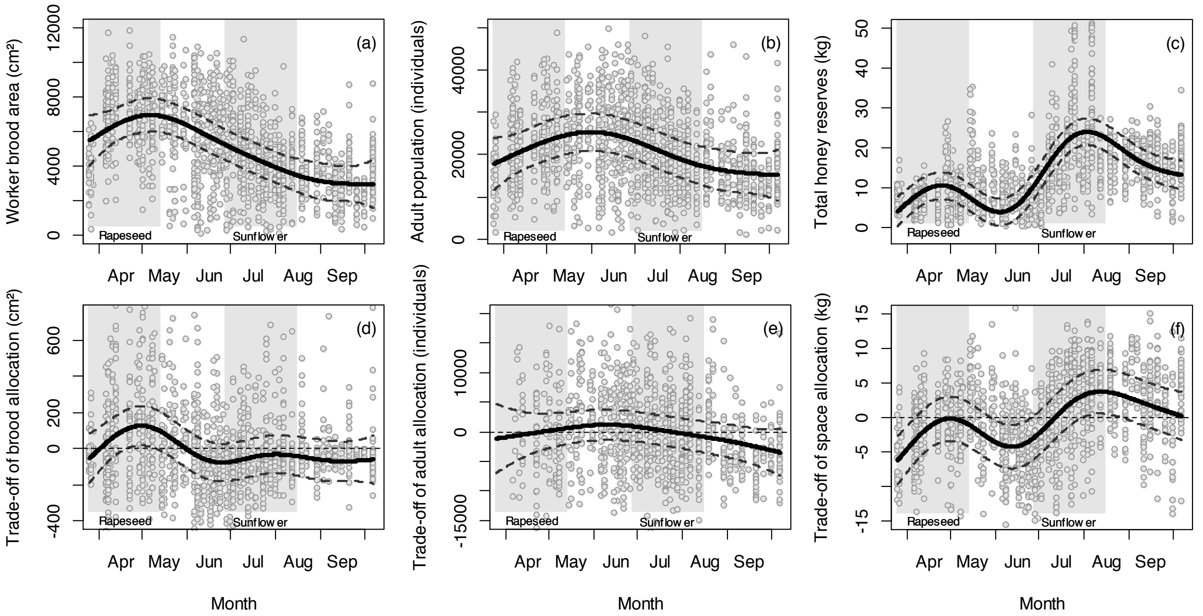

Figure 2 : Seasonal patterns of life history traits of honey bee colonies, (a) worker brood area, (b) adult population size, (c) total honey reserves, (d) trade-off in brood allocation, in which positive values represent preferential allocation to drone brood and negative to worker brood, (e) trade-off in adult allocation, above positive values represent preferential allocation to in-hive tasks and negative to out-hive tasks as foraging, (f) trade-off in space allocation in brood-chamber, where positive values represent preferential allocation to reserve and negative to brood. Each point represents an observed value, and thick line is the fitted model by the *GAMM* (see methods) with dashed lines showing confidence interval (CI 95%), n=1636.

Carry-over effects of pollen-food-shortage

For each of the 11 sampling dates available after the beginning of pollen supply depletion period, the response of worker brood area to pollen depletion varied significantly with time (*GAM*, $F_{4,6}$=11.07, p<0.01, Fig. 3a). During pollen supply depletion (May-June), colonies strongly decreased their brood surface in response to pollen scarcity (positive slope coefficients become strongly, and significantly, negative), i.e. they reacted without time lag to pollen depletion by decreasing reproductive effort. Then, bees apparently gradually compensated during and after sunflower blooming, though no coefficient was significant (Fig. 3a), and there was no evidence of a carry-over effect of pollen depletion in breeding intensity. Conversely, there was a strong signal suggesting a carry-over effect in brood allocation, since bees under severe pollen depletion produced relatively and significantly more drone (males; Fig. 3d). This temporal effect vanished later in the season (Fig. 3d). Another significant carry-over was apparent with the adult population size, with a lagged effect occurring after the sunflower period, i.e. about 2 months after the pollen supply depletion period (Fig. 3b). The reduction in adult population was about 2,000 individuals in August, i.e. a 10% decrease (Fig. 3b).

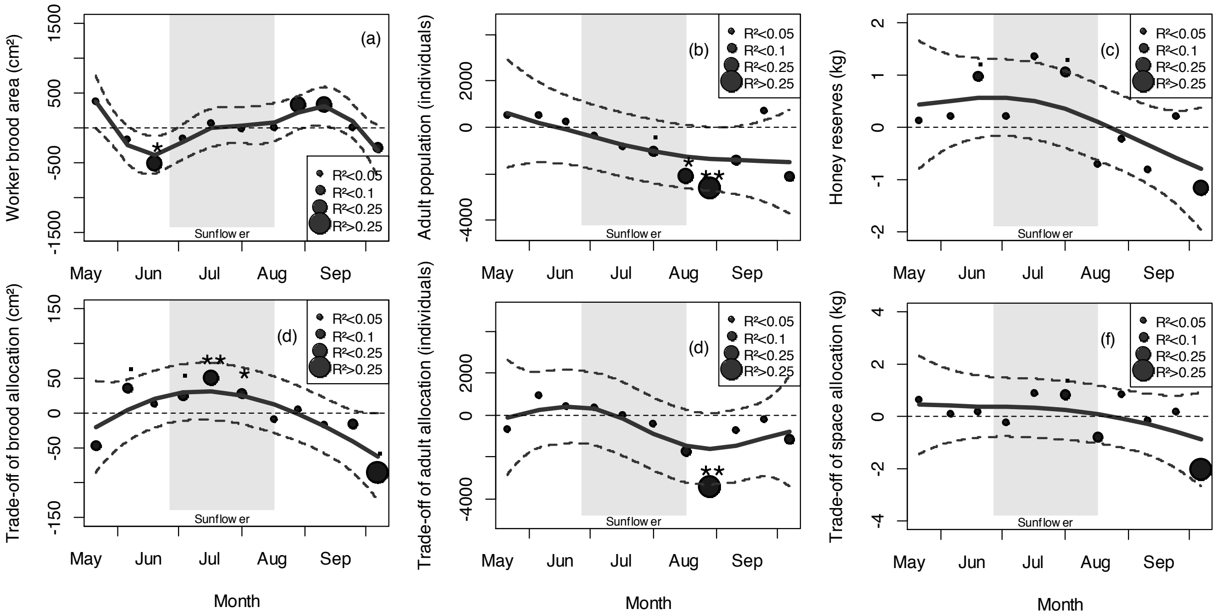

Figure 3 : Adaptation of life history traits in response to pollen supply depletion, (a) worker brood area, (b) adult population size, (c) total honey reserves, (d) trade-off in brood allocation, (e) trade-off in adult allocation, (f) trade-off in space allocation in brood-chamber. Zero horizontal line refers to the seasonal pattern of life history traits, indicates no effect of pollen depletion on life history traits. Positive values (slope coefficient of *LM* linear regression between *GAMM* model residuals (variability, see Fig. 2) of life history traits and pollen depletion intensity) represent an increase of the life history traits in response to pollen depletion occurred in May-June. Each point represents the *LM* for each sampling date, n=11, where the point size referred the R-squared of the model, .p<0.1, *p<0.05, **p<0.01, ***p<0.001. The thick line (temporal correlogram) is the fitted model by the *GAM* (see methods) with dashed lines showing confidence interval (CI 95%).

A similar pattern was observed in the behavioral cast allocation, with over-allocation of adult population to out-hive tasks (such as foraging) in August (Fig. 3e). No significant lagged effect could be detected with regard to reserve quantity or reserve allocation, though in both cases there was suggestion of a carry-over effect since negative temporal trend were observed for the two parameters (Fig. 3c and 3f). In particular, the honey reserve quantity was at a lower level than predicted in October when pollen supply depletion was severe ($F_{2,8}$=3.034, p=0.0997, Fig. 3c). Similarly, there was no indication of any carry-over effect of honey production for beekeepers (honey reserve stocked in super-chambers; $F_{1,48}$=1.237, p=0.2717, see ESM7).

Influence of colony life history on pollen supply depletion intensity

We further analyzed to which extant there was an influence of the life history traits expressed before pollen depletion (i.e., about one month earlier) on the severity of the pollen supply depletion (Fig. 4). A decrease in worker brood production during rapeseed mass-flowering blooming increased the severity of pollen depletion (Fig. 4a), suggesting that hives in which bees invested more in breeding (hence large brood size, and therefore large adult population size 20 days later) endured less severe pollen depletion effects.

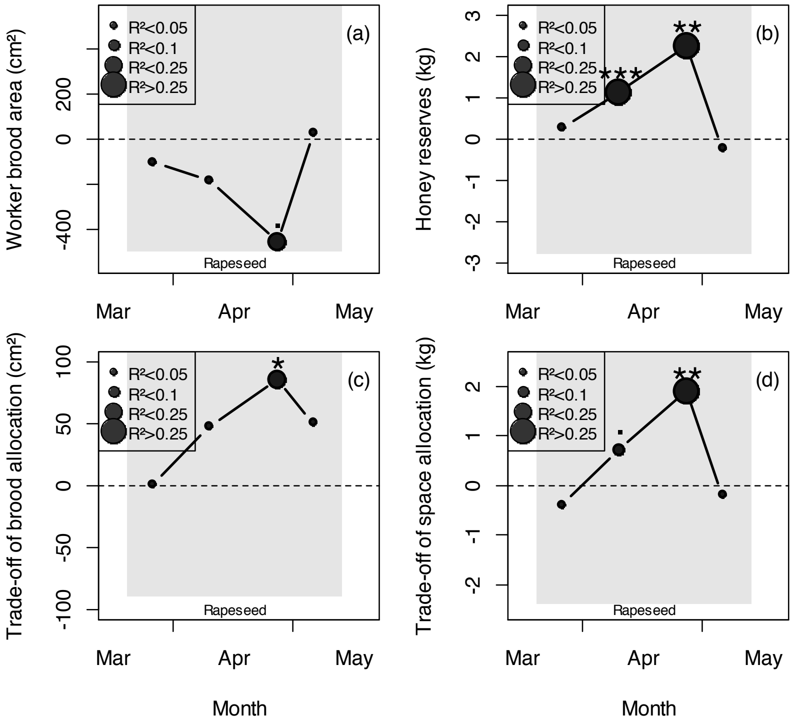

Figure 4 : Influence of colony life history on pollen supply depletion intensity, (a) worker brood area, (b) total honey reserves, (c) trade-off in brood allocation, (d) trade-off in space allocation in brood-chamber. Zero horizontal line refers to the seasonal pattern of life history traits, indicates no effect of life history traits on pollen depletion. Positive values (slope coefficient of *LM* linear regression between *GAMM* model residuals (variability, see Fig. 2) of life history traits and pollen supply depletion intensity) represent an increase of pollen depletion occurred in May-June in response to the previous life history traits. Each point represents the *LM* for each sampling date, n=4, where the point size referred the R-squared of the model, .p<0.1, *p<0.05, **p<0.01, ***p<0.001.

The same result was apparent with drone brood reproduction before pollen depletion: the less they invested in males, the smoother was the pollen supply depletion effect (Fig. 4c). Conversely, colonies which allocated more effort into honey production (food reserves) paid a higher cost, i.e. pollen depletion was more severe (Fig. 4b). This resulted from the fact that such hives stored honey in brood-chamber, therefore to the detriment of brood (Fig. 4d). The cost was even higher for bees that stored honey in super-chambers ($F_{1,48}$=3.958, p=0.05248, see ESM7), since this honey was harvested by beekeepers just before the pollen depletion period, in May. The two latter results suggested that there was a maladaptive trait since investment in reserves at the rapeseed blooming period was paid with cost during pollen depletion.

Seasonal patterns in *Varroa* pressure and influence of pollen supply depletion

Seasonal pattern of *Varroa* pressure showed non uniform pattern of temporal variation (*GAMM*, $F_{3,301}$=36.9, $p<0.001$, n=305, see Fig. 5a), being low until July (where *Varroa* pressure was minimal), then increasing strongly up to October (period of anti-*Varroa* treatment by beekeepers). The *Varroa* pressure was significantly affected by pollen supply depletion with a carry-over effect which occurred after sunflower mass-flowering blooming. This effect was characterized by a significant over-pressure of *Varroa* in colonies with high pollen depletion conditions (*GAM*, $F_{4,6}$=5.834, $p<0.05$, n=11, see Fig. 5b), until anti-*Varroa* treatment by beekeepers.

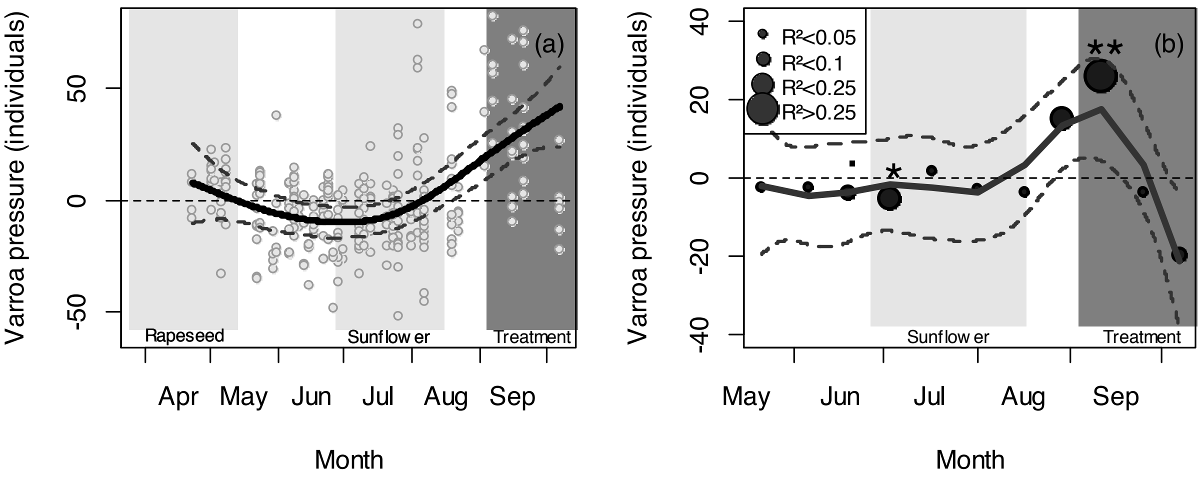

Figure 5 : Seasonal pattern in the pressure of the ectoparasitic mite *Varroa destructor*, (a) seasonal patterns of *Varroa* pressure in honey bee colonies, each point represents an observed value, and thick line is the fitted model by the *GAMM* (see methods) with dashed lines showing confidence interval (CI 95%), n=305. (b) Pollen supply depletion influence on *Varroa* pressure variability, each point represents the *LM* for each sampling date, n=11, where the point size referred the R-squared of the model, .p<0.1, *p<0.05, **p<0.01, ***p<0.001. The thick line (temporal correlogram) is the fitted model by the *GAM* (see methods) with dashed lines showing confidence interval (CI 95%).

Consequence of pollen supply depletion on honey bee colony survival

Colony survival in both summer and wintering was significantly and negatively influenced by pollen supply depletion intensity ($F_{2,238}$=22.86, $p<0.001$, Fig. 6a and $F_{3,195}$=15.53, $p<0.001$, Fig. 6b, respectively). Colony survival was rather high (about 60-80%) when pollen depletion was minimal (about 1.3 in the gradient of pollen depletion, see Fig. 1), but survivorship of colonies gradually decreased up to 20% survival probability when pollen depletion severity increased.

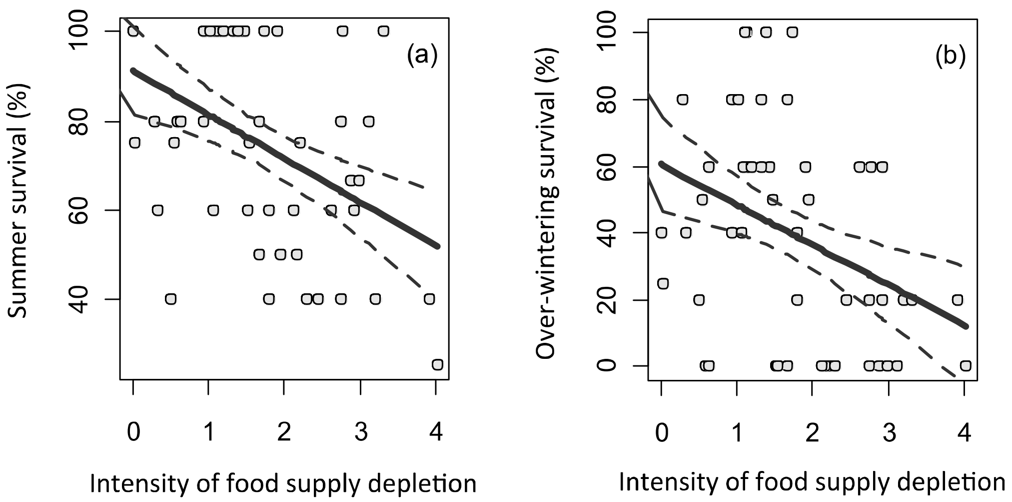

Figure 6 : Consequence of the life history adaptive carry-over effect of colonies in response to pollen supply depletion on (a) summer colony survival (April to October), and (b) over-wintering colony survival (November to March). Each point represents the observed survival of one colony, and thick line is the fitted model by the *GAMM* (see methods) with dashed lines showing confidence interval (CI 95%), n=241.

Discussion

Cascading carry-over effects in response to spring pollen supply depletion

The resource supply and foraging are two critical components driving the life history of any living organism (Stearns, 1989). In agricultural landscapes such as cereal farming systems, the honey bee undergoes a substantial pollen supply depletion during its demographical peak (Requier et al., in revision). Our study presents the first empirical evidence of the suspected impact of the floral resource scarcity on the honey bee in an intensive agricultural landscape (Naug, 2009, Decourtye et al., 2010). Our results support the hypothesis that honey bee colonies adjust their brood surface in response to pollen scarcity (Schmickl et al., 2003, Keller et al., 2005a), and actually, brood was the only life history trait significantly modulated during the pollen depletion. However, the brood constitutes the offspring of the colony and therefore the adult population size of the colony in the future, one or more generations later, and since pollen depletion in this intensive cereal farming system appeared during the peak of brood production (Requier et al., in revision), this presumably adaptive mechanism generated a carry-over effect on the adult population (Mattila and Otis, 2006) from in July but particularly marked in August. In addition, our results highlight a preferential allocation of adult workers to out-hive tasks (i.e., foraging tasks) particularly in August, thus when the adult population was found to be reduced. The adult population is mainly composed of workers – drones representing less than 15% of the total population (Winston, 1994) – and their activity within the colony can be allocated to larval rearing (in-hive task) or foraging (out-hive task, Seeley, 1982). Lifespan of honey bees, (i.e. an average of 7 days to foraging, about 800 km, Seeley and Visscher, 1985, Winston, 1994) is driven by age at first exit from the hive, the shift age between in-hive tasks and out-hive tasks (Feigenbaum and Naug, 2010, Toth et al., 2005). Indeed, honey bee death is almost exclusively related to external tasks, with predation risk and loss by disorientation (Visscher and Dukas, 1997), and to metabolic cost of foraging (i.e. an average of 7 days to foraging, about 800 km, Seeley and Visscher, 1985, Winston, 1994).

The observed preferential allocation of adult to out-hive tasks is thus likely to explain the decrease in adult population size, despite an increasing brood production. Furthermore, this carry-over effect happens just after honey extraction by beekeepers (sunflower honeydew), resulting in a decrease in the abundance of reserves in bee-hives, to which presumable honey bees respond by increasing their foraging effort in order to establish enough reserves before over-wintering (Schulz et al., 1998, Fewell and Winston, 1992). In addition, we detected another carry-over effect in the form of a trade-off in brood allocation. While, as expected, brood allocation was oriented to female workers during pollen scarcity (Wharton et al., 2008), we found that brood was allocated to drones in July (after pollen depletion period but during sunflower flowering). Drones contribute very little to the collective tasks in the colony, but ensure genetic dispersion of the colony through mating with offsite virgin queens (Winston, 1994). This preferential allocation to drone brood, occurring after pollen depletion period, suggests a survival mechanism of colonies in response to pollen depletion, in order to enhance genetic dispersal.

Disruptive adaptation to pollen supply depletion results in increased winter mortality in agricultural landscapes

Overall therefore we provide here strong empirical evidence for a disruptive adaptive mechanism of life history of honey bee colonies in response to pollen supply depletion, which causes a decrease in colony winter survival by carry-over effect of a significant decrease of adult population size and a decrease of honey reserves stored in the hive before the over-wintering period. Since these two traits are keystone correlates of the over-wintering survival of honey bee colonies (Winston, 1994), it is not surprising that we detected strong negative correlation between the severity of pollen depletion in spring and winter mortality of hives.

Furthermore, our results highlighted a strong influence of spring pollen depletion on *Varroa* pressure on the colonies at the end of the season (September). Despite we cannot disentangle whether *Varroa* pressure results from pollen shortage in spring or lower adult population size in august, a clear limit of correlational analyses, our empirical results nevertheless confirm the leading role of *Varroa* in precipitating the death of honey bee colonies (Moritz et al., 2007, Jaffe et al., 2009). Pollen supply depletion could promote the rise of opportunistic parasites and pathogens (Alaux et al., 2010, Mayack and Naug, 2009) and increase the sensitivity to pesticides (Wahl and Ulm, 1983), and therefore our results suggest that the spring pollen supply depletion which occurs in intensive cereal farming systems should be reconsidered into the syndrome of honey bee colony losses.

Implication for honey bee and pollination conservation in agricultural landscapes

Intensive beekeeping practices, which aim to maximize honey production, are tightly associated with intensive cereal farming systems owing to the yearly occurrence of two major honeyflows, i.e. rapeseed and sunflower (Louveaux, 1996, VanEngelsdorp and Meixner, 2010). Flowering of rapeseed happens early in the breeding season. Therefore local intensive beekeeping practices have custom to boost the colonies in order to promote the rapeseed honey production. However, the honey bee colonies favoring reserve storage at the expense of brood production during rapeseed will be more likely to experience difficulties during pollen food-shortage, and by carry-over effect, these colonies will produce less honey during sunflower and their winter survival probability will decrease. Our results therefore question to some extant beekeeping management practices in such systems, such as extracting honey in early spring (after rapeseed flowering), feeding the colonies, or multiplying livestock by dividing the colonies.

Alternatively, one may improve honey bee flower resource availability in such farmland landscapes, through a number of forms, such as restoring and promoting resource-rich semi-natural habitats, fallows and field margins sown with attractive flower mixes (Haaland et al., 2011, Wratten et al., 2012). Agri-Environmental Schemes (AES) implementation may be a satisfactory solution to the lack of food resources for honey bees in agricultural landscapes (Decourtye et al., 2011), with demonstrated effectiveness for wild bees and bumblebees (Sepp et al., 2004, Kleijn et al., 2004, Kleijn and Sutherland, 2003), but strong decrease in herbicide use may be an interesting alternative or complement since it will improve the availability of weeds. Weeds were found to account for a substantial part of the honey bee diet in this environment because their flowering diversity peak coincides with the food-shortage period, and species like poppy can make up to 60% of harvested pollen brought back to hive in June (Requier et al., in revision). Weed conservation could be possible by relaxing the use intensity of herbicides, as supported by the development of national action plans required by new European legislation on pesticides (Barzman and Dachbrodt-Saaydeh, 2011). These promising solutions are further consistent with the conservation of other groups involved in ecosystem services such as pest predator insects (Bianchi et al., 2006) and of the rare plant species themselves as well as their pollinators (Gibson et al., 2006).

Since we found that pollen food-shortage intensity tended to decrease with the local amount of grasslands (perennial grasslands into 1,000m) and forested elements (forest border and hedgerows within 500m), improving such elements in the landscape is an alternative solution to classical AES. Increasing semi-natural elements and their associated flora may strongly contribute to honey bee diet since they need a wide diversity of plant species which semi-natural habitats are able to provide (i.e., hedgerows, forest edges, meadows and roadside strips, Requier et al., in revision). In addition, a diversified pollen diet might help bees to reinforce immunocompetence against pathogens (Di Pasquale et al., 2013, Alaux et al., 2010). Moreover, protecting and restoring semi-natural habitats and plant diversity is also consistent with the conservation of native bees (wild bees and bumblebees) and can enhance overall biodiversity and ecosystem services (Wratten et al., 2012, Nicholls and Altieri, 2013).

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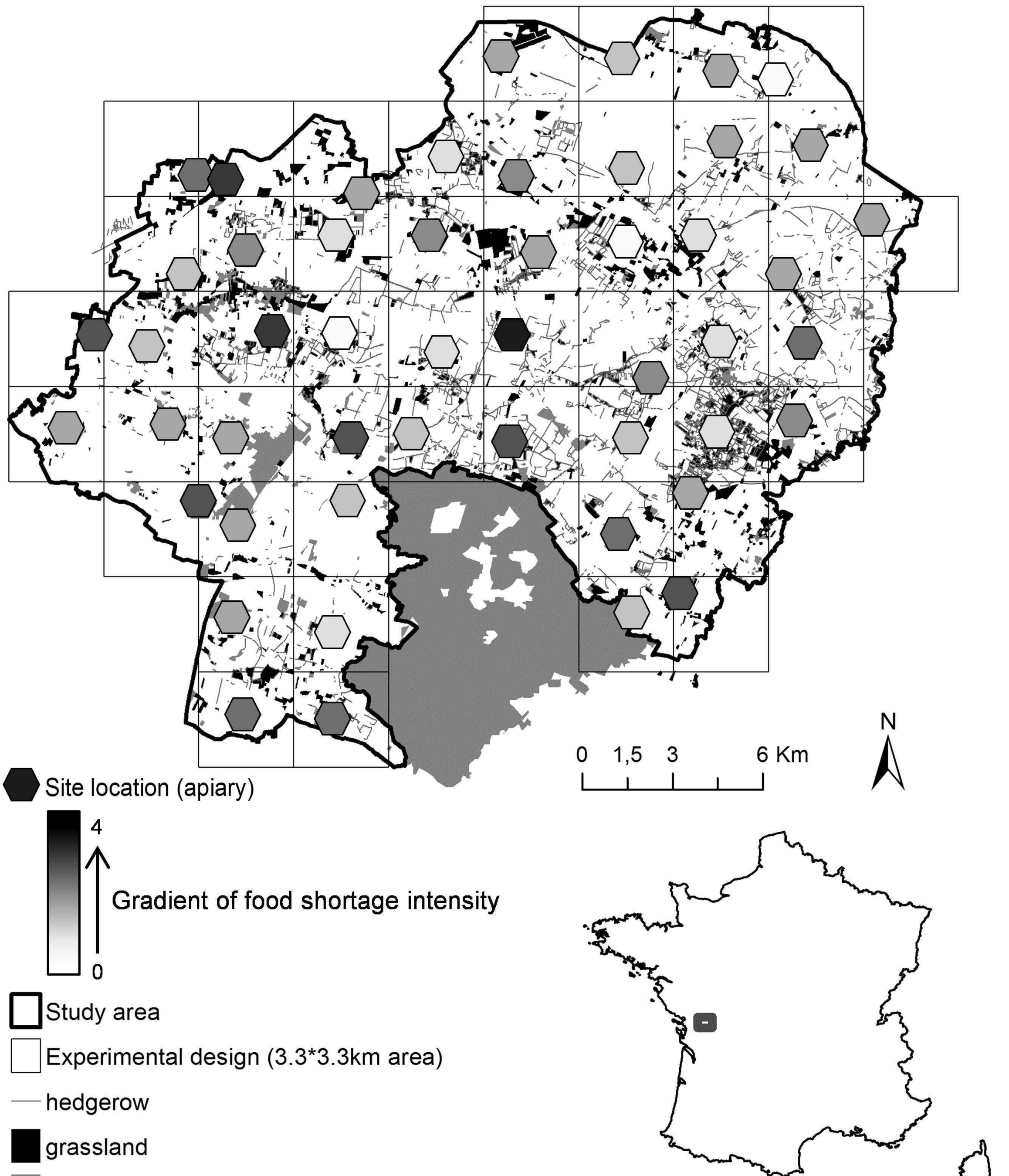
Supporting Information

Appendix S1: The study area and experimental design, the LTER "Zone Atelier Plaine et Val de Sèvre", France, showing location of the 50 squares (apiaries) and their respective pollen food-shortage intensity (gradient black and white). The main habitat types which contrast pollen food-shortage were presented, such as grassland (in gray) which consists of permanent grasslands, and forest (in black) which includes uniquely hedgerows and forest border.



Appendix S2:

Annual deviation of climatic condition

Substantial inter-annual variations in seasonal dynamics of colonies have already been identified (e.g. honey reserves, Odoux et al., 2014), which are likely to be caused by climatic conditions and plant phenology. The cumulative sum of temperatures above 0°C was used for each year between 2008 and 2012 to account for the potential effect of annual climatic conditions, as this parameter is well known to directly influence plant flowering phenology (Price and Waser, 1998, Cleland et al., 2007). The same calculation was made for the period 1986-2012 in order to derive an average value. We calculated the temperature × days dynamic for each year as for the average. The day difference of a given year to the average (i.e., the residual) provided a proxy of year deviation compared to mean (see appendix S3 in Requier et al., in revision), and we used this proxy as an annual phenological index in order to rescale the seasonal patterns of pollen and nectar harvests.

Estimation of harvested pollen

Harvested pollens from the five hives of an apiary were pooled for a given collection, and thus a total of 780 samples were available. Pollen traps were set only after ensuring that the queen was laying eggs and when weather conditions allowed foraging, i.e. when outside temperature was above 12°C. Pollen traps consist of a grid with three lines of 5.3 mm diameter holes through which foragers are forced to crawl and may lose their pollen loads. Such pollen traps retain about 10±5% of all the harvested pollen brought back to the hive (Lavie and Fresnaye, 1963), and we assumed it reflected pollen composition of daily pollen harvest, and, given that the retained percent is stable, also the weight of the harvest. From each sample, exogenous impurities were removed and samples were grouped by square and date, oven dried for 48-h at 45°C and weighted (Sartorius balance, d=0.01g).

Pollen food-shortage characterization with (figure 1) the correlation between the two estimators, depletion slope and last biomass of pollen, (table 1 & 2) the results of the influence of landscape composition on pollen depletion

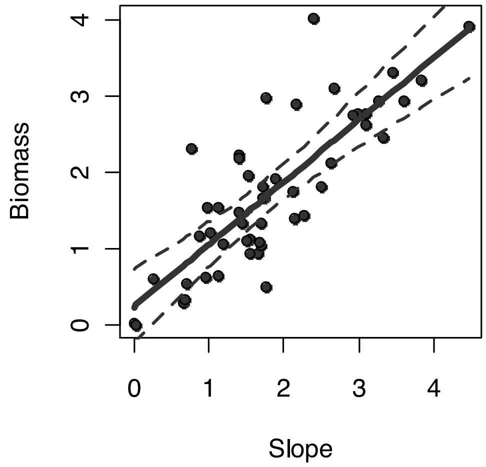


Figure 1 : Linear regression *LM* between slope of pollen depletion and last biomass of pollen depletion (*LM*, $F_{1,48}$=96.36, p<0.001). Each point represents the observed pollen food-shortage value, and thick line is the fitted model by the LM with dashed lines showing confidence interval (CI 95%), n=50.

Model_i	K_i	L_i	AIC_i	Δ_i (AIC)	w_i (AIC)
Grassland within 1,000m	3	1	143.3699	0	0.10
Grassland within 1,500m	3	0.56	144.5216	1.15	0.06
Forest within 1,000m	3	0.46	144.9264	1.56	0.05
Null model	2	0.41	145.1390	1.77	0.04

Table 1 : Landscape influence on the slope of pollen depletion, results of AIC Analysis for model selection (LM), L *i* = maximum likelihood for model *i*, Δ *i* (AIC) = [AIC*i* – min(AIC)], K *i* = number of estimated parameters for model *i*, *wi* (AIC) = the rounded Akaike weights, n=50.

Model_i	K_i	L_i	AIC_i	Δ_i (AIC)	w_i (AIC)
Forest within 500m	3	1	144.71	0	0.08
Null model	2	0.81	145.14	0.42	0.06

Table 2 : Landscape influence on the last biomass of pollen depletion, results of AIC Analysis for model selection (LM), L *i* = maximum likelihood for model *i*, Δ *i* (AIC) = [AIC*i* – min(AIC)], K *i* = number of estimated parameters for model *i*, *wi* (AIC) = the rounded Akaike weights, n=50.

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Appendix S3: The number of data samples for (table 1) the life history traits observed in honey bee colonies, and (table 2) the *Varroa* pressure

	2008	2009	2010	2011	2012	Total
Rapeseed		**97**	**100**	**54**	**108**	**359**
1					40	40
2		50	30	27	30	137
3		30		27	11	68
4		17	70		27	114
Food shortage	**73**	**82**	**88**	**104**	**65**	**412**
5		22	29	42	17	110
6	39	28	30	31	24	152
7	34	32	29	31	24	150
Sunflower	**112**	**110**	**89**	**121**	**87**	**519**
8	39	30	15	30	31	145
9	52	30	29	30	26	167
10	21	50	45	61	30	207
Pre-wintering			**79**	**139**	**128**	**346**
11			15	30	28	73
12			15	15	30	60
13			49	14	30	93
14				31	40	71
15				49		49
Total	**185**	**289**	**356**	**418**	**388**	**1636**

Table 3 : Number of data samples for life history traits observed in honey bee colonies, according years and sampling sections

	2008	2009	2010	2011	2012	Total
Rapeseed	**17**	**42**	**40**	**9**		**108**
1		8	5			13
2		15	12			27
3		19	5			24
4	17		18	9		44
Food-shortage	**51**	**28**	**50**	**44**		**173**
5	14	10	17	9		50
6	18	1	17	15		51
7	19	17	16	20		72
Sunflower	**61**	**70**	**39**	**47**		**217**
8	17	21	18	20		76
9	22	21	9	10		62
10	22	28	12	17		79
Pre-wintering	**70**	**48**	**31**	**39**		**188**
11	27	29	10	29		95
12	43	19	21	10		93
Treatment	**58**	**45**	**45**	**40**		**188**
13	10	10	27	10		57
14	28	27	18	20		93
15	20	8		10		38
Total	**257**	**233**	**205**	**179**		**874**

Table 4 : Number of data samples for *Varroa* pressure, according years and sampling sections

Appendix S4: Life history traits of honey bee colonies

Brood area

Brood area, on both side of each brood frame, were assimilated to ellipses (Fresnaye, 1961, Mallet and Charles, 2001) and therefore approximated using length L and width W measurements (cm) following the formula $S\ (cm^2) = ¼\ (\pi \times L \times W)$. Brood surfaces were then added across all the frames of each colony. Isolated empty cells within brood surfaces were ignored. Honey bee colonies could operate a trade-off in sexual brood reproduction, with especially a preferential allocation to worker brood in food-shortage conditions, regulated by a cannibalism behavior significantly oriented to drone brood (Wharton et al., 2008). So, each of the 1,636 observations included one measure of worker brood area and one other measure for drone brood area, in order to evaluate the trade-off in brood allocation (see Statistical analyses section for method).

Adult population

The adult population size (number of adult individuals) was assessed by weighting each brood frame with and without bees, as well as the hive bottom and the honey super-chambers. Adult population size was then assessed considering an average of 10-4 kg/bee. The resulting values slightly underestimated real adult population size because it did not take into account the portion of foraging bees that had left the colony at the time of measurement. Indeed, adult population included dominantly worker bees, but also a few number of drone individuals (<15% maximum, Winston, 1994). Each worker bee is able to perform many tasks into the colony, including larval rearing and foraging. However the polyethism-age theory predicts that a same bee cannot perform simultaneously brood rearing and foraging (Seeley, 1982). Therefore, there is a trade-off in adult allocation between these two main tasks (see Statistical analyses section for the evaluation method).

Honey reserve

Honey reserve mass includes the supply of honey, nectar, and pollen. For each hive, frames were weighted without honey bees both from the main brood-chamber and the honey super-chambers, as the difference between total frame mass and the mass of empty frames before introduction in the hive. Whenever necessary, brood mass was also removed from the resulting mass difference. Brood mass m values were derived from brood surfaces S, using the formula m (kg) = mmax × S / Smax, Smax indicated total frame surface (1,632 cm^2) and mmax represented the total brood mass expected for a frame surface entirely covered by brood (0.6385 kg, predicted from the linear regression between 6,000 brood surface values and the corresponding full-empty frame mass differences, Odoux et al., 2014). Similar to local beekeeping techniques, honey reserves in super-chambers were extracted twice a year, just after the rapeseed and sunflower honeyflows (May and August, respectively).

Colony survivorship

An important parameter in the life history trade-off of any organism is its survival, and each adaptive mechanisms of life history are able to modulate the probability of survival (Stearns, 1989). In order to investigate the potential impact of the adaptive evolutionary mechanisms of the honey bee colonies in front of pollen food-shortage, we have monitored the survivorship of 241 colonies (not all the 257 previously stated because of methodological limit of survival classification). For this we have informed at each the 1636 colony observations if the honey bee colony were dead or alive. We considered a dead colony when the hive is empty or if the colony is queenless drone laying (i.e. without queen and no possibility to supersedure colony reproduction).

Varroa pressure

The life cycle of *Varroa* consists of a female phoretic stage as a parasite on adult bees and a reproductive stage inside the sealed brood cells of the bee larvae/pupae (Martin, 1998). The reproductive stage is the most problematic stage of *Varroa* parasitism in honey bee because of the transmission of several viruses which modulated dramatically life history of bees (e.g. deformed wing virus, DWV, Martin et al., 2010). We used a usual method used by beekeepers in order to monitor the growth dynamic of *Varroa* into honey bee colonies. At the beginning of the season, we placed a sticky board in the bottom of one of the five hives per square, and every two week, we counted all the fallen *Varroa* (mortality). Therefore, 878 data samples were collected according to this systematic counting of *Varroa* adult mortality (colonies monitored in 2012 do not be sampling, see appendix S3 for the complete data sampling). This *Varroa* monitoring informs of the abundance of *Varroa* presence into the honey bee colony, such as a parasitic pressure on host. Similar to local beekeeping techniques, an anti-*Varroa* treatment were applied into each honey bee colonies, after sunflower honeyflow (September).

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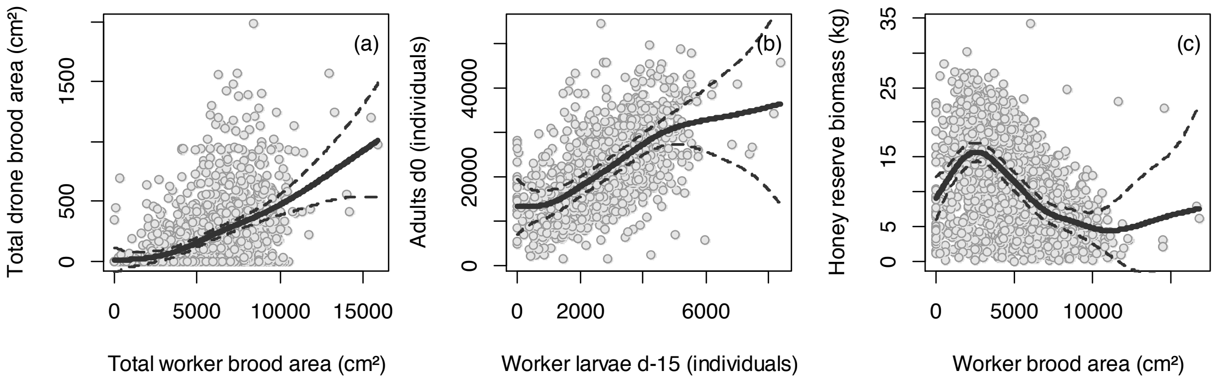
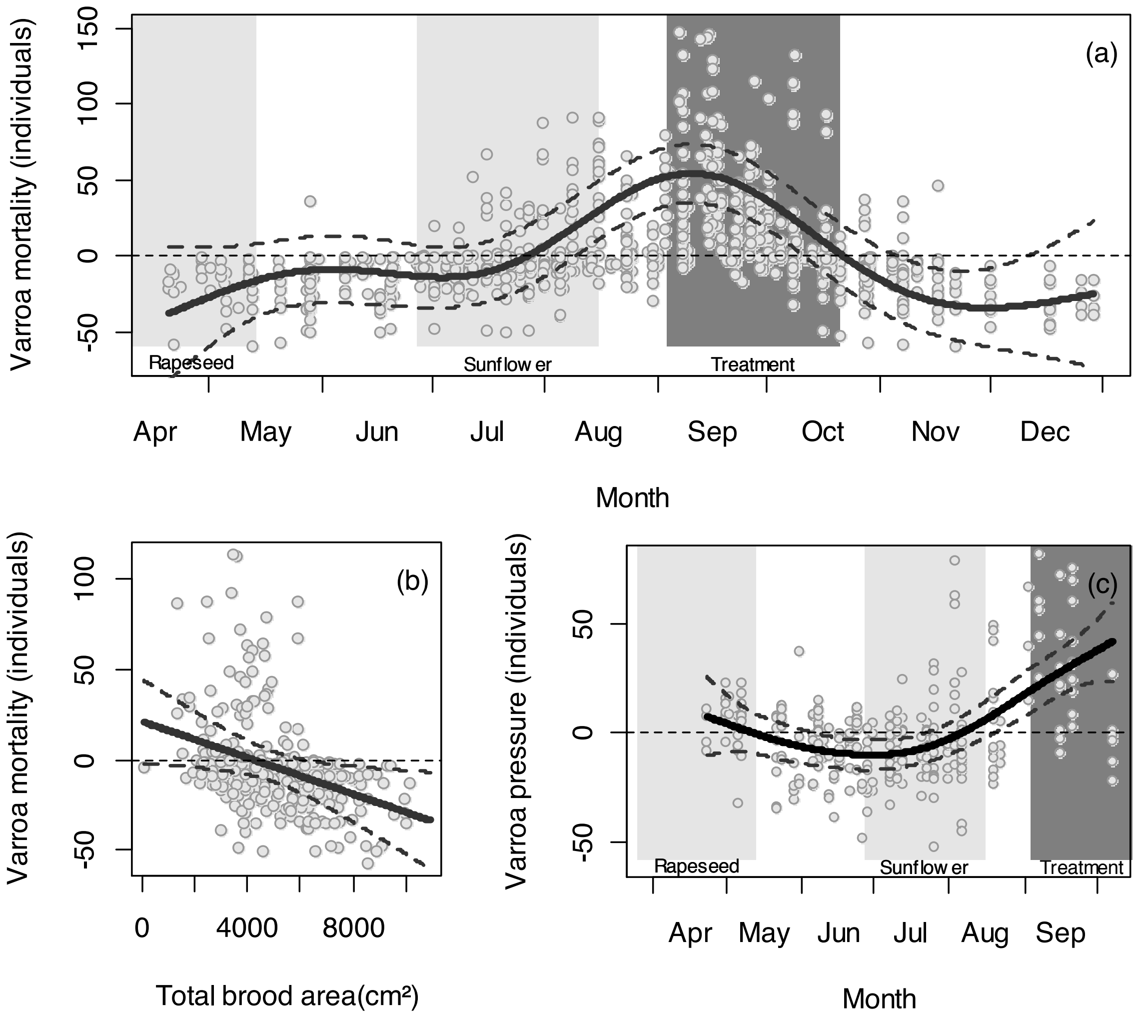
Appendix S5: Estimation of the life history trade-offs in honey bee colonies: (a) trade-off in brood allocation, (b) trade-off in adult allocation, (c) trade-off in space allocation



(a) Trade-off in brood allocation (*GAM*, $F_{4,1630}$=112.9, p<0.001)
(b) Trade-off in adult allocation (*GAM*, $F_{4,1148}$=207.7, p<0.001)
(c) Trade-off in space allocation (*GAM*, $F_{6,1629}$=83.47, p<0.001)

Appendix S6: *Varroa* pressure: (a) seasonal pattern of *Varroa* mortality, (b) estimation of *Varroa* pressure on honey bee brood, and (c) seasonal pattern of *Varroa* pressure



(a) (*GAM*, $F_{4,1631}$=156.6, p<0.001)
(b) (*LM*, $F_{1,303}$=49.1, p<0.001)
(c) (*GAMM*, $F_{3,301}$=36.9, p<0.001)

Appendix S7: Honey production by beekeeping and pollen food-shortage

The storage of honey reserves in super-chambers has a very high trend to increase the intensity of food-shortage in pollen (*LM*, $F_{1,48}$=3.958, p=0.05248, see Fig. a). These honey reserves will be harvested by beekeepers just before the pollen depletion period, in May. Pollen food-shortage does not have any significant carry-over effect on honey production for beekeeping industry (honey reserve stocked in super-chamber), only a slight trend in decrease of honey production is visible (*LM, $F_{1,48}$*=1.237, p=0.2717, see Fig. b).

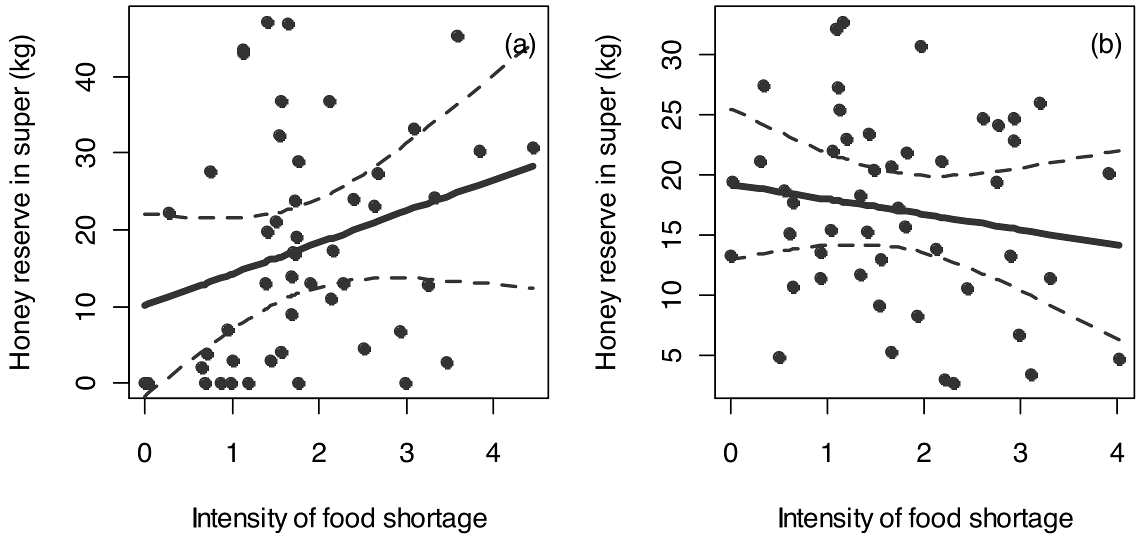


Figure: Linear regression *LM* between honey storage in super for beekeeping industry and pollen food-shortage intensity, (a) in spring during rapeseed mass-flowering blooming, (b) in summer during sunflower mass-flowering blooming. n=50.

DISCUSSION

Synthèse et discussion

Cette thèse présente des résultats originaux sur l'écologie de l'abeille domestique en paysage céréalier intensif. Les principaux résultats (résumés dans la figure 1 ci-dessous) démontrent, pour la toute première fois de manière empirique, les conséquences de l'intensification de l'agriculture et de la dynamique des ressources florales sur l'approvisionnement alimentaire et l'histoire de vie des colonies d'abeille domestique. En effet, les dynamiques du régime alimentaire en pollen et en nectar suivent un patron temporel bimodal comprenant une période de déplétion d'approvisionnement. De façon très surprenante, le patron bimodal en pollen n'est pas dirigé uniquement par la floraison massive des cultures de colza et de tournesol (ce n'est pas le cas en début de saison avec le colza, Fig. 1a), ces cultures oléagineuses représentent moins de 30% du butin annuel pollinique (Fig. 1b), contrairement au nectar dont la bimodalité est dirigée exclusivement par ces deux cultures, qui à elles seules représentent 80% du butin annuel. En effet, ce sont les espèces semi-naturelles qui contribuent de façon dominante au régime alimentaire pollinique (plus de 60% du butin annuel, Fig. 1b) et garantissent l'apport de ressource de haute qualité nutritionnelle. De plus, ces espèces sont utilisées avec une très importante richesse et diversité florale, en relation avec la composition et la structure paysagère environnant les ruchers. Deux types de ressources d'une importance inattendue ont un rôle prépondérant dans le régime alimentaire pollinique, à savoir les ressources arborées (forêts, lisières forestières et haies) en début de saison pendant le démarrage des colonies, et les adventices des cultures (comprises dans les plantes herbacées, Fig. 1b) garantissant l'aquisition pollinique des colonies en période de déplétion d'approvisionnement alimentaire. Cette période de déplétion d'approvisionnement alimentaire printanière (mai-juin) apparait par ailleurs pendant le pic démographique des colonies.

La seconde étude démontre les conséquences reportées de cette déplétion d'approvisionnement alimentaire pollinique printanière sur l'histoire de vie des colonies. En effet, lors de la restriction en pollen, les mécanismes adaptatifs des colonies opèrent un compromis d'allocation des ressources entre la survie (réserve) et la croissance (couvain), favorisant le maintien des réserves au détriment du couvain. Cette régulation de la consommation engendre des coûts reportés en chaine sur les différents traits de vie de la colonie. Tout d'abord une répercussion reportée sur un affaiblissement de la population adulte, résultant de la diminution de la progéniture, puis une répercussion reportée sur la quantité de réserve, résultant de la diminution de la population butineuse. Cependant, ces deux traits d'histoire de vie (réserve et population adulte) sont déterminants pour la survie de la colonie pendant l'hivernage. Finalement, la déplétion d'approvisionnement alimentaire pollinique printanière diminue le taux de survie des colonies, pendant la saison de développement des colonies, mais également pendant l'hivernage (Fig. 1c). La restriction de disponibilité florale dans les paysages céréaliers semble être perçue par les colonies d'abeilles comme une disette alimentaire affaiblissant leur fitness, phénomène sans doute lié aux syndromes de dépopulation des colonies d'abeilles.

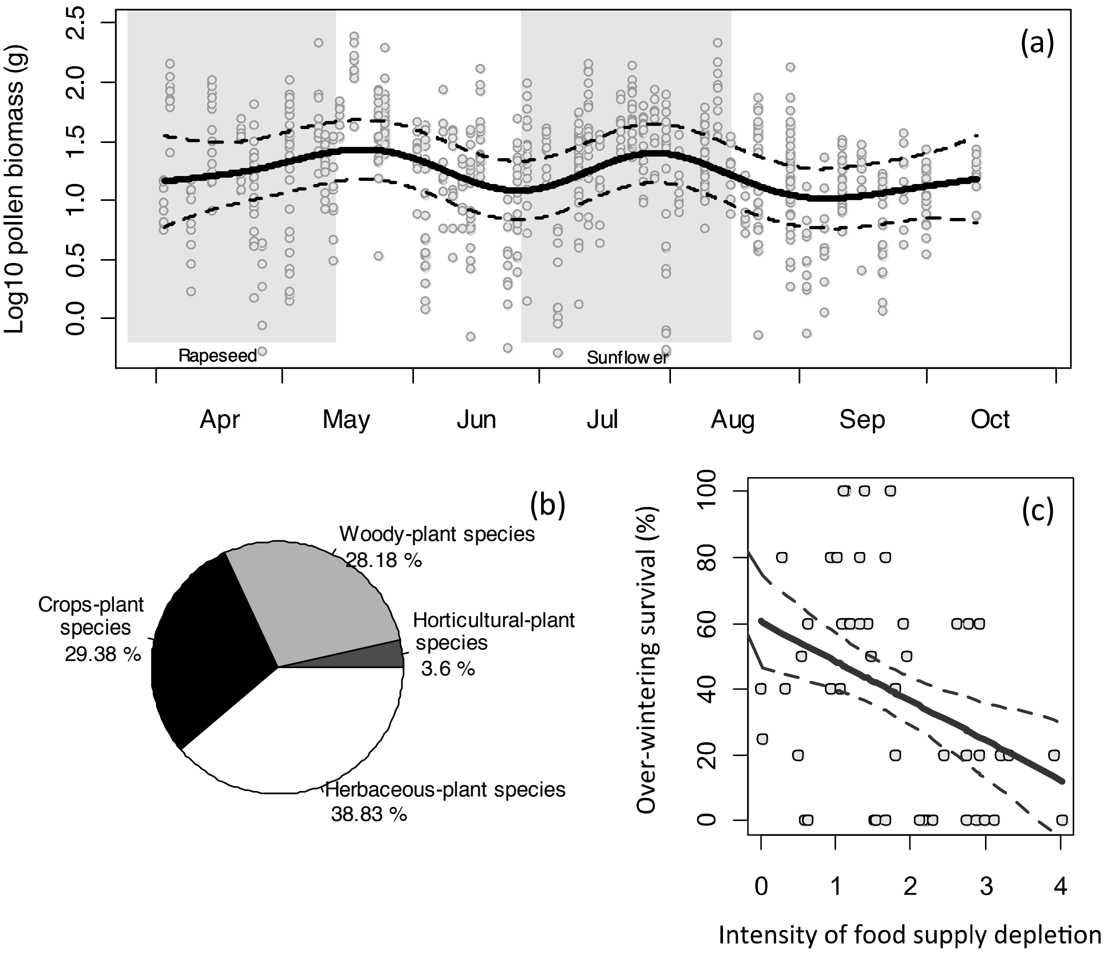


Figure 1 : Principaux résultats de la thèse, (a) dynamique temporelle de récolte de pollen, (b) origine botanique du butin annuel pollinique, (c) conséquence de la disette pollinique printanière sur le taux de survie des colonies pendant l'hivernage.

Limites de cette étude

La première inévitable question que l'on peut se poser, comme dans toute étude empirique, provient du caractère unique du site d'étude. En effet, notre étude n'a pas été répliquée spatialement à proprement dit, les 50 sites d'étude pouvant être interprétés comme des pseudo-réplicas spatiaux au sein d'une seule vaste zone d'étude. L'important investissement technique et humain pour la réalisation de ce suivi à grande échelle spatiale et temporelle rend sa réplication difficile, d'où son caractère pionnier, fournissant des résultats empiriques particulièrement originaux. Cependant, la large dimension de la zone d'étude (à savoir 450km^2), typique des paysages céréaliers intensifs de France et comprenant une importante variabilité de configuration paysagère, nous permet d'envisager le caractère générique de nos résultats.

La Zone Atelier « Plaine et Val de Sèvre » héberge depuis une vingtaine d'années de nombreuses études scientifiques sur de nombreux taxons (plantes, insectes, oiseaux), qui ont toutes révélé une étonnante richesse spécifique. Pour exemple, un inventaire des abeilles sauvages a été réalisé entre 2010 et 2012 selon le même design spatial que notre étude, révélant la présence de 191 espèces, richesse totale estimée à 250 espèces par l'indice de

Chao (sur un total de 45,040 abeilles enregistrées sur 1,286 sites échantillonnés), sur les 1000 espèces vivant en France (Rollin et al., 2013). Dans la continuité de cette étude, j'ai également pu mettre en place des inventaires botaniques afin d'évaluer la richesse et diversité floristique disponibles essentiellement dans les habitats semi-naturels (bord de route, prairies, haies et lisières forestières), révélant également une étonnante richesse floristique de 332 espèces botaniques à fleur d'un potentiel intérêt pour l'abeille (provenant de 500 inventaires botaniques, 317 en 2011 et 183 en 2012, travaux réalisés dans le cadre de cette thèse). D'autres inventaires d'adventices au sein des parcelles agricoles confirment ce caractère conservé de la Zone Atelier « Plaine et Val de Sèvre », avec la présence de plus de 250 adventices (Meiss et al., 2010). La très grande proximité entre les chercheurs écologues et les agriculteurs sur cette Zone Atelier n'est sans doute pas étrangère à cette richesse biologique, après plusieurs dizaines d'années de sensibilisation aboutissant à des pratiques agricoles plus raisonnées, favorable à la biodiversité en milieu agricole.

Ce constat est d'ailleurs susceptible de sous-estimer le réel manque de ressource alimentaire pour l'abeille en paysage agricole, en prenant en exemple le caractère encore plus intensif des paysages agricoles de Beauce, en France. Il serait donc fortement souhaitable de répliquer ce monitoring de suivi des colonies dans d'autres localités. Dans ce sens, une étude a été menée entre 2010 et 2012 au sein des paysages agricoles du Gers (32, France), suivant un monitoring des colonies très similaire à celui présenté dans cette thèse. Malgré un effort d'échantillonnage nettement inférieur à celui de notre étude (à savoir 6 ruchers et 43 ruches), les conclusions de cette étude dans le Gers tendent à confirmer nos observations sur l'utilisation des ressources et la dynamique du régime alimentaire. En effet, Rhone et al. (in press) observent également une période de restriction alimentaire en paysage agricole sur les colonies d'abeilles domestiques, coïncidant temporellement avec nos résultats (à savoir Mai-Juin). Cette étude confirme le rôle prépondérant des ressources arborées et des adventices dans le régime alimentaire des colonies d'abeilles, permettant de garantir une importante diversité et qualité nutritionnelles, mais permettant également d'offrir un approvisionnement pendant la restriction alimentaire.

Disponibilité et sélection des ressources florales

Notre évaluation de l'utilisation de l'habitat par les abeilles est basée sur l'étude du régime alimentaire. Par conséquent, il aurait été très intéressant de mettre en relation la disponibilité des ressources autour des ruchers avec la collecte de celles-ci. Autrement dit, la collecte d'une ressource par les colonies d'abeilles peut-elle être simplement expliquée par la disponibilité de cette ressource dans l'environnement des colonies ou, de manière alternative, les abeilles pratiquent-elles une sélection des ressources? La sélection des ressources, en ce qui concerne l'abeille domestique, est une question complexe et délicate à analyser au vu des caractéristiques biologiques de cette espèce (mais voir Visscher and Seeley, 1982, Houston et al., 1988, Van Nest and Moore, 2012). En effet, les colonies d'abeilles domestiques, avec leurs 2,000 butineuses journalières (Seeley, 1985), un rayon de butinage de plus de 2km en moyenne (Visscher and Seeley, 1982, Beekman and Ratnieks, 2000, Steffan-Dewenter and Kuhn, 2003), et plus de 15 espèces polliniques collectées journellement, jusqu'à 271 espèces annuellement, rendent l'étude de la stratégie de butinage très complexe méthodologiquement.

Cependant, connaitre la stratégie de butinage des abeilles est indispensable pour pouvoir concevoir des mesures de gestion afin de ré-enrichir le paysage en ressources florales pour l'abeille (Decourtye et al., 2010). Dans un nouvel article en préparation, nous avons étudié la stratégie de butinage des abeilles, sur deux espèces clés dans l'alimentation pollinique des colonies, à savoir le coquelicot *Papaver rhoeas* et le bleuet *Centaurea cyanus*, dont nous connaissions la distribution exhaustive dans toute la Zone Atelier. Sous l'hypothèse d'un compromis de sélection entre la distance et la quantité de la ressource, basée sur le caractère de 'central-place forager' et de 'optimal foraging' de l'abeille domestique (Van Nest and Moore, 2012), nous avons testé la corrélation entre la biomasse collectée et la quantité disponible (nombre de patchs) pour chaque disque de distance (de 100m à 5,000m par pas de 100m) autour des 50 ruchers (Fig. 2a).

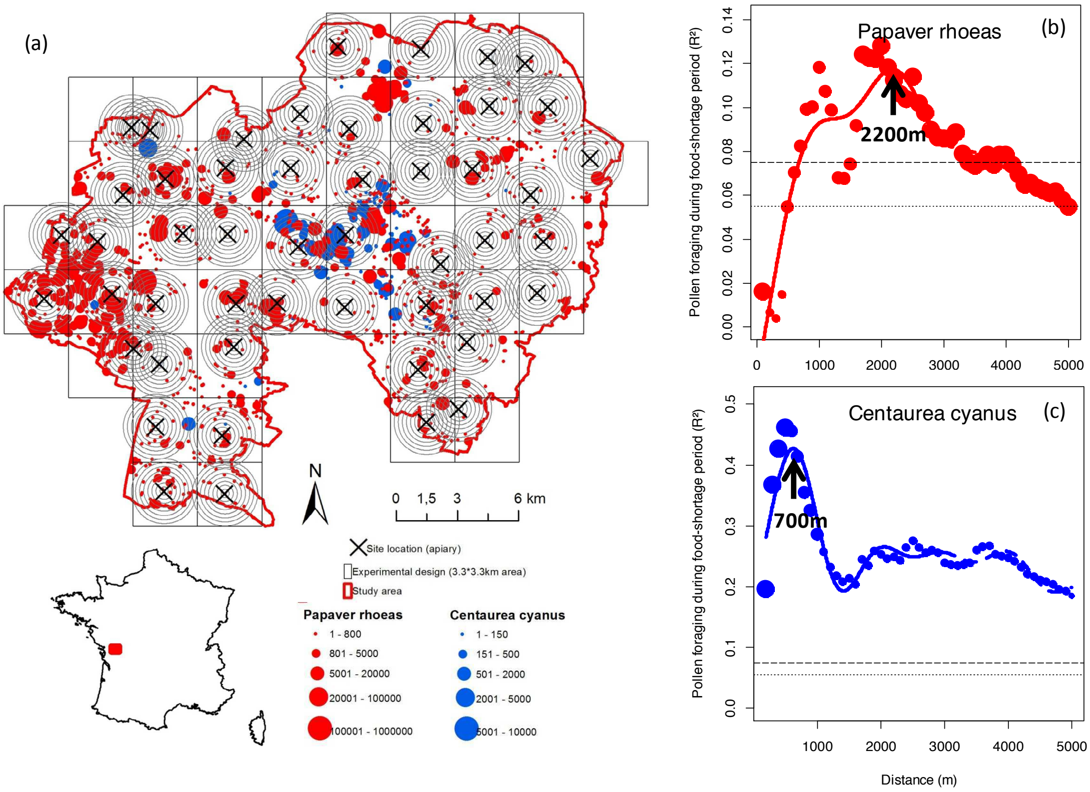


Figure 2 : Sélection des ressources par l'abeille domestique selon un compromis distance-quantité, (a) distribution spatiale des ressources coquelicot et bleuet, résultats des corrélations récolté-disponible par pats de distance pour (b) le coquelicot et (c) le bleuet. Les lignes horizontales renseignent les seuils de significativité des corrélations, ---<0.05, …<0.1. Chaque point résulte du R^2 de la corrélation entre le nombre de patchs disponibles (transformé en log10) et la biomasse de pollen maximale collectée (transformé en log10) par pat de distance, n=50. La taille des points renseigne sur la valeur de la pente de corrélation (toutes positives, variant de 0.13 à 0.83). Le patron du corrélogramme de distance est représenté par un GAM, n=50.

Les résultats de cette étude montrent, lors de la disette alimentaire printanière, que la corrélation maximale pour le coquelicot intervient aux environs de 2,200m (*GAM*, $F_{7,42}$=25.49, p<0.001, Fig. 2b), et à 700m pour le bleuet (*GAM*, $F_{8,41}$ =31.8, p<0.001, Fig. 2c). Etonnamment, les distances les plus proches des ruchers ont des coefficients de corrélation moindre dans la relation disponible-récolté, confirmant que les abeilles butinent rarement à proximité de leur nid (Seeley, 1985, Beekman and Ratnieks, 2000). En tenant compte de l'estimation moyenne de la distance de butinage des abeilles de 2km (Visscher and Seeley, 1982, Beekman and Ratnieks, 2000, Steffan-Dewenter and Kuhn, 2003), nous constatons que le bleuet est sélectionné à une distance nettement inférieure : 700m. Le bleuet serait donc utilisé uniquement s'il est proche des colonies. A l'inverse, le coquelicot présente une distance de sélection optimale de 2,200m, renforçant le rôle de cette ressource pour l'abeille par ce caractère sélectif à longue distance.

Histoire de vie des abeilles

Nous avons mis en avant un mécanisme adaptation complexe des colonies d'abeilles domestiques en réponse à déplétion d'approvisionnement en pollen. Cette adaptation a engendré son affaiblissement tant sur la quantité de réserve que sur la taille de la population, deux traits de vie indispensables pour garantir la survie hivernale des colonies. Parmi les traits de vie de la colonie étudiés, nous avons suggéré la présence de compromis, dont le compromis d'allocation des adultes entre des taches internes et des taches externes telles que le butinage. Cette estimation de compromis a été basée sur plusieurs hypothèses successives qui au final rendent l'interprétation de ce compromis délicat. Dans une deuxième étude en préparation abordant l'étude de l'histoire de vie des ouvrières par l'outil RFID, nous avons manipulé expérimentalement la restriction alimentaire printanière en ajoutant autour d'une colonie 1ha de phacélie (culture mellifère réputée pour l'abeille domestique), destinée à fleurir en mai-juin (période de pénurie). Par comparaison avec un site témoin sans ajout de ressource, nous avons étudié l'influence de l'intensité de la disette sur l'histoire de vie des abeilles ouvrières. En particulier, nous enregistrons la longévité, l'âge de première sortie et l'âge de butinage de 130 abeilles (62 dans le site enrichi en ressource et 68 dans le site témoin), afin de valider ou infirmer l'existence d'un compromis d'allocation des individus face à la restriction alimentaire. Les résultats obtenus avec cette étude au niveau individuel confirment ceux exposés au niveau de la colonie, à savoir, lorsque la restriction alimentaire est forte, les abeilles seront préférentiellement allouées à des taches internes au détriment des taches externes (G*LM*, $F_{1,128}$=7.657, $p<0.01$, et $F_{1,128}$=0.3172, $p=0.574$, respectivement pour les Fig. 3a et 3b). Nous pouvons donc confirmer la présence d'un mécanisme de régulation des colonies face à la restriction alimentaire par régulation de l'allocation des individus adultes, à la suite de cette seconde mise en évidence avec des données et des méthodes différences.

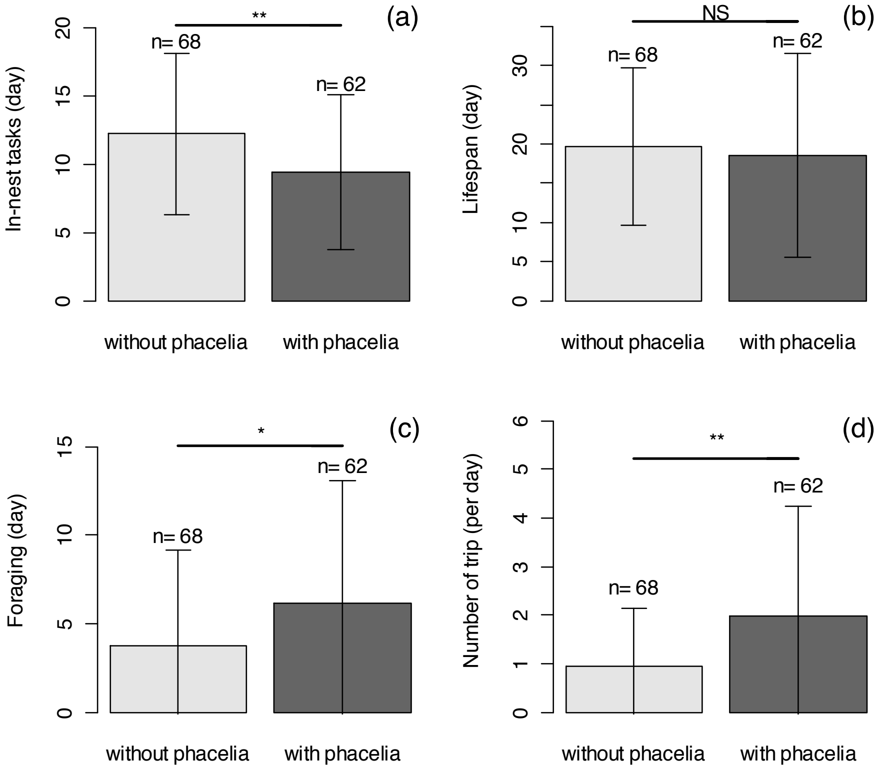


Figure 3 : Traits d'histoire de vie des individus ouvrières, (a) âge de première sortie représentant le temps passé aux taches internes, (b) longévité, (c) durée de butinage, (d) nombre moyen de vol de butinage journalier, n= 130.

Ces nouvelles données ajoutent une information originale sur l'influence de la restriction alimentaire sur le comportement de vol des abeilles et l'activité de butinage. En effet, une forte intensité de restriction alimentaire réduit la durée de butinage (G*LM*, $F_{1,128}$=5.082, p<0.05, Fig. 3c) et le nombre de vols journaliers de butinage (G*LM*, $F_{1,128}$=10.9, p<0.01, Fig. 3d) sans modifier la durée des vols (G*LM*, $F_{1,128}$=0.1876, p=0.6657), résultant donc en une diminution de l'activité de butinage par individu. La restriction alimentaire pourrait donc diminuer les capacités d'approvisionnement des butineuses et expliquer la déplétion d'approvisionnement alimentaire constaté dans les résultats de l'étude du régime alimentaire. Ces nouveaux résultats montrent également tout l'intérêt de notre adaptation de l'outil RFID pour mesurer les traits d'histoire de vie des abeilles en conditions naturelles. La seule mesure de la longévité des individus (variable majoritairement étudiée) aurait induit un non-effet de l'ajout de ressource sur les abeilles, alors qu'il existe un effet sousjacent, beaucoup plus complexe et significatif, d'adaptation comportementale des individus à cet ajout de ressource, qui par compensation n'agit pas sur la longévité. Dans cette thèse, j'ai également suivi des individus ouvrières par l'outil RFID à grande échelle spatiale et temporelle, avec plus de 5,500 abeilles équipées (2100 en 2011 et 3412 en 2012) et suivies automatiquement tout au long de la saison et dans 18 sites paysagers contrastés. Plusieurs objectifs de recherche étaient visés. Par manque de temps, ils n'ont pas pu être présentés dans ce manuscrit, au profit d'autres axes de recherche :

(i) analyser l'influence de la dynamique temporelle des ressources (empiriquement et expérimentalement) sur l'histoire de vie des ouvrières et leur implication dans leur activité de butinage (*article in prep.*),

(ii) étudier l'influence de la composition de la structure paysagère et des Mesures Agri-Environementales (MAE) sur l'histoire de vie des ouvrières et leur implication dans leur activité de butinage, en dynamique temporelle des ressources,

(iii) analyser l'hypothèse d'un double effet de la dynamique en ressource sur l'histoire de vie des abeilles, à savoir l'influence sur l'histoire de vie adulte (cf. points i et ii) et l'influence probable de l'alimentation larvaire sur l'histoire de vie de l'adulte,

(iv) étudier la concordance méthodologique d'évaluation des compromis d'allocation des individus entre des mesures sur les colonies et sur les individus,

Cependant, le monitoring RFID ne renseigne que sur les passages d'une abeille sous un lecteur. Cette méthode proposée permet donc d'obtenir une estimation de temps alloué au vol. Mais l'outil RFID ne permet pas de suivre les individus comme le ferait le radar harmonique. Nous ne sommes donc pas en mesure d'affirmer qu'une estimation de sortie d'une abeille équivaut à un vol. Dans l'objectif d'étudier ce potentiel biais méthodologique, nous avons mis en place une surveillance vidéo des envols et atterrissages des abeilles (Fig. 4a). En collaboration étroite avec le laboratoire L3I de la Rochelle et un de ses doctorants, nous avons adapté une méthode d'analyse trajectométrique pour les vols des abeilles (Fig. 4 b-c, voir aussi l'article détaillé en annexe 2) et développé une méthode de détection des abeilles équipées de puces afin de faire concorder les données de vidéo et les données RFID (*article in prep*.). Cette méthode permettra dans un premier temps d'étudier l'impact de la pose de puce RFID sur le comportement de vol des abeilles. Ensuite, les analyses trajectométriques permettrons de valider ou infirmer le modèle de caractérisation d'histoire de vie des ouvrières, à savoir la caractérisation de leur polyéthisme d'âge entre le stade d'apprentissage du vol et le stade de butinage.

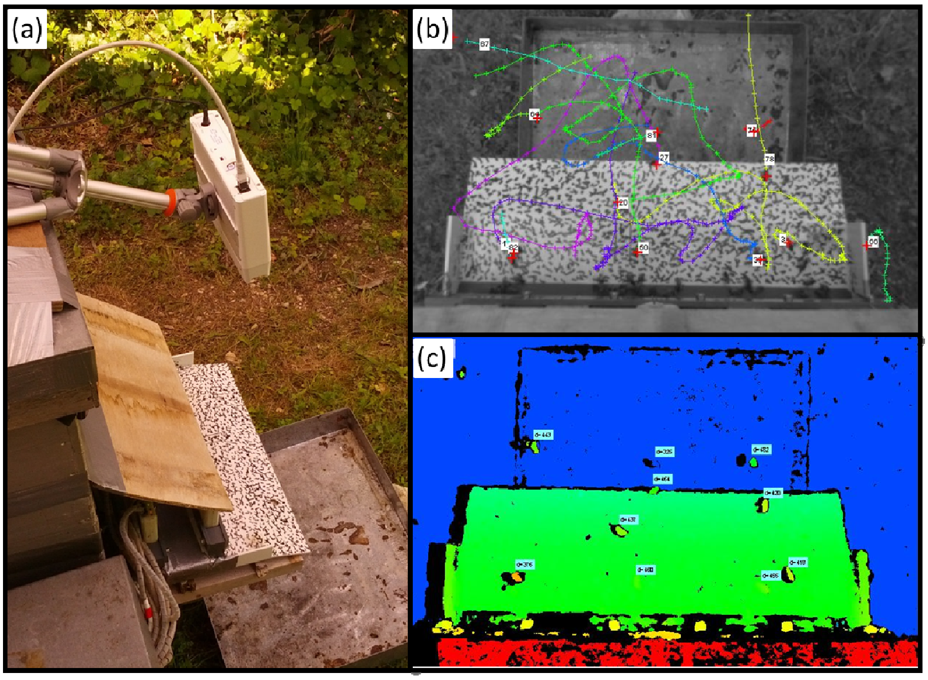


Figure 4 : Mise en place d'une surveillance vidéo des envoles et atterrissages des abeilles, (a) les deux outils RFID et vidéo conjointement utilisés, (b et c) analyses trajectométrique de vol des abeilles en 3 dimensions.

Conclusion et perspectives

L'article de revue introductive dans ce manuscrit de thèse a exposé une lacune de connaissance dans l'écologie de l'abeille domestique en milieux agricoles, pouvant potentiellement induire une sous-évaluation du rôle de l'intensification de l'agriculture et de la diminution des ressources florales dans le déclin des colonies d'abeilles, et préemptant la mise en place de mesures de conservation efficaces. Dans cette optique, des travaux de recherche ont été engagés, analysant successivement l'utilisation des ressources florales et l'histoire de vie des colonies d'abeilles en paysage céréalier intensif. Les résultats obtenus, basés sur un vaste jeu de données empiriques, confirment la présence soupçonnée d'une déplétion d'approvisionnement alimentaire en pollen et en nectar entre les floraisons massives du colza et du tournesol. D'autre part, les résultats obtenus mettent en avant un mécanisme complexe d'adaptation de la colonie face à cette déplétion d'approvisionnement en pollen. Cette adaptation de la colonie engendre un affaiblissement de la colonie tant sur la quantité de réserve que sur la taille de la population, deux traits de vie indispensable pour garantir la survie hivernale des colonies. Ces deux résultats présentent la première preuve empirique de l'impact présumé du manque de ressources florales sur l'abeille en paysage agricole intensif (Naug 2009, Decourtye et al., 2010), et démontre la présence d'une disette alimentaire printanière réduisant la fitness des colonies. Notre étude suggère donc de reconsidérer le rôle de la diminution des ressources florales disponibles dans l'environnement au sein des causes de déclin des abeilles. En réponse à ce constat, la mesure adoptée par les apiculteurs consiste à ajouter artificiellement de la nourriture dans la colonie, à l'aide de sirop de sucre et/ou pain de pollen. Cependant, ces aliments artificiels sont connus comme étant de moins bonne qualité nutritionnelle que la ressource florale présente dans l'environnement (DeGrandi-Hoffman et al., 2008). Il est donc nécessaire de mettre en place des mesures conservatoires visant à ré-enrichir les paysages agricoles en ressources florales pour la conservation de l'abeille domestique, de l'apiculture et du service de pollinisation dans ces paysages.

L'enrichissement des paysages agricoles en ressources florales pour l'abeille est susceptible de prendre plusieurs formes, bien que les techniques actuelles de gestion les plus souvent utilisées consistent à restaurer et promouvoir les habitats semi-naturels riches en ressources et les jachères, tel que les bordures de champs semés avec des mélanges de fleurs mellifères (Haaland et al. 2011, Wratten et al., 2012). La première forme de mesure comprend la réintroduction de cultures entomophiles dans les systèmes culturaux, comme les légumineuses (*Medicago sp., Onobrychis sp., Trifolium sp.*), cultures abondement visitées par les abeilles domestiques (Rollin et al., 2013). Ces cultures sont également préférées parmi les Mesures Agri-Environementales (MAE) choisies par les agriculteurs, par le coût moindre des graines et leurs facilités d'intégration dans les systèmes de rotations des cultures (Decourtye et al., 2011, voir également l'article en annexe 4 pour plus de détail sur les innovations culturales envisageables, orientées sur la conservation des abeilles en paysage agricole intensif). Mais cette production fourragère a un réel intérêt durable d'installation que dans les régions où l'élevage est encore présent (Van Buskirk et Willi, 2004).

La restauration et la conservation des éléments semi-naturels permettrait l'apport en diversité de ressource florale dont les colonies d'abeilles sont dépendantes pour assurer leur survie (Schmidt, 1984, Schmidt et al., 1987) et l'immunocompétence des ouvrières adultes

(Di Pasquale et al., 2013, Alaux et al, 2010). En outre, cette conservation et restauration des habitats semi-naturels est également compatible avec la conservation des abeilles sauvages (abeilles solitaires et bourdons) et améliore la biodiversité et les services écosystémiques dans les paysages agricoles (Wratten et al. 2012, Nicholls et Altieri, 2013). Une troisième alternative est axée sur la conservation des adventices. Les adventices se révèlent être une ressource importante dans le régime alimentaire pollinique des abeilles, un rôle d'autant plus important que cette ressource représente la source d'approvisionnement majoritaire en pollen pendant la période de disette alimentaire printanière, survenant en pic démographique de la colonie. La conservation des adventices peu préjudiciables pour les cultures serait possible par une réduction de l'intensité de certaines pratiques agricoles, y compris l'utilisation d'herbicides, comme requis par les plans d'actions nationaux dans le cadre de la nouvelle législation européenne sur les pesticides (Barzman et Dachbrodt-Saaydeh, 2011). Ces solutions prometteuses sont également compatibles avec la conservation d'autres groupes impliqués dans les services écosystémiques tels que les insectes prédateurs des ravageurs (Bianchi et al., 2006), et les espèces végétales rares associées à leurs pollinisateurs (Gibson et al., 2006).

D'autres mesures peuvent aussi être envisagées par une adaptation locale de la gestion apicole. En effet, nous avons pu démontrer que lors de la floraison du colza (pré-disette), les colonies ayant choisies préférentiellement de créer des réserves au détriment du couvain subissaient de façon beaucoup plus aggravante la disette alimentaire. Pourtant cette stratégie des abeilles est incitée par les apiculteurs professionnels, à leur profit puisque garantissant une forte production de miel pendant la floraison du colza. Cependant, par 'carry-over effect', ces colonies produiront moins de miel pendant la floraison du tournesol et, encore plus grave, auront un taux de survie diminué pendant l'hivernage. Il nous semblerait donc plus judicieux de proscrire cette technique de gestion de la part des apiculteurs en sortie d'hivernage, technique apicole incitant le comportement de création massive de réserve pendant la floraison du colza. Mais la filière apicole doit également être conservée, et la commercialisation du miel en est l'un des principaux enjeux. A cet égard, nous pouvons imaginer des innovations techniques orientées sur la création d'un design de ruches plus approprié aux conditions d'histoire de vie des colonies en paysage agricole. Ce nouveau design devrait considérer le besoin en réserve dans le corps de ruche pour subsister à la disette alimentaire entre colza et tournesol. Une seconde solution déjà utilisée par certains apiculteurs locaux pourrait consister à diviser les colonies très populeuses en début de saison, permettant à la fois d'augmenter le cheptel et de réduire la population des colonies afin qu'elles aient moins besoin de ressources pendant la période de pénurie.

Nous avons pu mettre en évidence une diminution du taux de survie des colonies d'abeilles en réponse à la disette alimentaire pollinique printanière. L'intensification de l'agriculture a engendré deux grands types de stress environnementaux pour l'abeille domestique, à savoir la diminution des ressources florales (étudiée dans cette présente thèse) et l'augmentation de l'utilisation des pesticides-insecticides (Tilman et al., 2001). De récentes études évoquent l'influence de l'exposition aux pesticides (le thiaméthoxame, un néonicotinoïde en semence enrobée) sur la diminution du retour à la ruche des butineuses (article en annexe 5), pouvant engendrer un affaiblissement conséquent de la colonie et diminuant sa probabilité de survie (article en annexe 6). Il a également été démontré expérimentalement qu'une pénurie en ressource pollinique réduit la sensibilité des abeilles aux pesticides, augmentant le risque d'intoxication (Wahl and Ulm, 1983). Dans ce sens nous émettons l'hypothèse d'un effet conjoint entre l'action de la disette alimentaire et des insecticides sur les pertes de colonies d'abeilles en plaines céréalières intensives. En effet, il est probable qu'il existe une interaction spatiale entre l'intensité de la disette alimentaire provoquée par le manque de ressource florale en mai-juin, et la pression d'intoxication, par exemple provoquée par la distribution spatiale du maïs (autorisation de semences enrobées contenant du thiaméthoxame jusqu'en 2012), qui par effet interactif ou additif provoquerait une réduction de la probabilité de survie hivernale. Du même ordre, nous pouvons emettre l'hypothèse d'une intéraction temporelle entre l'impact de l'exposition aux pesticides pendant la floraison massive des cultures systémiques (colza, tournesol, maïs), et l'impact de la disette alimenraire printanilère, affaiblissant succéssivement et additivement la dynamique démographique des colonies. Cette succession temporelle de stress et d'affaiblissement des colonies pourait dépasser le seuil de résilience des abeilles. Cette hypothèse de synergie spatiale et temporelle entre disette alimentaire et stress écotoxicologique fait émerger la nécessité d'investiguer de mesures de conservation réunissant le ré-enrichissement des paysages agricoles en ressource et la diminution de l'utilisation des pesticides.

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ANNEXES

Composition paysagère et dynamique des colonies

Book chapter in press in Quae Edition

Title: Abeilles domestiques dans une plaine céréalière intensive: la composition du paysage influence la dynamique des colonies.

Authors : Henry, M., Odoux, J. F., Requier, F., Rollin, O., Tamic, T., Toullet, C., Le Mogne, C., Peyra, E., Decourtye, A., Aupinel, P. & Bretagnolle, V.

ABEILLES DOMESTIQUES DANS UNE PLAINE CÉRÉALIERE INTENSIVE: LA COMPOSITION DU PAYSAGE INFLUENCE LA DYNAMIQUE DES COLONIES

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Résumé

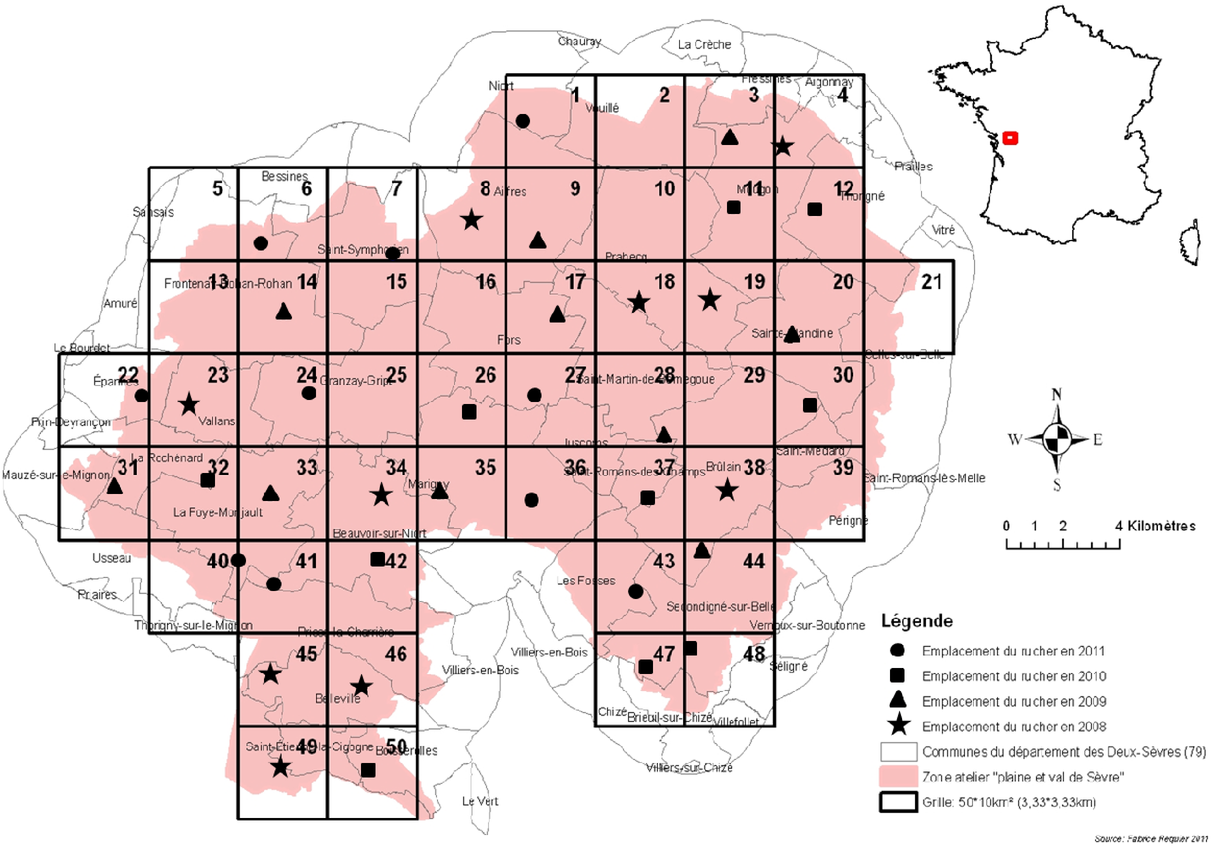
Un programme de suivi de ruchers a été initié en 2008 dans la Zone Atelier Plaine & Val de Sèvre (Poitou-Charentes), avec pour objectif de caractériser l'effet de la composition du paysage sur la dynamique des colonies d'abeilles domestiques dans un contexte de plaine céréalière intensive. Dans cet agrosystème, les ressources florales disponibles pour les abeilles concernent d'une part les cultures fleuries et d'autre part les ressources florales spontanées. Les abeilles butineraient préférentiellement dans les grandes cultures du fait de leur mode de vie social, mais il existe des périodes de disette, pendant lesquelles seules les ressources spontanées demeurent disponibles. Des indicateurs de la dynamique des colonies ont été mis en relation avec les données d'assolement relevées dans l'environnement des ruchers. Les indicateurs biologiques retenus sont les quantités de couvain, de réserves et d'individus des colonies. Conformément aux hypothèses, des corrélations significatives ont été établies entre certains indicateurs de la dynamique des colonies et la composition du paysage, révélant un effet de complémentarité écologique entre les compartiments cultivés et semi-naturels. Cet effet est observé en début de saison et conditionne potentiellement la survie des colonies plus tard dans la saison, ainsi que leur productivité en miel.

Summary

A monitoring program was launched in 2008 in the Study Area Plaine & Val de Sèvre (Poitou-Charentes, France), with the aim of characterizing the effect of landscape composition on the dynamics of honey bee colonies in a context of intensive cropping system. In this agrosystem, honey bees rely on two kinds of floral resources, namely the mass-flowering crops (mostly oilseed rape and sunflower) and the spontaneous floral resources in semi-naturel habitats. Bees forage preferentially in mass-flowering crops, partly because their social way of life imposes them to store large amounts of honey to sustain colony development. However we have identified periods of food shortage, between mass-flowering periods, during which wild flowering plant species in semi-natural habitat remnants are the only available floral resources. Descriptors of colony dynamics were analysed in relation with landscape metrics describing the environment of experimental apiaries. Biological descriptors were the brood area, the adult population size and the honey reserve mass. Significant correlations were established between some descriptors of colony dynamics on one hand and landscape metrics on the other hand, revealing an effect of ecological complementarity between mass flowering crops and semi-natural habitats early in the beekeeping season, with possible implications for colony sustainability and honey production later on.

Mots-clés : Abeille domestique ; paysage agricole ; développement de colonie; ressource florale ; colza; habitat semi-naturel

Un programme de suivi de ruchers a été initié en 2008 dans la Zone Atelier Plaine & Val de Sèvre (Poitou-Charentes) et baptisé ECOBEE, avec pour objectif de caractériser l'effet de la composition du paysage sur la dynamique des colonies d'abeilles domestiques (*Apis mellifera* L.) dans un contexte de plaine céréalière intensive (Odoux et al., 2009). La zone d'étude couvre une superficie de 45000 ha, comprenant environ 19000 parcelles agricoles dont les rotations culturales sont documentées chaque année depuis 1995 (Bretagnolle et al., 2011). Dans cet agrosystème intensif, les ressources florales disponibles pour les abeilles sont de deux types : d'une part des cultures fleuries attractives, et d'autre part les ressources florales spontanées dans les habitats semi-naturels résiduels. Les cultures fleuries occupent des surfaces conséquentes, représentées principalement par le tournesol (environ 12% de l'assolement total), le colza (10%), le maïs (9%) ou la luzerne (4%). Hormis les prairies (6% de l'assolement), les espaces dits semi-naturels, ou interstitiels, occupent des surfaces plus restreintes et difficiles à estimer: jardins et vergers (3%), milieux boisés (3%), bords de champs et de routes (3%), réseaux de haies (en moyenne 5km par km^2, source : archives SIG locales et fonds cartographiques IGN). Outre sa grande taille et l'importance des informations agronomiques qui y sont collectées, la Zone Atelier donne accès à divers gradients environnementaux pertinents : complexité paysagère (taille et occupation des parcelles, présence d'éléments semi-naturels), sols, hydrographie, activités humaines et pratiques agricoles.



Source: Fabrice Requier 2011

Figure 1. Représentation du découpage de la zone atelier en 50 secteurs carrés, et des secteurs sélectionnés pour les suivis annuels de colonies.

Les données palynologiques et comportementales collectées par ECOBEE (Odoux et al., 2012 ; Requier et al., 2012 ; Rollin et al., 2012) indiquent que les abeilles domestiques utilisent massivement les cultures de masse fleurie lorsque celles-ci sont disponibles, principalement le colza, le tournesol et les légumineuses. Ce comportement peut notamment s'expliquer par leur mode de vie social nécessitant la constitution d'importantes réserves communes de nectar et de pollen (Tscharntke et al., 2005, Decourtye et al., 2011). Nous formulons donc l'hypothèse que la dynamique saisonnière des colonies d'abeilles serait en partie déterminée par l'abondance des cultures fleuries dans leur domaine vital. Cependant, bien qu'abondantes dans l'environnement, les cultures fleuries ne sont disponibles que temporairement. Il existe en particulier des périodes dites de *disette*, pendant lesquelles seules les ressources florales spontanées (adventices comprises) demeurent disponibles (Decourtye et al., 2006). Lors de ces périodes, les habitats semi-naturels peuvent donc jouer un rôle de compensation écologique nécessaire au maintien des colonies (Albrecht et al., 2007).

Pour tester ces hypothèses, des indicateurs de la dynamique des colonies ont été mis en relation avec les données d'assolement relevées à différentes échelles spatiales dans l'environnement des ruchers expérimentaux. Les indicateurs biologiques retenus sont les quantités de couvain, de réserves et un estimateur de la quantité d'individus adultes peuplant les colonies, mesurés grâce à une routine de visites bimensuelles d'un réseau de 50 colonies par an. Les colonies ont été conduites dans les conditions habituelles de l'apiculture professionnelle locale. Les ruches sont ouvertes, chaque cadre est pesé avec et sans abeilles, la surface de couvain qu'ils portent est mesurée sur chaque face. Afin de couvrir une grande variété de contextes paysagers, les 50 ruches sont disposées en 10 ruchers de 5 ruches, répartis à travers l'ensemble du territoire. Pour ce faire, le territoire a été subdivisé en 50 secteurs carrés de 3km de côté. Chaque année, entre 2008 et 2011, 10 nouveaux secteurs ont été choisis aléatoirement et sans remise (Figure 1). Les suivis saisonniers (Avril à Septembre) comprennent donc un total 200 colonies réparties sur 40 des 50 secteurs que compte le territoire.

Dans un premier temps la cinétique moyenne des indicateurs biologiques a été décrite et modélisée pour les quatre années de suivis. Ils permettent de conclure à un patron saisonnier marqué de la dynamique des colonies d'abeilles, avec une forte répétabilité d'une année sur l'autre (Figure 2). La quantité de couvain est maximale en début de saison apicole (période de floraison du colza), puis diminue progressivement jusqu'à atteindre un minimum en fin d'été. La population adulte, elle, atteint un maximum 3 à 4 semaines après le pic d'abondance du couvain, ce qui paraît cohérent avec la durée théorique de développement larvaire (21 jours). Le pic de population est donc atteint en période de disette (27.000 individus en moyenne), puis diminue de façon constante jusqu'à 17.000 individus en fin de saison. Finalement, la production de miel suit un patron bimodal, correspondant à la miellée de colza et à la miellée de tournesol, la seconde étant plus productive. La période intermédiaire, en juin, correspondant à une absence de masses florales dans le milieu et se traduit par une réduction des réserves en miel, alors que la population d'ouvrières adultes connaît son pic démographique.

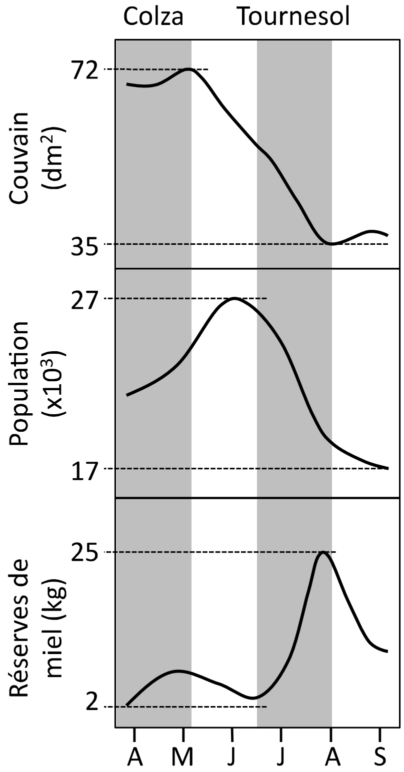


Figure 2. Cinétique temporelle moyenne des 200 colonies suivies pendant les quatre saisons apicoles (avril-septembre) de 2008 à 2011. Les suivis de colonies comprenaient la mesure des surfaces totales de couvain sur les cadres, la taille de la population adulte, et la quantité de miel mis en réserves. Les périodes approximatives de floraison des principales cultures de masses florale sont indiquées par les zones grisées (colza, tournesol).

Dans une seconde étape, nous avons cherché à identifier les entités paysagères, cultures fleuries ou habitats semi-naturels, dont l'occupation du sol pourrait influencer la dynamique des colonies d'abeilles domestiques. Après avoir envisagé différents scénarios possibles intégrant l'intensification agricole, la complémentarité des habitats, ou aucune de ces hypothèses, des corrélations significatives ont été établies entre certains indicateurs de la dynamique des colonies et la composition du paysage. Les résultats soulignent l'influence conjointe des ressources florales très abondantes du colza et celles apportées au même moment par les éléments fixes du paysage (pic de floraison de nombreux ligneux) sur la production du couvain en début de saison (Figure 3). Cette interaction entre deux types d'éléments paysagers au mois d'avril (surfaces de colza et surfaces boisées) peut être interprétée comme une complémentarité écologique, chaque habitat ayant un effet positif et additif sur la production totale de couvain. Cet effet est transitoire (période du colza), mais est partiellement répercuté durant le reste de la saison, puisque les colonies placées dans un environnement plus boisé voient leur population décroitre moins rapidement que les autres. Cet enchaînement temporel peut *in fine* influencer la production des ruches en miellée de tournesol.

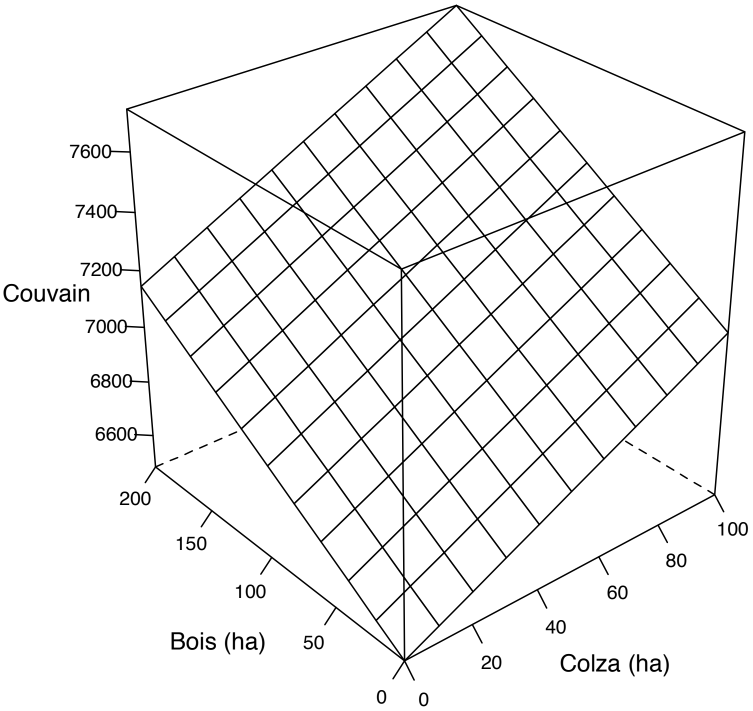


Figure 3. Variation des quantités moyennes de couvain dans les ruches (cm^2), en fin de période de floraison du colza, selon le contexte paysager. Les prédictions du modèle statistique montrent un effet complémentaire « additif » entre la sole de colza et la quantité d'habitats boisés mesurés dans un rayon de 1,5 km autour des ruches.

En conclusion, la dynamique temporelle des colonies a d'abord été influencée par la production de couvain en début de saison, qui correspond à la floraison du colza et de nombreux arbres présents dans les haies et les éléments boisés. Toutefois, les populations d'abeilles bénéficiant des surfaces en colza les plus élevées ont connu ensuite de plus grandes difficultés de développement pendant la période de disette. A cette période, les colonies situées dans une zone où les cultures de céréales ont été majoritaires, ont moins investi dans la production de couvain. De futures investigations permettront d'évaluer la dynamique des réserves alimentaires dans les ruches et la survie hivernale des colonies.

Ce dispositif de suivi de colonies a d'ores-et-déjà été mis à profit dans le cadre du projet pluridisciplinaire POLINOV (2009-12) ayant rassemblé agronomes, apidologues et écologues autour du défi de la conception de systèmes de culture innovants conciliant à la fois les enjeux de protection des abeilles, de durabilité de l'apiculture et de l'agriculture. En particulier, des leviers d'action ont pu être identifiés (l'importance des cultures de masses florales et de certains types d'habitats semi-naturels à différentes périodes de la saison apicole) et pris en compte dans la conception de nouveaux systèmes de culture. Les innovations incluent une allocation spatiale des cultures plus adaptée aux besoins complémentaires des abeilles en termes de cultures de masse florale et de milieux semi-naturels, mais aussi une adaptation des successions culturales et des aménagements des bordures de champs.

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Trajectométrie d'envol des abeilles

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Title: 3D Tracking of Honeybees Enhanced by Environmental Context.

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3D Tracking of Honeybees Enhanced by Environmental Context

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Abstract. This paper summarizes an approach based on stereo vision to recover honeybee trajectories in 3D at the beehive entrance. The 3D advantage offered by stereo vision is crucial to overcome the rough constraints of the application (number of bees, target dynamics and light). Biologists have highlighted the close scale influence of the environment on bees dynamics. We propose to transpose this idea to enhance our tracking process based on Global Nearest Neighbors. Our method normalizes track/observation association costs that are originally not uniformly distributed over the scene. Therefore, the structure of the scene is needed in order to compute relative distances with the targets. The beehive and especially the flight board is the referent environment for bees, so we propose a method to reconstruct the flight board surface from the noisy and incomplete disparity maps provided by the stereo camera.

Keywords: stereo vision, honeybees 3D tracking, surface reconstruction, beehive monitoring

1 Introduction

Forced to observe the worldwide decline of honeybees (Apis mellifera), biologists began to study different hypotheses that could explain the phenomenon. Recently, the authors of [1] highlighted the evidence of behavioral alterations caused by pesticides. In that study, entrances and exits data was collected by a Radio-frequency identification monitoring device placed at the beehive entrance. So far, no biological study has been conducted at a big scale on flight behaviors. In cause, the lack of suitable methods to collect trajectories of honeybees in flight. The only method used by biologists (harmonic radar) is intrusive and suffers from biases. We believe that computer vision can effectively achieve this task with the respect of the application constraints.

Our challenging application of tracking bees in 3D at the beehive entrance has been studied in [2], laying down the application constraints and a detect-before-track approach based on Kalman Filter coupled with Global Nearest Neighbor. In this current paper, we focus our attention on the weakness of our tracking algorithm nearby the flight board. As a main contribution, we propose to enhance the tracking process by taking in consideration the targets relative to environment. This is achieved by a normalization of track/observation association costs relying on the distance of the target from the flight board. Also, as a second contribution, we introduce a method to recover the flight board surface, which is not adequately represented by individual disparity images provided by the stereo camera.

This paper is organized as follows. First, Section 2 summarizes the base of our monitoring system, detailing the acquisition method, the segmentation, and the tracking processes. Then, Section 3 introduces in first, our normalization method of track/observation association costs, and then our method to recover and model the flight board surface in 3D. Results are shown and discussed in Section 4. And finally, Section 5 concludes and opens promising perspectives.

1.1 Related Work

In the following are discussed papers dealing with trajectometry based on video related to insects or animals. The process of recovering trajectories can be split into two distinct parts which are presented below: detection and tracking.

For target detection, several methods have been proposed. Detect-before-track approaches are generally based on a segmentation process, and then the observations are associated in order to know tracks using an assignment method. Many methods based on that approach use a more or less advanced background subtraction (e.g. [3, 4]). In [4], potential false alarms are filtered using a shape (ellipsis based) matching process. In contrast, some methods introduced do not require any background subtraction. The authors of [5] detect bees using the well-known Viola-Jones method [6], and the authors of [7] introduce an approach based on vector quantization which is able to detect individual bees among hundreds of walking bees. In [8], flying bats are detected taking the advantage of multiple cameras by directly applying Direct Linear Transform. In case of track-before-detect approaches, the position of the target is first estimated, and a probability for that estimation to correspond to the target drives the next estimation. For that kind of approach, a likehood function based on appearance models (pre-computed "eigenbees" in [9], or adaptive models in [10, 11]) are used.

Many methods have been proposed for tracking. When following one clearly detected target moving along a simple trajectory, approaches such as those used in [12], near neighbor or mean shift may be sufficient. But in the case of tracking multiple targets, assignment problems arise due to missed detections and occlusions and thus cause false alarms. The authors of [3, 4] use Global Nearest Neighbor (GNN) for track assignment, instanciation and destruction. In [8] the authors track multiple flying targets thanks to a multiple hypotheses tracker (MHT). Compared to GNN, MHT integrates the time in the assignment decision process. In [9, 10, 11, 13], a nonlinear motion model is considered for their targets and they base their tracking on a particle filter [14], which corresponds to a track-before-detect approach. In [13], the authors introduced a MRF-augmented particle filter for multiple targets with reasonable computational cost, contrasting with the three other methods used in [9, 10, 11] that are less suitable in term of performance when working on multiple interacting targets.

When dealing with many flying targets in natural conditions (uncontrolled light and background), the range of application remains narrow and methods remain to be explored. Compared to most approaches mentioned above, our stereo vision based system brings in this paper the advantage of the the 3^{rd} dimension. This work extends the preliminary study made in [2] by considering the spatial environment into the tracking process.

2 Detect-before-track Based on Stereo Vision

This section presents our stereo vision acquisition system and summarizes how we recover trajectories from a sequence of couples of intensity/disparity images. More details on the acquisition system and our segmentation are given in [2].

2.1 Acquisition System and Constrains

The following constraints make our application especially challenging: Up to 15 simultaneous flying targets with chaotic dynamics, uncontrolled lighting, and gradual soiling of the background. Among potential suitable cameras (stereo vision and time of flight), we chose the TYZX G3 EV stereo vision camera which seemed to satisfy the best our requirements, with the following configuration: 3 cm baseline and 62 degree HFOV lenses. The camera targets the flight board and is located 50 cm above it. This system acquires a couple of intensity/disparity images of 752 × 480 px at an average frame rate of 47 fps (unstable rate). Figure 1 shows a capture sample. The quality of the disparity map provided by the camera depends on lighting and texturing conditions. Bees standing on the flight board measure about 10 pixels on those images, and during high activity periods, the flight board can be crowded of bees, making the board difficult to discern.

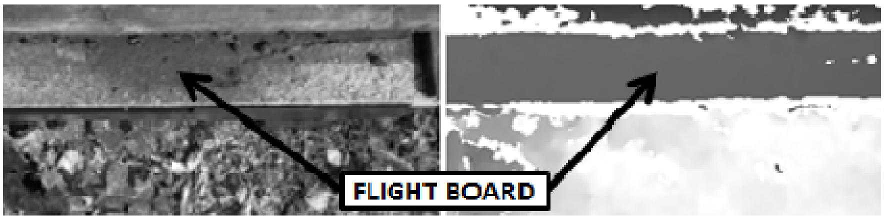

Fig. 1: Couple of intensity (left) / disparity (right) images provided by the stereo vision camera. The beehive is visible on the top. Bees take off/land on the flight board.

2.2 Segmentation of Flying Targets

Under the constraints listed above, classical motion detection methods based on background modeling fail. Our system detects flying bees thanks to an hybrid segmentation that takes advantage of both intensity and disparity images provided by the stereo camera. Our segmentation relies on an adaptive background model for the intensity, and combined mathematical morphologies and adapted thresholding for the disparity images. As a result, we obtain a mask containing regions corresponding to our targets. A target is defined by (u,v) the center of mass of the region, and d the median value of the depth values in the disparity image. So far, targets are expressed in the image reference coordinates. To ensure later a coherent tracking, targets are rather defined by (x,y,z) in the camera coordinate space (3D Euclidian space) using a projection based on stereo-camera calibration parameters. Our segmentation is globally robust but still returns in rough conditions up to three false alarms per image around the flight board. Also relatively few miss detections occur for too fast, too close or too far targets from the camera.

2.3 Multi-target Tracking in 3D

In our detect-before-track approach, each target is tracked by a Kalman Filter [15]. Despite the apparent rough dynamic of bees, we acquire frames at a sufficiently high frequency (about 47 fps) so that we can assume a constant speed model. Noise matrices (Q for the model, R for the measure) are tuned to prevent the model from derivation. Let us suppose $Y_{1:n}$ the series of observations corresponding to a target from time 1 to n. A Kalman Filter is instantiated with Y_1 and later destroyed when the step $k > n$. For a given step k, an observation is defined by the vector $Y_k = [x,y,z]^T$, and the estimated state of a target is defined by the vector $X_k = [x,y,z,\dot{x},\dot{y},\dot{z}]^T$ combining its 3D position and velocity. Figure 2 lays down the recursive mechanism of the Kalman Filter for the estimation of the state vector X_k and the prediction X_{k+1} given an sequence of observation from step 1 to k.

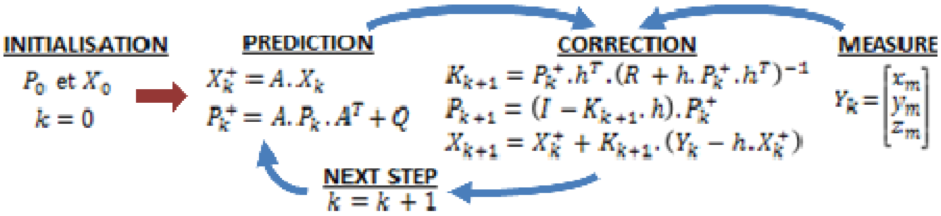

Fig. 2: Recursive mechanism of the Kalman Filter for the estimation of X.

In our application, up to 15 targets can be observed at each step, which requires a multi-target tracking approach. The commonly used Global Nearest Neighbor (GNN) [16] method handles observation to track associations, track instantiations and destructions. In GNN, the assignment matrix $A[c_{i,j}]$ represents all the possible associations and the costs generated by those associations. A includes the possibility for each observation to be associated to an existing track, not to be associated to any track or to be associated to a new track. $c_{i,j}$ is the cost for the observation i to be assigned to the possibility j. The best configuration of associations is the solution that minimizes the global cost (e.g. solved by the hungarian method). The association cost between an observation and a track is computed by the Mahalanobis distance given by:

$$\begin{gathered} d^2 = (Y - MX^+)'S^{-1}(Y - MX^+) \\ S = ME^+M' + Em \end{gathered} \tag{1}$$

where Y is the measure vector corresponding to the observation, M the measurement matrix, X^+ the predicted a priori position, S the innovation covariance with Em as the measure noise matrix, and E^+ the predicted noise covariance matrix.

3 Tracking with Environmental Consideration

In GNN, the multi-target assignment process relies on comparisons of association costs d defined by (1). For a track/observation association, if the minimum cost exceeds a fixed threshold later called Association Cost Limitation (ACL), the association is not considered and the observation becomes a false alarm or a new track. The global chaotic dynamics of flying bees forces the adoption of a tolerant threshold. In our application the problem is, in addition to an high frequentation, most of wrong detections (false alarms and miss detections) are located near the flight board. Moreover, targets near the flight board tend to progress nearly on the same plan resulting in the lost of the 3D advantage. So when tracking a bee near the flight board, in case of miss detection the loose threshold allows the track to be associated with a nearby false alarm or other candidate observations, which causes the degeneration of the track. The magnitude of association costs is not uniformly spread over the scene, so adapting the association costs to the situation is needed.

3.1 Association Costs Normalization

As reference, a classic approach consists in adapting the uncertainty at the track initialization. Bees entering the scene from the outside generate tracks with a big initial uncertainty, which has the effect of decreasing *d* defined by (1). In contrast, bees taking-off from the flight board have a low velocity and thus can generate tracks with a small initial uncertainty, which has the effect of increasing *d*. In this way, tracks are less vulnerable to wrong associations nearby the flight board and more able to be associated to relatively far observations when entering the scene. The problem is only partially solved because, the convergence of the uncertainty (in the Kalman Filter) is naively driven by the elapsed time, which does not corresponds well to the random chaotic trajectories exhibited by honeybees.

The authors of [17] explain the relation between honeybees' speed and surrounding objects. Using our classic tracker based on [2], we effectively observed lower speeds and also lower association costs nearby the flight board. The lower association costs are explained by the more stable targets' dynamic resulting in easily predictable positions. As a contribution, we propose to normalize association costs all over the scene relying on targets' relative distances from the board. Figure 7 shows an example of association cost distribution, within which we can identify the two following distinct effects. The primary growing effect ($l < 50$) potentially corresponds to the gradual stability in the dynamic adopted by bees when approaching the flight board in the last centimeters. The second effect ($l > 50$) is less perceptible, and potentially corresponds to changes in bees' dynamic at a larger scale (e.g. approach of the beehive). Relying on the modeling (functions α and β) of those effects, we propose the normalized cost c_f based on d^2 that has the advantage to penalize potential wrong detections near the board and thus limits the degeneration of the track. c_f is given by:

$$c_f = \begin{Bmatrix} d^2/\alpha_f & \text{if } f \leq l \\ d^2/\beta_{f-l} & \text{if } f > l \end{Bmatrix} \tag{2}$$

where *f* is the closest distance of the target from the flight board, *l* the limit of strong influence of the environment, and α and β are respectively the quadratic and affine normalization functions of *f* relative to the application. Coefficients of α and β can be estimated by quadratic and linear regressions from a set of association costs relative to the board distance taken from well recovered tracks.

The relative distance target/flight board can be computed only with the knowledge of the structure of the flight board surface. Therefore, the following section proposes a method to reconstruct the flight board surface under the constraints of our application.

3.2 Surface Reconstruction in Cluttered Conditions

Individual disparity images provided by the stereo camera are noisy, incomplete and do not represent the flight board surface as needed. Therefore, we need to compute from a sequence of disparity images a sample of pixels that represent the surface as complete as possible and without any bee on it. As a paradox, a medium activity constitutes an optimal condition to retrieve the structure of the flight board. Indeed, zero activity means that untextured parts remain untextured over all the sequence, which is not good for the disparity computation. In contrast, an overcrowded flight board makes it hard to distinguish the surface. In the following we propose a method that iteratively filters pixels from a sequence of disparity images that represent the best the flight board structure.

A region of interest corresponding to the flight board is defined manually, and the flight board is isolated from the rest of the image. A median filter is applied on each disparity image in order to filter inconsistent disparities due to stereo matching errors. A model is initialized with the first image of the sequence. Then image after image, we update each pixel of the model with the greater disparity value between the model and the current image. Figure 3 shows step by step the flight board being extracted. *N* is the number of disparity images used. It has to be high enough to include a sample of complementary depth maps that could be robust to the clutters by passing through it. But, due to the maximization of the disparity, an overestimation of *N* increases the chances to add noise (wrong disparity values that have not been filtered by the median filter). An optimal number of iterations (or number of disparity images to use) can be estimated by finding the minimum standard deviation of the depth values on the board over iterations. Figure 5 shows an example with 180 as the optimal number of iterations which corresponds to a flight board approximately located at 380 mm from the camera.

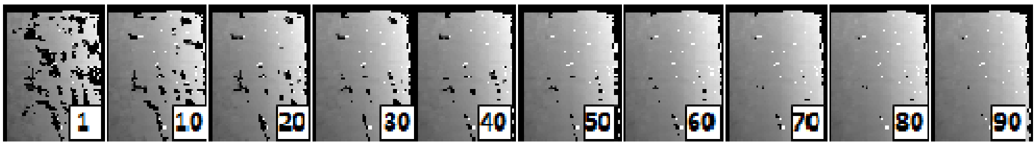

Fig. 3: Step by step flight board reconstruction (90° rotated, partial top view). The numbers are the number of disparity images used. The darker the closer from the camera. Black areas correspond to unavailable disparity information.

As the model still contains holes and inconsistencies, we then apply a fitting based on the locally weighted scatterplot smoothing method (LOWESS) regression modeling method [18] with the Tukey's bisquared function for outliers resistance. Figure 4 shows a mesh reconstructed from the surface model representing a curved flight board.

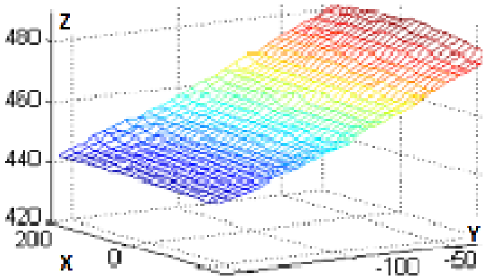

Fig. 4: Reconstructed surface model representing a curved flight board.

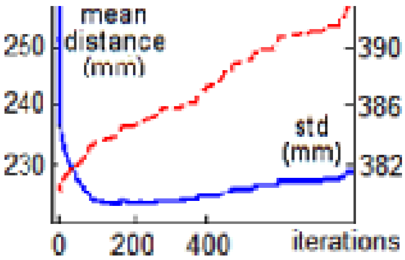

Fig. 5: Standard deviation (blue line) and mean values (red dashed line) of depth values of the flight board under reconstruction.

4 Results and Discussion

Since no trajectory ground truth is available for our application, we based our evaluation on simulated bee trajectories using a multi-agent approach. Our simulator is based on the following assumptions: Bees tend to slow down nearby the flight board and also when changing direction. Bees located at the limit of the field of view can reach a maximum speed of 3 cm/step. We simulated 200 trajectories (100 landings, 100 takeoffs) with a constant number of 15 bees in the field of view. At each step of the simulation (or frame), 3 false alarms are added, and each bee has 10% changes to be undetected. Those conditions of simulation result in similar data obtained from the terrain under challenging conditions. Figure 6 shows a set of simulated/recovered trajectories with respect to a real reconstructed flight board. The shape of the trajectoires are unique but still tend to follow a similar pattern.

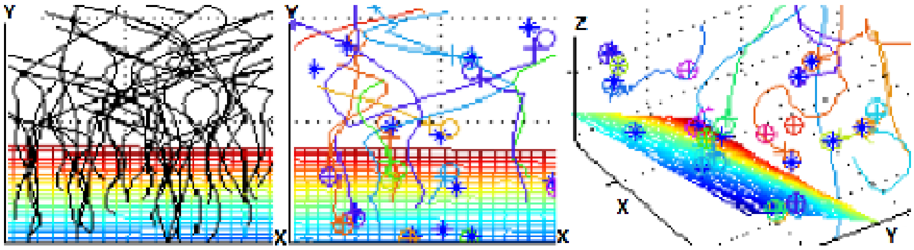

Fig. 6: Top view of simulated trajectories (left) based on a real recovered flight board, and simulated bees tracked with our normalized method (center). 3D view of bees tracked from a real capture with our normalized method (right). Crosses and circles respectively correspond to observations and predicted targets position.

Figure 7 illustrates the effect of our normalization on the relation association cost / distance from the flight board. The normalization model is tuned as followed: Area A with a polynomial function having as coefficients 0.00048, -0.0029 and 0.038 and Area B with an affine function having as coefficients 0.0013 and 1. Therefore, normalized costs can be compared to a constant ACL.

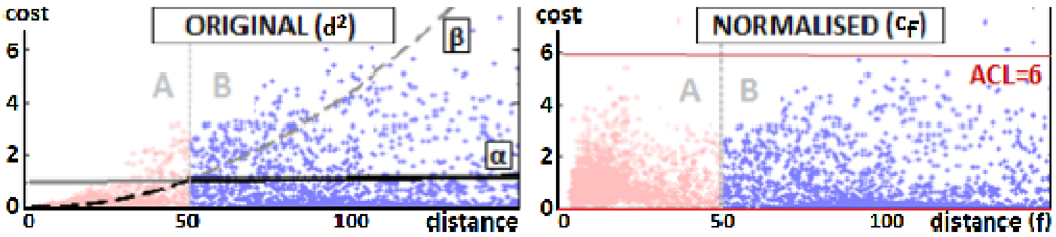

Fig. 7: Association cost / distance to the flight board relation between the original (unormalized) and our normalized method. The lower original costs nearby the board are explained by easily predictable moves. Areas A and B are separated at the distance of l=50 mm. The quadratic function α (dashed curve) and the affine function β (plain line) are obtained respectively by fitting of points of A and B. As an example, ACL=6.

Figure 8 shows comparative results between the original and our normalized method according to different ACL. The classic method recovers at most 60% of the tracks (ACL=7), where our normalized method recovers 71% (ACL=13). Figure 9 shows the importance of recovering accurately the flight board when using our normalized method. Considering an optimal ACL of 13, the result decreases when adding a positive or negative error to the depth of the flight board surface.

If the flight board surface can not be well recovered (e.g. too much activity), it is preferable to manually lower the flight board estimation (positive depth error in Figure 9) to take in consideration the layer of walking bees. This layer measures up to 15 mm, which does not affect results drastically. Without automatic feedback on the quality of the recovered flight board surface, it would be acceptable to consider by default the 15 mm layer of walking bees (being there or not). The use of a simulation to evaluate tracking results is debatable. Despite our efforts to create a truthful simulator, there will always be differences between simulated and real data. Nevertheless, we confirmed (without quantification) the benefit of our normalization method on real trajectories (see Figure 6). Further studies involving real data and comparisons of simulation methods have already been started.

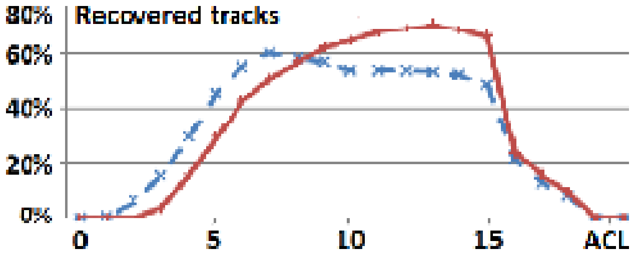

Fig. 8: Comparative results between the unnormalised (dashed line) and our normalized (plain line) methods using different Association Cost Limitation (ACL).

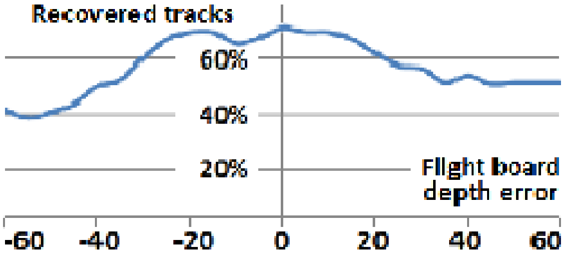

Fig. 9: Effect of a potential wrong flight board recovery (delta depth) on the normalized method based on the optimal ACL, which is 13.

5 Conclusion

In addition to our global tracking system based on stereo vision partially presented in [2], we introduced in this article the idea of taking into account targets' environment into the tracking process. The accurate reconstruction of the flight board surface made possible the computation of the relative distance of the bee from its landing/takeoff area. Then our normalization method for association costs made in case of miss detections the tracking process less vulnerable to wrong associations coming from false alarms. Our normalization method provides an improvement of 17.5% on simulated trajectories, which shows the importance taking in account the difference magnitude of association costs when the application allows it. As an example, it works well with our honeybees which tend to follow a typical behavior nearby the flight board.

Comparing costs is a common step in other tracking methods such as MHT, it would be interesting to check the portability of our method. Concerning the flight board reconstruction, our method introduced in Section 3 works with a camera located above the flight board. It could be also interesting to study the possibility of recovering the flight board surface from a non-vertical view.

Concerning long term perspectives, biologists are interested in high level applications such as abnormal behavior detection. On the one hand, behavior models could focus on individual bee trajectories. We can imaging a tracker that takes in consideration more parameters (e.g. activity, weather, time of the day) to adapt motion models. On the other hand, more global models could focus on the colony activity. We can imagine abnormal colony behavior detector based on some basic rules (e.g. low activity during a sunny day).

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POLINOV pour des systèmes de cultures favorables aux abeilles

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Title : Projet Polinov, épisode 1: pour des systèmes de cultures favorables aux abeilles.

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Projet Polinov, épisode 1 pour des systèmes de cultures favorables aux abeilles

Mieux comprendre les interactions entre abeilles et zones agricoles céréalières avant de proposer de nouveaux systèmes de cultures.

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L'utilité des abeilles pour la production végétale est reconnue, et l'apiculture bénéficie en retour de grandes surfaces en cultures mellifères. Mais les objectifs des filières agricoles et apicoles, ainsi que des acteurs de la conservation des abeilles sauvages, peuvent créer des antagonismes. Les agriculteurs ont besoin, pour garantir leur production, de stratégies de protection des plantes efficaces contre les ravageurs mais qui peuvent être dommageables à l'état de santé des abeilles. Les apiculteurs doivent exploiter leur cheptel apiaire en dépit de son état de santé fragile.

Il faut des approches innovantes porteuses de solutions capables de résoudre ces tensions en tenant compte des différentes exigences. C'est le but de Polinov.

Pourquoi Polinov

Le contexte favorise des solutions nouvelles

Le contexte actuel favorise le développement de nouvelles solutions bénéficiant aux divers acteurs d'un même territoire. En effet, les systèmes de cultures sont face à des évolutions radicales liées aux objectifs environnementaux (Ecophyto), et la filière apicole est dorénavant mieux organisée pour pouvoir co-construire avec les organismes de recherche et les filières agricoles des systèmes de culture répondant aux problèmes touchant les colonies d'abeilles.


Photo : ACTA

Abeille domestique **sur fleur de mélilot, espèce végétale utilisée en jachère fleurie.**

Limites des approches « simples »

Les solutions couramment mises en œuvre pour protéger les abeilles dans les agrosystèmes sont liées soit à l'établissement d'un diagnostic du risque lié à l'usage de pesticides, soit à une gestion raisonnée des aménagements territoriaux (jachères, bandes enherbées ou haies fleuries). Mais ces approches simples, investies indépendamment l'une de l'autre,

RÉSUMÉ

CONTEXTE - L'apiculture et l'agriculture (productions végétales) coexistent, avec des services mutuels mais aussi des risques de tensions.

Le contexte actuel favorise la recherche d'une évolution tenant compte des interactions entre facteurs économiques et environnementaux, et des échelles des parcelles cultivées, de leurs abords et de toute l'exploitation (système d'exploitation).

Tel est l'objet du programme Polinov.

ENQUÊTE ET ANALYSE - L'étude a été menée sur la zone atelier Val-de-Sèvre. Elle a consisté d'abord en un état des lieux des systèmes existants, décrits par enquête puis évalués grâce au logiciel DEXi-Abeilles, selon leurs perfomances :

- économiques pour l'apiculture comme l'agriculture ;
- sociales pour les agriculteurs, apiculteurs et autres acteurs ;
- environnementales pour les pollinisateurs (abeilles domestiques, abeilles sauvages, bourdons) et la biodiversité.

L'article décrit DEXi-Abeilles et son fonctionnement, ainsi que les quatre systèmes types étudiés.

OBSERVATOIRE - En parallèle, l'observatoire Ecobee, coordonné par le CNRS de Chizé, l'INRA du Magneraud et L'Unité mixte technologique d'Avignon, a suivi sur le terrain des colonies d'abeilles domestiques et les populations de butineurs (méthodologie dans l'article).

- Cela a permis de faire l'inventaire faunistique des abeilles domestiques, sauvages et des bourdons et leur diversité selon les sites (dans et hors des parcelles cultivées) et les saisons ;
- on a pu évaluer les ressources alimentaires réellement utilisées par les abeilles domestiques, avec des résultats attendus (importance du tournesol) et des surprises (régime plus varié qu'attendu).

PAR AILLEURS - Les connaissances acquises ont été utilisées pour les étapes suivantes du projet. Celles-ci, allant jusqu'à la conception de systèmes innovants et à leur évaluation *ex ante* (pour choisir les plus prometteurs à tester ensuite sur le terrain), feront l'objet d'un prochain article.

MOTS-CLÉS - Abeilles domestiques, abeilles sauvages, bourdons, biodiversité, environnement, Polinov, systèmes de culture, enquête, analyse multicritère, DEXi-Abeilles, observatoire, Ecobee.

ont leurs limites. Elles négligent les interactions entre facteurs agronomiques et environnementaux :
- la réduction durable de l'usage des insecticides ne peut s'affranchir de l'évolution globale des systèmes de culture et d'exploitation, pour concilier réduction des risques pour les abeilles et maîtrise des bioagresseurs des cultures ;
- vu la diversité des situations locales, il faut identifier un panel de solutions et disposer d'outils d'aide à la décision suffisamment génériques pour s'adapter à toutes les situations ;
- la mobilité des abeilles dans le milieu oblige à tenir compte des surfaces hors production en plus de celles en production.

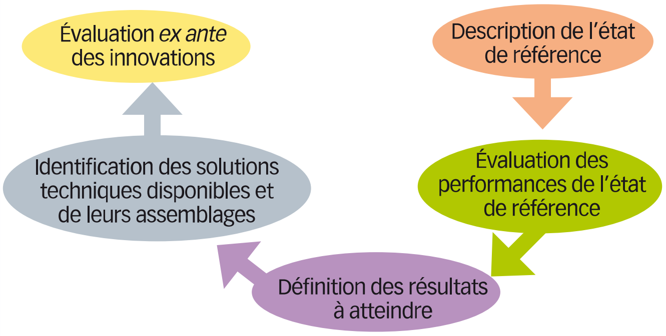
Polinov agrandit l'échelle

C'est pourquoi une démarche à plus grande échelle, celle du « système de culture » est nécessaire. C'est celle du projet Polinov, dont les objectifs étaient :
- acquérir des données de terrain pour analyser l'influence des systèmes de cultures actuels, et de l'organisation du paysage, sur les abeilles (observatoire Ecobee), en étudiant en particulier la disponibilité des ressources trophiques dans le milieu ;
- adapter les méthodes et outils d'analyse multicritère à l'enjeu de préservation des abeilles pour évaluer les performances des systèmes de culture en faveur de la biodiversité des abeilles sauvages et de l'état de santé du cheptel apicole, comme sur d'autres enjeux majeurs liés à la production agricole ;
- concevoir et évaluer des systèmes de culture innovants répondant aux enjeux de protection des abeilles et de durabilité de l'apiculture, en évaluant l'incidence sur la durabilité des productions végétales.

Fig. 1 : Démarche de Polinov pour concevoir des prototypes de systèmes d'exploitation agricole.

L'état de référence, à savoir les principaux systèmes d'exploitations agricoles sur la zone atelier Plaine et Val-de-Sèvre et l'organisation du paysage ont tout d'abord été décrits.
Les performances de ces systèmes ont été évaluées par analyse multicritère (DEXi-Abeilles) considérant la durabilité sociale, économique et environnementale (voir Figure 2). Les ressources disponibles pour les abeilles dans la zone ont été évaluées en parallèle par l'observatoire Ecobee grâce à une analyse fine du comportement alimentaire.

Des résultats à atteindre ont été définis.
Un inventaire des solutions techniques a ensuite été réalisé. Puis elles ont été assemblées dans des prototypes de systèmes d'exploitation (prototypages réalisés à partir des systèmes de référence).
Ces systèmes innovants ont, enfin, été évalués *ex ante*, c'est-à-dire avant mise en œuvre sur le terrain, par l'analyse multicritère. Celle-ci nous a permis de sélectionner les prototypes les plus prometteurs face aux résultats à atteindre.



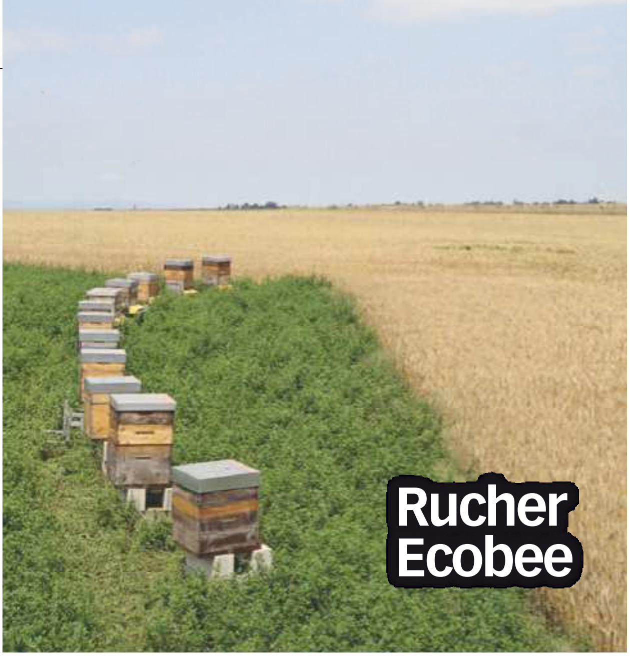


Photo : ACTA

Démarche retenue

Choisir les échelles d'espace et temps

Nous avons adapté la démarche décrite par le Réseau mixte technologique (RMT) *Systèmes de culture innovants* pour concevoir et évaluer les systèmes de cultures, afin de mieux prendre en compte la vaste aire de butinage des abeilles (supra-parcellaire) et leur cycle de vie annuel lié aux périodes de floraison (Figure 1). Nous nous sommes situés aux échelles de :
- la succession végétale en incluant les intercultures et les itinéraires techniques associés ;
- l'environnement proche de la parcelle soumis à l'influence du système de culture : espaces interchamps, haies, fossés ;
- l'exploitation qui associe plusieurs systèmes de culture dans différents îlots de productions.

Définir « abeilles » et « systèmes de culture »

Par « abeilles », on entend ici l'ensemble des hyménoptères apiformes. Il y en aurait 20 000 espèces dans le monde et 1 000 espèces environ en France. Le « système de culture » est l'ensemble des modalités techniques mises en œuvre sur les parcelles traitées de manière identique.

Description du territoire et de l'état de référence

Une zone atelier de 650 exploitations

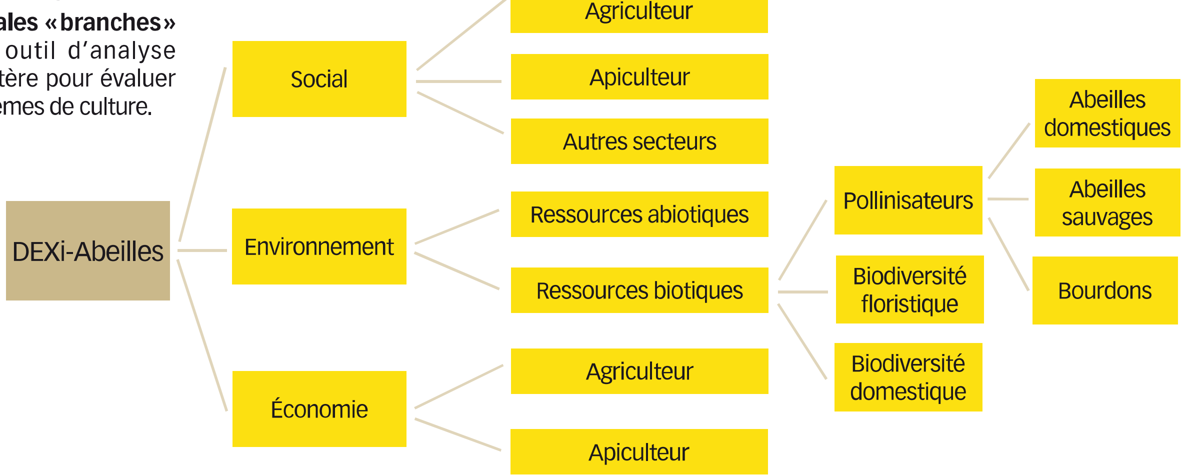
La zone atelier Plaine et Val-de-Sèvre, coordonnée par le Centre d'études biologiques de Chizé (CNRS), est une plaine céréalière au sud de Niort. Sur 450 km^2, elle comprend environ 650 exploitations agricoles divisées en 19 000 parcelles.
L'occupation des sols est inventoriée depuis 20 ans : plus de 30 % de céréales à paille, 10 % de tournesol, 8 % de colza, 8 % de maïs et 3 % de luzerne.
La moitié de la zone est sous statut de « zone de protection spéciale » avec un site Natura 2000. Annuellement, près de 9 000 ha de contrats agro-environnementaux sont signés (MAE Biodiversité, eau et agriculture biologique).

Informations recueillies

Tout d'abord, nous avons décrit les principaux systèmes d'exploitation agricole présents sur le territoire, à l'aide du logiciel Systerre développé par Arvalis-Institut du végétal.
Nous avons détaillé les systèmes au travers d'informations telles que le parc matériel, la main-d'œuvre, les cultures, le parcellaire, les interventions sur chaque parcelle, les prix des intrants et des extrants, les éventuelles mesures et les observations, les règles de décisions, les analyses de sol, etc. Les données ont

Fig. 2 : DEXi-Abeilles, structure générale.

Principales « branches » de cet outil d'analyse multicritère pour évaluer les systèmes de culture.



été fournies par des experts locaux de la chambre d'agriculture départementale, du Cetiom, d'Arvalis et de l'Institut de l'élevage. Les traitements phytosanitaires (date, fréquence, produit utilisé) ont été précisés grâce à l'enquête sur les pratiques d'une centaine d'agriculteurs de la zone atelier réalisée par l'ITSAP-Institut de l'abeille.
Les systèmes de référence 2009-2010 ont été mis à jour pour 2010-2011 en intégrant les rendements et prix de vente des deux campagnes.

Quatre systèmes ou « exploitations types »

Quatre systèmes d'exploitation ont été choisis pour représenter l'agriculture de la zone : céréalier non irrigué, céréalier irrigué, polyculture-élevage, agriculture biologique (Tableau 1).

Les trois premiers agrosystèmes de référence sont caractéristiques de la zone. Le système « bio », moins fréquent, nous a semblé à étudier vu les enjeux liant abeilles et pesticides. Il a été décrit par l'Institut technique de l'agriculture biologique (ITAB).

Évaluation de l'état de référence

Deux outils d'évaluation ont été développés :
- analyse multicritères des systèmes de culture (DEXi-Abeilles, voir Figure 2),
- observatoire de l'influence du paysage et des ressources alimentaires disponibles sur l'écologie des abeilles (Ecobee).

Notre but fut double. Il s'agissait d'abord d'évaluer les performances des systèmes de culture sur les critères d'intérêt, à savoir la santé de l'environnement et des abeilles ainsi que

Tableau 1 : Description succincte des systèmes d'exploitation pris comme références.

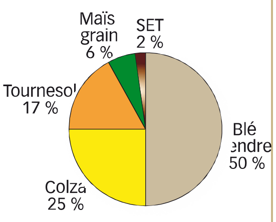
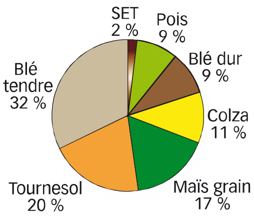
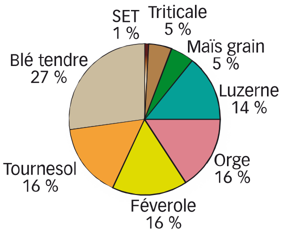
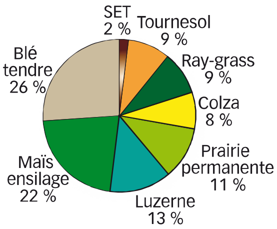
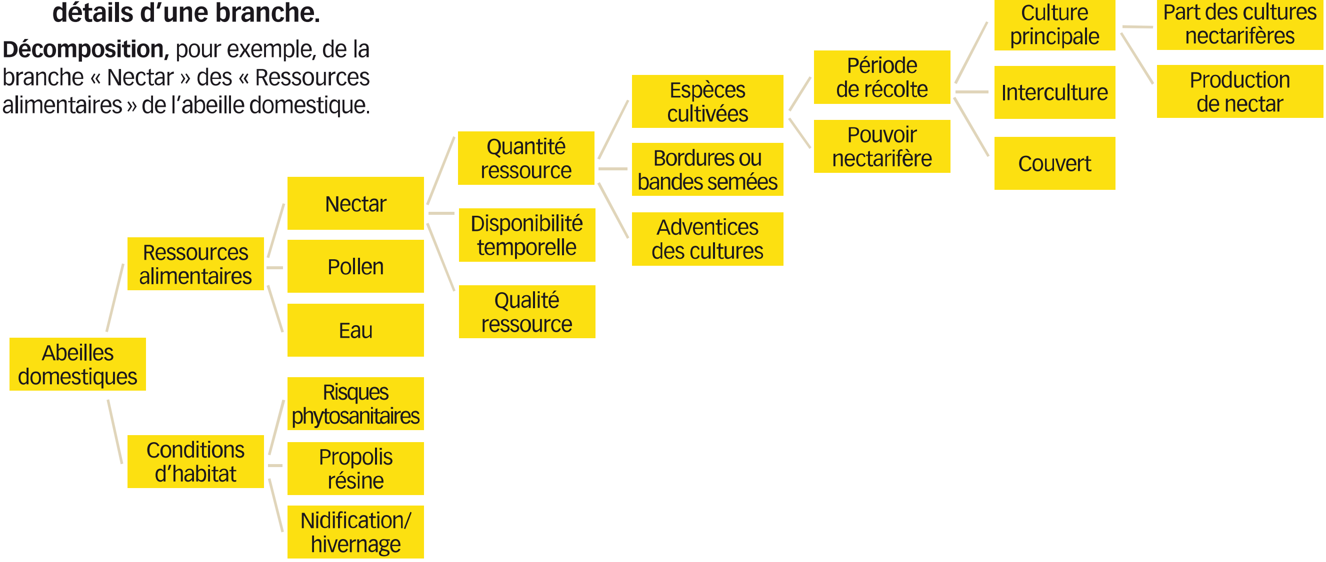
Systèmes	Céréalier sec en conventionnel	Céréalier irrigué en conventionnel	Céréalier AB (agric. biologique)	Polyculture élevage
SAU (ha)	120	110	100	115
Assolement				
(N.B. : SET = surface équivalente topographique)				
UTH (unité de trav. humain)	1	1	1	1
Type de sol	Groies superficielles	Groies superficielles	Groies superficielles	Groies superficielles
Cheptel	0	0	0	60-70 Vaches laitières
Ilots & Rotations *(N.B. : BTH = blé tendre d'hiver ; PP = prairie permanente ; Maïs ens. = maïs ensilage).*	**Ilot 1 (30 ha) :** Colza - BTH - BTH **Ilot 2 (80 ha) :** Colza - BTH-(moutarde) Tournesol - BTH **Ilot 3 (7,8 ha) :** Maïs grain	**Ilot 1 (40 ha)** : Tournesol - Blé dur - Pois - BTH **Ilot 2 (50 ha) :** Colza - BTH-(moutarde) Tournesol - BTH **Ilot 3 (18,1 ha) :** Maïs grain	**Ilot 1 (58,4 ha) :** Féverole - BTH - Orge - (moutarde) Tournesol - BTH **Ilot 2 (45 ha) :** Luzerne 1-Luzerne 2 - Luzerne 3 - BTH - (moutarde) Maïs grain - Féverole - Triticale - (moutarde) Tournesol - Orge	**Ilot 1 (15 ha) :** Colza - BTH - BTH **Ilot 2 (12,94 ha) :** PP **Ilot 3 (15 ha) :** (moutarde) Maïs ens. **Ilot 4 (35 ha) :** Luzerne 1 - Luzerne 2 - Luzerne 3 - BTH - Colza- BTH - (moutarde) Tournesol **Ilot 5 (35 ha) :** (moutarde) Maïs ens.- Ray grass 1 - Ray grass 2 - (moutarde) Tournesol - BTH - (moutarde) Maïs ens. - BTH
Offre en miel	9 147 kg	6 501 kg	7 605 kg	8 420 kg
IFT mars-sept insecticides	0,79	0,61	0	0,48
Indice toxicité abeilles	146,7	118,9	0	69

Fig. 3 : DEXi-Abeilles, détails d'une branche.

Décomposition, pour exemple, de la branche « Nectar » des « Ressources alimentaires » de l'abeille domestique.



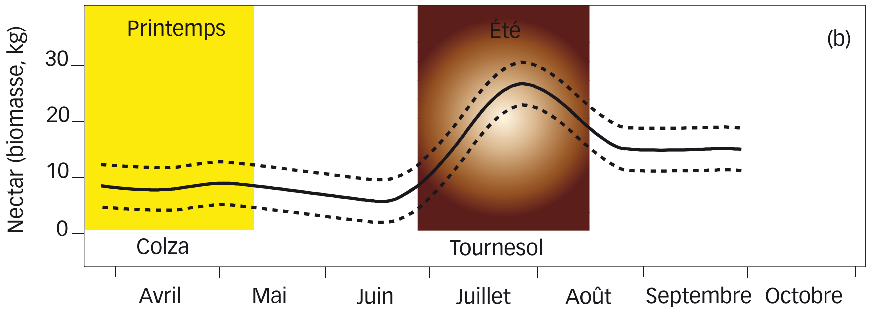


Fig. 4 : Époques à miel.

Réserves en miel mesurées dans les ruches en fonction du temps.
Ce graphique établi à partir de cinq années de suivi des ruchers d'observation indique deux périodes où les réserves en miel sont significativement supérieures. L'une, en début de saison lors de la floraison du colza, est concomitante à l'accroissement des populations. L'autre, beaucoup plus importante, a lieu lors de la floraison du tournesol.

la durabilité de l'apiculture et de l'agriculture; ceci pour pouvoir ensuite juger de l'évolution de ces performances dans les prototypes de systèmes innovants.

Notre deuxième but était de préciser où l'innovation doit porter ses fruits.

DEXi-Abeilles, l'analyse des systèmes

Onze critères pour trois intérêts

DEXi-Abeilles est un outil permettant d'évaluer les systèmes de culture sur trois bases :

– intérêt économique (pour l'agriculteur et l'apiculteur),
– intérêt environnemental,
– acceptabilité sociale.

Il a l'avantage de pouvoir considérer des critères multiples et parfois contradictoires, grâce auxquels il est possible de choisir, classer ou trier les stratégies décrites dans les différents scénarios de systèmes de culture.

Onze critères agrégés permettent l'évaluation à l'échelle de la rotation, en tenant compte des pratiques agricoles intraparcellaires et de la gestion des bords de champ.

L'impact des systèmes de culture est évalué sur les abeilles sauvages et domestiques.

Un arbre dans l'ordinateur

DEXi-Abeilles a été développé par le laboratoire Agronomie et Environnement (UMR 1121 INRA-université de Lorraine) sur la base d'un outil d'aide à la décision multicritères, DEXi. Son élaboration a consisté d'abord à décrire et décomposer l'évaluation de la durabilité des systèmes de culture sous forme d'un arbre. La Figure 2 page précédente représente les principales « branches » de l'arbre.

Chaque élément terminal de l'arbre a lui-même été décomposé (voir Figure 3) pour évaluer la valeur des systèmes de cultures vis-à-vis des abeilles domestiques.

À chaque élément de l'arbre a été associé un classement (ex : valeur du système pour les abeilles domestiques très favorable, favorable, moyenne, défavorable ou très défavorable).

Structure, règles, pondérations...

Pour agréger les éléments, des règles de décision ont été formalisées sous la forme *« [si ... alors] »* et des pondérations ont été attribuées à chaque élément.

La structure de l'arbre, les règles de décisions et pondérations ont été établies à partir de la littérature scientifique et technique, de bases de données (ex : valeur pollinifère et nectarifère des cultures et plantes spontanées) et d'avis d'experts apidologues, écologues et agronomes. Les éléments terminaux de l'arbre sont les variables d'entrée dont DEXi-Abeilles a besoin pour évaluer la durabilité des systèmes de culture.

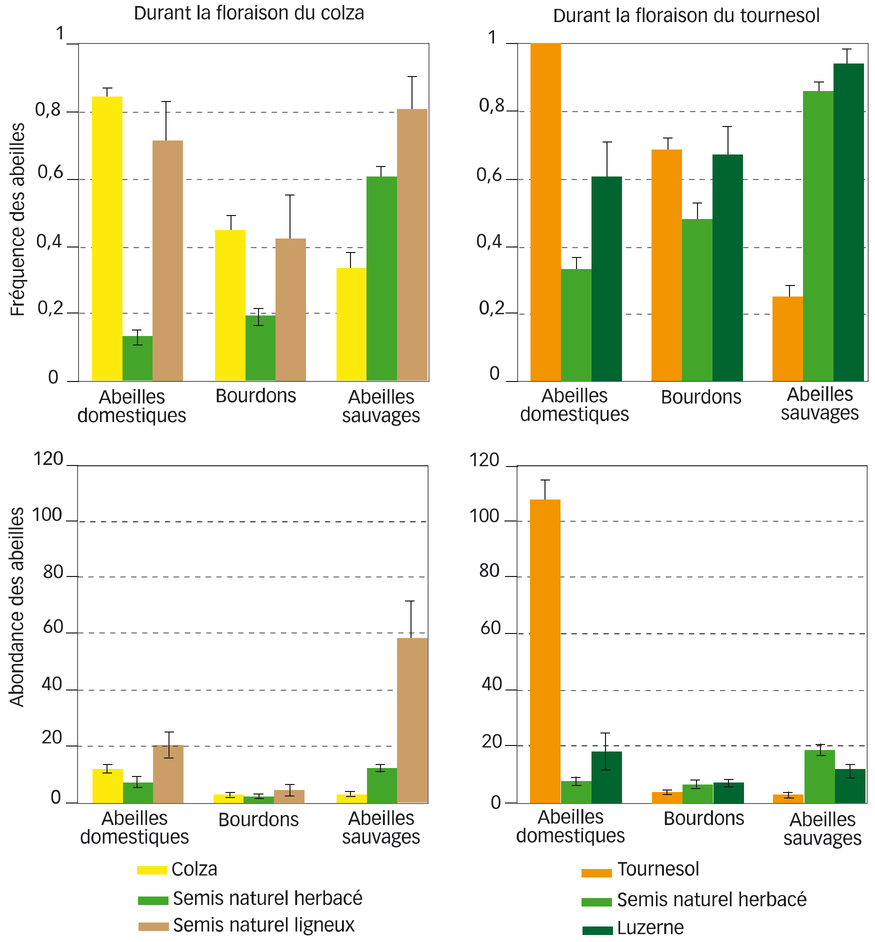
... et 89 variables

Au final, l'outil intègre 89 variables. La plupart sont des informations d'enquête facilement accessibles sur le système de cultures (ex : proportion de cultures principales nectarifères), et moins de 10 % issues de *Systerre* et d'*Indigo*.

Certaines branches de l'arbre sont issues de la procédure d'évaluation de l'outil *Masc* (ex : impact sur les ressources abiotiques).

Fig. 5 : Comportement des pollinisateurs.

Présence des pollinisateurs sur différents couverts végétaux en fleurs. Il y a de nettes différences dans l'utilisation des ressources fleuries entre les groupes d'abeilles durant la floraison du colza et du tournesol. Les abeilles domestiques, et dans une moindre mesure les bourdons, préfèrent butiner les fleurs de colza et de tournesol que les fleurs sauvages des prairies et des bordures, alors que c'est l'inverse chez les abeilles sauvages.



Ecobee, l'observatoire

Des ruches au centre des carrés

L'observatoire Ecobee vise à mieux connaître l'écologie des abeilles domestiques et sauvages en zone céréalière.

Le site d'étude a été divisé en 50 carrés de 10 km². Chaque année, 10 carrés ont été tirés aléatoirement, sans remise, pour faire l'objet d'une expérimentation. Un rucher de 5 ruches a été placé au centre de chaque carré, puis suivi d'avril à octobre. Nous avons mesuré 4 variables principales :

1) taille de la population des adultes,

2) surface occupée par les stades larvaires et nymphaux,

3) surface occupée par le couvain mâle (c'est un indice de l'allocation à l'effort reproducteur),

4) poids des réserves alimentaires.

Chaque ruche a été équipée d'une trappe à pollen, mise en service pendant 24 heures tous les 10 jours.

Au printemps **69 % du pollen** des ruches Ecobee vient de plantes **arbustives.**

Pollen de trappe, irrégularité confirmée

Chaque échantillon fut pesé, puis un prélèvement a fait l'objet d'analyses palynologiques. Celles-ci ont permis l'identification botanique des pollens jusqu'à l'espèce ou au genre. Puis ces espèces ont été regroupées en 5 catégories : ornementales, forestières et des haies, prairiales, cultivées, adventices des cultures. Cela nous a permis de confirmer l'hypothèse souvent avancée de la forte irrégularité aux cours du temps des ressources issues de la flore en zone céréalière.

La quantité de pollen et de nectar récoltée varie en effet fortement. On note des pics de récolte de pollens en juin et en août, et des pics de récolte de nectars durant la floraison des cultures oléagineuses, colza et tournesol.

Pollens analysés, résultat surprise : un régime plus varié que ce que l'on croyait

Contrairement aux attentes, le pollen issu des cultures ne représente qu'un tiers du butin annuel et est collecté essentiellement en fin d'été (tournesol et maïs). Ainsi, le régime alimentaire de l'abeille domestique dans ce paysage agricole céréalier est beaucoup plus varié qu'attendu.

Une part conséquente est liée aux plantes adventices des cultures et aux plantes arbustives (lisières forestières et haies). Au printemps, 69 % du pollen récolté provient des massifs boisés et des haies.

De mi-juin à mi-juillet, les quantités de pollen collectées se réduisent de plus de 50 % alors que la taille des populations atteint son maximum (en moyenne 27 500 ouvrières adultes). Bon nombre d'apiculteurs doivent donner des compléments alimentaires à leurs colonies durant cette période.

Si la part des récoltes de pollen sur les cultures oléagineuses a été plus faible qu'attendu, le rôle stratégique des miellées issues du colza, et surtout du tournesol, a été confirmé (Figure 4). Les résultats des inventaires faunistiques réalisés sur fleurs en témoignent (Figure 5).

Inventaires faunistiques sur fleurs, 30 000 « abeilles » identifiées

Ces inventaires, réalisés d'avril à août 2010, 2011 et 2012, ont consisté à capturer et identifier à l'espèce près de 30 000 abeilles butinant sur les fleurs, et ce sur plus de 800 couverts végétaux.
Ces abeilles ont été classées en trois groupes : abeilles domestiques, abeilles sauvages et bourdons.
Les abeilles sauvages ont préféré butiner les fleurs sauvages des prairies et bordures plutôt que celles du colza ou du tournesol. Ainsi, la diversité en abeilles sur la flore herbacée naturelle a été quatre fois supérieure à celle rencontrée sur le colza.
Au contraire, les bourdons ont été plus présents sur les fleurs des cultures oléagineuses que sur les autres couverts fleuris, mais de façon moins marquée que l'abeille domestique.

Les miellées de colza et surtout de tournesol ont confirmé leur rôle stratégique.

Esquisse du paysage idéal

Les connaissances issues de l'observatoire Ecobee nous amènent à imaginer une esquisse du paysage idéal pour la conservation des abeilles et pour l'apiculture.
Il devrait présenter des cultures à l'importante masse florale appréciée des abeilles domestiques et des apiculteurs (colza, tournesol, luzerne). Mais il devrait comporter aussi des surfaces à flore plus variée, préservée dans la durée, offrant ainsi des apports alimentaires plus réguliers dans le temps : haies, bois, bosquets, bandes enherbées, lisières, bords de champs et de routes, etc.

Autre rucher Ecobee. **Noter le paysage plus diversifié que celui entourant le rucher p. 42 : jachère (premier plan) mais aussi arbres, haie…**

Photo : ACTA

Si l'on traduit ces connaissances en termes d'effet de mesures agro-écologiques favorisant une flore spontanée et diversifiée, on s'attend à ce que ce type de couverts attire peu d'abeilles domestiques et de bourdons durant les floraisons du colza, du tournesol et de la luzerne. Mais il devrait être très utile aux abeilles sauvages, sauf durant la floraison de la luzerne, aussi attirante pour les abeilles sauvages que les couverts herbacés spontanés.
Ces connaissances acquises grâce à nos inventaires ont été intégrées pour concevoir les techniques innovantes.

Prise en compte du risque d'intoxication

La forte dépendance des abeilles domestiques envers les cultures oléagineuses, ainsi que la récolte significative du pollen de maïs (plus de 30 % du pollen récolté de mi-juillet à mi-août), poussent à prendre en compte le risque d'intoxication lié aux pesticides agricoles.
En effet, cela a été détaillé lors de la description des itinéraires techniques, le colza et le tournesol s'inscrivent dans des systèmes de cultures céréaliers. Or ces systèmes, dans leur grande majorité, utilisent une protection chimique conventionnelle des plantes.
Parmi les traitements usuels sur le territoire, les experts apidologues ont jugé particulièrement *« à risque »* : *Karaté K* (à base de lambda-cyhalothrine) sur colza et tournesol, *Décis Expert* (à base de deltaméthrine) sur maïs, *Gaucho TS31* (à base d'imidaclopride) sur blé tendre, *Cruiser TS* (à base de thiaméthoxam) sur colza et maïs.
Ce risque peut être lié à l'application d'un insecticide à proximité ou durant la période de floraison de la culture, ou à l'emploi de semences traitées avec un insecticide systémique.
Des résidus de ces derniers peuvent être présents dans les ressources que les abeilles récoltent sur la plante traitée ; par ailleurs, certains experts évoquent un risque de contamination de plantes mellifères cultivées après une plante traitée avec un insecticide systémique (ex : colza d'hiver après blé tendre d'hiver traité *Gaucho*).
Ces différents niveaux de risque d'exposition des abeilles ont été pris en compte dans la conception des systèmes innovants. Un prochain article évoquera cela.

POUR EN SAVOIR PLUS

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POLINOV vers des systèmes de cultures favorables aux abeilles

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Projet Polinov, épisode 2
Vers des systèmes de cultures favorables aux abeilles

Proposition de nouveaux systèmes de cultures basés sur la compréhension des interactions abeilles/zones céréalières.

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Polinov est un projet de recherche pluridisciplinaire mené sur la zone d'étude Plaine et Val-de-Sèvre, près de Niort (Deux-Sèvres). Il visait à concevoir et préévaluer des systèmes d'exploitation agricoles qui soient favorables à l'apiculture, aux abeilles domestiques et sauvages, mais aussi durables techniquement et économiquement pour les agriculteurs. Un précédent article a raconté les premières étapes du projet. Voici la suite.

Rappel de l'épisode précédent

État de référence décrit

Le travail comprenait cinq étapes. Les deux premières ont été décrites dans l'article précédent. La première étape fut la description de « l'état de référence » : quatre systèmes d'exploitation agricoles caractéristiques de cette zone céréalière ont été définis.

Performances évaluées

Deuxième étape, ils ont ensuite été évalués pour leurs performances selon plusieurs critères : économiques pour les agriculteurs et les apiculteurs, sociaux pour les agriculteurs, les apiculteurs et les autres secteurs, et enfin environnementaux, notamment (mais pas seulement) vis-à-vis des abeilles domestiques, des abeilles sauvages et des bourdons.

Outre les statistiques disponibles et les enquêtes et entretiens auprès des agriculteurs de la zone, deux outils ont été utilisés : l'outil d'analyse multicritère DEXi-Abeilles et l'observatoire des abeilles domestiques Ecobee, et ses ruchers.

Les détails méthodologiques sont dans l'article précédent.

Conception de systèmes d'exploitation agricole innovants

Abeilles, sept buts pour leur santé : définition des résultats à atteindre

Forts de notre connaissance de l'état de référence (systèmes d'exploitation actuels, organisation du paysage) et de ses répercussions sur les populations d'abeilles (irrégularité des ressources et risques liés aux insecticides), nous avons défini les sept résultats à atteindre pour améliorer la production de miel, la biodiversité et la santé des abeilles (Tableau 1).

Ce dernier objectif en regroupait deux principaux :

1) augmenter la quantité, la qualité et la disponibilité des ressources alimentaires des abeilles domestiques et sauvages,

2) diminuer les risques d'intoxication liés à l'application de pesticides.


Photo : M. Decoin

Jachère semée. **Les pollinisateurs les fréquentent notamment entre les floraisons du colza et du tournesol.**

Évaluation des besoins que cela implique

Les besoins alimentaires et en production de miel ont été simulés en estimant, de façon simplificatrice, qu'à chaque système d'exploitation était associé un rucher sédentaire de 50 ruches qui dans l'état de référence produisait deux miellées par an, celles de colza et de tournesol, chacune de 1 000 kg. À partir de bases de données attribuant une quantité de miel poten-

RÉSUMÉ

CONTEXTE - Le projet Polinov consiste à concevoir et évaluer *ex ante* des systèmes de cultures innovants favorables à l'apiculture et aux abeilles domestiques et sauvages, tout en évaluant leurs effets technico-économiques agricoles.

Les deux premières étapes, présentées dans un article précédent, ont consisté à :

– décrire l'état de référence (situation initiale) de quatre systèmes agricoles d'une zone géographique (Plaine et Val-de-Sèvres, Deux-Sèvres),

– évaluer leurs performances vis-à-vis des abeilles et d'autres critères (évaluation multicritère par le logiciel DEXi-Abeilles, étude des abeilles grâce à l'observatoire Ecobee basé sur le suivi de ruchers).

ÉTUDE - L'étape suivante a consisté à définir sept buts à atteindre.

La quatrième étape a été la conception de systèmes de cultures innovants en :

– choisissant les techniques estimées les plus performantes par DEXi-Abeilles,

– assemblant ces techniques (diverses combinaisons entre elles et avec les systèmes de référence) pour bâtir des prototypes de systèmes de cultures innovants.

La dernière étape a été l'évaluation multicritère, de nouveau par DEXi-Abeilles, des prototypes ainsi élaborés.

RÉSULTATS - Selon cette évaluation, les systèmes innovants améliorent l'état des abeilles et/ou des autres pollinisateurs, mais tous sont coûteux pour l'agriculteur. Il reste à vérifier cela sur le terrain.

MOTS-CLÉS - Abeilles domestiques, abeilles sauvages, bourdons, biodiversité, environnement, Polinov, systèmes de cultures, conception, systèmes innovants, prototypes, évaluation multicritère, DEXi-Abeilles.

tiellement produite par unité de surface implantée par telle ou telle plante, nous avons simulé la production en miel du rucher virtuel pour une année.

Deux indices ont été utilisés pour estimer la réduction de la pression exercée par les pesticides, l'un évaluant l'usage des produits (indice de fréquence de traitement ou IFT), l'autre évaluant plus spécifiquement l'impact sur les abeilles (QSA = dose appliquée de substance active (SA) par hectare/dose Létale de la SA tuant 50 % des effectifs ou DL50 abeille).

Partenaires et actions

L'atelier de conception fut constitué de représentants des structures partenaires œuvrant pour le développement des productions végétales (Chambre d'agriculture des Deux-Sèvres, Acta, Cetiom, Arvalis), des productions apicoles (ITSAP-Institut de l'abeille, ADA Poitou-Charentes), ainsi que de représentants des équipes de la recherche (Inra). Cette constitution a permis d'établir un état des lieux des connaissances disponibles sur l'influence du paysage et des systèmes de cultures sur les abeilles et de rechercher les solutions techniques à mobiliser.

La démarche a été la suivante :

1) constitution d'un groupe de travail rassemblant des agronomes et des spécialistes des abeilles,
2) réalisation d'un inventaire des solutions techniques innovantes,
3) définition de combinaisons des solutions techniques constituant les systèmes d'exploitation innovants,
4) évaluation multicritère des techniques élémentaires puis des systèmes innovants,
5) analyse comparative des résultats pour sélectionner les meilleurs prototypes de systèmes d'exploitation innovants.

Techniques innovantes mobilisées

Pour améliorer les ressources alimentaires des abeilles, quatre moyens testés

Dans le but d'améliorer les ressources alimentaires disponibles pour les abeilles dans les systèmes d'exploitation, nous avons envisagé la mise en place et la gestion de couverts végétaux produisant du nectar et du pollen sous quatre formes différentes :

- surfaces équivalentes topographiques (SET),
- cultures intermédiaires pièges à nitrates (Cipan),
- bandes fleuries,
- rotation culturale présentant des plantes mellifères et pollinifères.

Pour les 4 % de SET par SAU, nous avons introduit la composition de haies à partir d'espèces ligneuses d'intérêt (érable champêtre, cornouiller sanguin, viorne lantane, alisier torminal, troène), accompagnées de jachères implantées à l'automne d'un mélange à base de trèfle hybride, sarrasin, minette, sainfoin, lotier corniculé et phacélie. Cette jachère peut être broyée au début de la floraison du tournesol afin d'apporter une seconde floraison en fin de saison.

L'aménagement des SET avec des espèces végétales produisant du nectar et du pollen se révèle être une mesure insuffisamment efficace pour atteindre les objectifs en termes d'apport en ressources trophiques pour les abeilles.

D'après les évaluations multicritère réalisées, l'introduction de cultures mellifères et pollinifères dans les terres arables sont les mesures présentant les performances les plus élevées.

Tableau 1 : Les résultats attendus des systèmes d'exploitation innovants

I.	**Santé des abeilles**
	Assurer l'autoconsommation des abeilles domestiques.
	• Obtenir une continuité des apports en nectar et en pollen durant minimum 3 mois (fin mars à fin juillet, ou fin juin à fin septembre).
	• Obtenir une continuité des apports en nectar et en pollen durant 6,5 mois (fin mars à fin septembre).
	Réduire les risques d'intoxication liés aux pesticides.
	• Ne pas utiliser de pesticides avec une toxicité QSA* supérieure à 50 en début de période d'activité des abeilles (avril à juin).
	• Diminuer de 30 % l'indice de fréquence de traitements (IFT) des insecticides en période d'activité des abeilles (mars à septembre).
II.	**Production de miel**
	• Obtenir une troisième miellée de 1 000 kg de miel minimum entre celle du colza et celle du tournesol.
III.	**Biodiversité**
	Diversité de l'assolement.
	• Doubler, au minimum, la richesse spécifique des espèces semées qui fournissent du nectar et/ou du pollen.
	Diversité de la flore.
	• Augmenter la richesse spécifique des plantes sauvages ou semées apportant du nectar et/ou du pollen et qui ne reçoivent pas de pesticides, sur au minimum 5 % de la surface agricole utile (SAU).

*QSA = dose appliquée de substance active (SA) par hectare/dose létale de la SA tuant 50 % des effectifs (DL50 abeille).

Pression des pesticides, stratégies testées

Pour réduire la pression liée à l'usage des pesticides s'exerçant sur les populations d'abeilles, nous avons mobilisé plusieurs stratégies qui vont d'un remplacement des produits les plus à risque par d'autres connus comme étant moins risqués, à la suppression des traitements à base d'insecticides sur une partie (avril-juin) ou sur l'ensemble de la période d'activité des abeilles (mars-septembre), en passant par la diminution de la fréquence de certains traitements.

Quelques solutions techniques innovantes ont été testées pour réduire l'utilisation des pesticides, comme une réduction de la densité de semis du colza et de sa fertilisation, ou encore son association avec une plante gélive pour limiter l'usage des herbicides. Sur le tournesol, nous avons considéré la possibilité d'effectuer un désherbage mécanique ou des applications d'herbicides localisées lors du semis.

Techniques les plus efficaces choisies puis assemblées dans des prototypes

Pour la suite du travail, nous avons sélectionné les techniques innovantes les plus efficaces, à savoir la mise en place de bandes fleuries, l'introduction de plantes produisant du nectar et du pollen dans la rotation, ainsi que le remplacement ou l'élimination des insecticides à risque durant la période printemps-été et la réduction de l'usage des insecticides en automne.

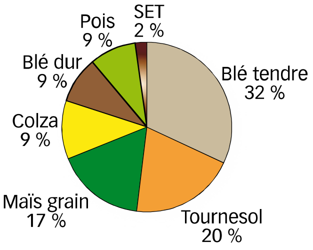
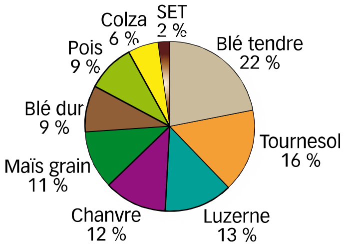
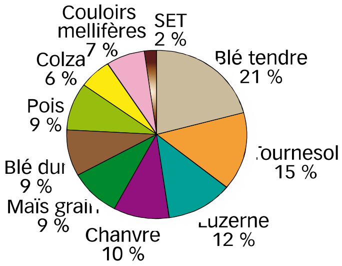
Ensuite ces techniques ont été assemblées dans des prototypes de systèmes d'exploitation (qui eux-mêmes sont des assemblages de plusieurs îlots ayant chacun un système de culture). De nombreux prototypes ont ainsi été testés, seuls les plus performants sont présentés dans la suite de l'article.Comme pour les systèmes d'exploitation de référence, les systèmes innovants ont été décrits grâce à l'outil Systerre.

Description des prototypes de systèmes d'exploitation agricole innovants

Céréalier irrigué avec introduction de luzerne, chanvre et Cipan : les cultures

Le premier prototype conçu est le système céréalier irrigué avec changement de succession végétale sur les îlots 2 et 3, consistant à introduire de la luzerne et du chanvre (Tableau 2). La luzerne est porte-graine durant deux ans, puis produit du fourrage durant un an. Cette culture apporte une rupture

Tableau 2 : Description succincte des prototypes de systèmes d'exploitation céréaliers avec irrigation

Systèmes	**Céréalier irrigué de référence**	**Céréalier irrigué innovant** **Changement de rotation et réduction des pesticides**	**Céréalier irrigué** **Changement de rotation et bandes fleuries**
Assolement			
Ilots & Rotations	**Ilot 1 (40 ha) :** Tournesol - Blé dur – Pois - Blé tendre d'hiver **Ilot 2 (50 ha) :** Colza - Blé tendre d'hiver (moutarde) - Tournesol - Blé tendre d'hiver BTH **Ilot 3 (18,1 ha) :** Maïs grain	**Ilot 1 (40 ha) :** Tournesol - Blé dur – Pois - Blé tendre d'hiver **Ilot 2 (50 ha) :** Luzerne porte-graine (2 ans) - Colza - Blé tendre d'hiver (sarrasin) – Chanvre - Blé tendre d'hiver (trèfle hybride/phacélie) - Tournesol **Ilot 3 (18,1 ha) :** Maïs – Maïs - Chanvre	**Ilot 1 (40 ha) :** Tournesol - Blé dur – Pois - Blé tendre d'hiver **Ilot 2 (45,1 ha) :** Luzerne porte-graines (2 ans) – Colza - Blé tendre d'hiver (sarrasin) – Chanvre - Blé tendre d'hiver (trèfle hybride/phacélie) - Tournesol **Ilot 2 bis (4,9 ha) :** Bandes fleuries **Ilot 3 (15,1 ha) :** Maïs – Maïs - Chanvre **Ilot 3 bis (3 ha) :** Bandes fleuries
Offre en miel	6501 kg	12166 kg	13573 kg
IFT mars-sept. insecticides	0,61	0,38	0,59
Indice de toxicité QSA	118,9	43,5	100,9

dans la rotation. Et nos inventaires faunistiques des abeilles ont montré qu'elle est une plante appréciée par les abeilles domestiques, sauvages et par les bourdons.

Le chanvre est une plante très pollinifère et très économe en intrants. Il a également été utilisé pour casser la monoculture de maïs sur l'îlot positionné en fond de vallée.

Entre la récolte du blé tendre et le semis du tournesol, nous avons intégré une Cipan avec du trèfle hybride et de la phacélie (à la place de la moutarde). Une Cipan à base de sarrasin a été prévue entre une culture de blé tendre et le chanvre. Semées sur 20 ha, ces plantes doivent assurer une floraison en septembre et octobre et, par conséquent, améliorer les stocks alimentaires dans les ruches avant l'hiver.

Actions sur les insecticides

Les applications des pesticides jugés « à risque » ont été remplacées de la façon suivante : Karaté K remplacé par Mavrik Flo (à base de tau-fluvalinate) sur colza et tournesol ; Décis Expert remplacé par du Coragen (à base de chlorantraniliprole) sur maïs grain ; Gaucho TS31 remplacé par du Decis Expert (à base de deltaméthrine) appliqué en novembre (hors période de butinage) sur blé ; Cruiser TS remplacé par du Belem (à base de cypermethrine) sur maïs.

Un passage avec Karaté K (à base de lambda-cyhalothrine) a été retiré de l'itinéraire sur blé.

La fréquence des autres traitements à base d'insecticides a été réduite sur le colza : Mageos (à base d'alphaméthrine) en septembre, Mavrik flo (tau-fluvalinate) fin mars.

Nos évaluations des performances de ce système indiquent une production de miel presque doublée et une réduction de la pression liée aux pesticides : IFT insecticide réduit de 37 % et indice de toxicité abeilles réduit de plus de moitié (Tableau 2).

Système irrigué avec bandes fleuries

Dans le second système céréalier irrigué innovant, nous avons appliqué les mêmes modifications de rotation et nous avons ajouté la création de bandes fleuries (Tableau 2).

Nous leur avons consacré une partie de l'îlot 2 (10 % de la surface) et de l'îlot 3 (6 % de la surface) en semant à l'automne un mélange associant trèfle hybride, sarrasin, minette, sainfoin, lotier corniculé et phacélie.

Les performances de ce système au niveau de la production de miel sont supérieures au premier prototype innovant. En revanche, la pression liée aux pesticides est quasi équivalente à celle estimée pour le système de référence.

Prototypes « céréaliers non irrigués » et AB

Les systèmes céréaliers non irrigués présentent les mêmes modifications que celles détaillées précédemment pour les systèmes irrigués : luzerne et chanvre dans la succession culturale, réduction des traitements insecticides, Cipan mellifères et pollinifères, implantation de bandes fleuries.

À ces techniques s'ajoute, sur un îlot, de l'orge suivi d'un tournesol en dérobé recherchant ainsi un étalement de la floraison dans la saison.

On enregistre également dans ces systèmes innovants une nette diminution de l'IFT insecticide dans la période d'activité des abeilles, de mars à septembre. Il en est de même pour l'indice estimant la toxicité des insecticides sur l'abeille domestique.

Dans le système en agriculture biologique, les Cipan mellifères et pollinifères ainsi que les bandes fleuries augmentent les ressources disponibles pour les abeilles.

Évaluation multicritère des systèmes innovants

Comparaison avec les références : mieux pour les abeilles

L'utilisation de DEXi-Abeilles nous a permis de comparer les performances des systèmes innovants avec celles des systèmes de référence actuellement existants sur la zone.

Comme attendu, les critères relatifs à la santé des populations de bourdons, d'abeilles sauvages et d'abeilles domestiques sont plus élevés dans les systèmes innovants que dans le système de référence. Par exemple, en céréaliculture irriguée (Figure 1, page suivante), le système avec réduction de l'usage des insecticides, associée à une nouvelle rotation, améliore nettement le score attribué à la santé des populations d'abeilles domestiques.

En revanche, la santé des autres abeilles, celles sauvages et les bourdons, est moins influencée par ces innovations car les sites semi-naturels pour nidifier avec une connectivité paysagère ne semblent pas suffisamment présents.

Ainsi, parmi les deux systèmes céréaliers irrigués innovants

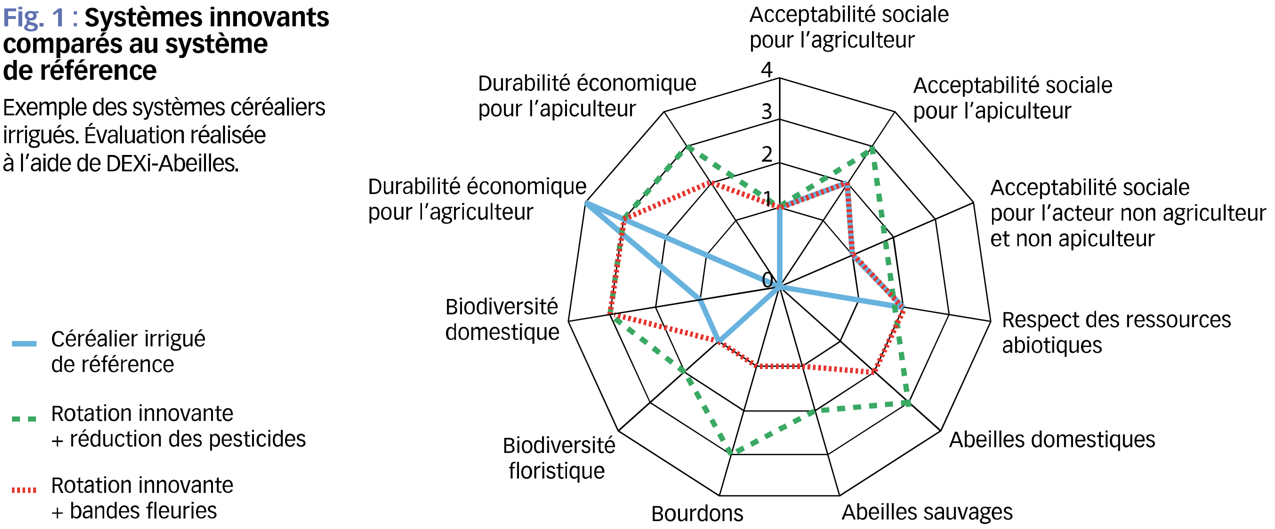


Fig. 1 : Systèmes innovants comparés au système de référence

Exemple des systèmes céréaliers irrigués. Évaluation réalisée à l'aide de DEXi-Abeilles.

présentés ici, c'est celui intégrant un changement de rotation et les bandes fleuries qui a les meilleures performances globales.

Un surcoût pour l'agriculteur

Mais la durabilité économique pour l'agriculteur des deux systèmes innovants est plus faible. Plus précisément, la mise en place des systèmes innovants provoque une diminution de la marge nette avec aides, donc un surcoût d'entre 4 000 et 5 000 € par exploitation et par an.

Pour améliorer l'acceptabilité économique de ces innovations, il peut être envisagé de les répartir sur un ensemble d'exploitations couvrant l'aire de butinage d'un rucher sédentaire.

Conclusion

Dans le temps, diversifier les rotations et changer les traitements

Pour conclure, nos travaux sur l'écologie des différentes espèces d'abeilles, prenant en compte les enjeux technico-économiques de l'apiculture et de l'agriculteur, dictent une gestion du territoire intégrant les dimensions spatiales et temporelles.

En effet, les techniques innovantes aux meilleures performances ont pour but de limiter l'irrégularité des ressources alimentaires dans le temps et, de ce fait, les répercussions sur les populations d'abeilles et les productions de l'apiculteur.

Les systèmes de cultures innovants qui associent ces techniques favorisent la santé des abeilles, de par leurs successions végétales riches en plantes produisant du nectar et du pollen. Ces mêmes plantes présentent l'intérêt non seulement de diversifier la rotation et de régulariser les ressources pour les abeilles mais encore d'être peu dépendantes de traitements phytosanitaires.

Nos simulations indiquent que la santé des abeilles est significativement améliorée si la réduction des traitements insecticides concerne la totalité de la période d'activité des abeilles, et pas seulement la période de floraison de telle ou telle culture.

Dans l'espace, diversifier le paysage

L'utilisation des infrastructures agroécologiques par les abeilles est modulée par la composition du paysage dans lequel ces infrastructures s'inscrivent. Nous montrons que la conservation de la flore sauvage est plus ou moins bénéfique aux abeilles sauvages selon sa proximité avec les systèmes de cultures et la nature des plantes cultivées.

Qu'il s'agisse des populations d'abeilles domestiques ou sauvages, elles se retrouvent renforcées lorsque nous proposons de diversifier les paysages agricoles.

Performances abeilles et coût pour l'agriculteur, prototypes à tester sur le terrain

À ce jour, les innovations les plus performantes pour les abeilles ont un coût économique et social pour l'agriculteur qu'il ne semble pas prêt à assumer dans le contexte de production actuel. La poursuite de la démarche passera par une mise à l'épreuve des innovations dans des exploitations agricoles (projet DEPHY-Abeilles).

Même si cette future étape nous permettra de réajuster certains critères économiques et sociaux liés aux innovations, il paraît évident aujourd'hui que l'optimisation du service de pollinisation sur un territoire implique un solide accompagnement d'un collectif d'acteurs agricoles (agriculteurs, apiculteurs), voire d'acteurs non agricoles (collectivités, chasseurs, naturalistes…).

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POUR EN SAVOIR PLUS

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Vol de retour des butineuse après intoxication

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Title: A common Pesticide Decreases Foraging Success and Survival in Honey Bees.

Authors: Henry, M., Beguin, M., Requier, F., Rollin, O., Odoux, J. F., Aupinel, P., Aptel, J., Tchamitchian, S. & Decourtye, A.

A Common Pesticide Decreases Foraging Success and Survival in Honey Bees

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Nonlethal exposure of honey bees to thiamethoxam (neonicotinoid systemic pesticide) causes high mortality due to homing failure at levels that could put a colony at risk of collapse. Simulated exposure events on free-ranging foragers labeled with a radio-frequency identification tag suggest that homing is impaired by thiamethoxam intoxication. These experiments offer new insights into the consequences of common neonicotinoid pesticides used worldwide.

Colony collapse disorder (CCD) is a recent, pervasive syndrome affecting honey bee (*Apis mellifera*) colonies in the Northern hemisphere, which is characterized by a sudden disappearance of honey bees from the hive (*1*). Multiple causes of CCD have been proposed, such as pesticides, pathogens, parasites, and natural habitat degradation (*2*–*4*). However, the relative contribution of those stressors in CCD events remains unknown. Some scientists and beekeepers suspect pesticides to hold a central place in colony-weakening processes (*1*) or at least in interaction with other stressors (*5*, *6*). In modern cereal farming systems, honey bees are readily exposed to pesticides because they rely heavily on common blooming crops, such as oilseed rape (*Brassica napus*), maize (*Zea mays*), or sunflower (*Helianthus annuus*), that are now routinely treated against insect pests (*3*). Systemic pesticides in particular diffuse throughout all the tissues as plants grow up, eventually contaminating nectar and pollen (*7*). Foraging honey bees are therefore directly exposed, but so is the rest of the colony as returning foragers store or exchange contaminated material with hive conspecifics (*7*, *8*). Those exposure pathways are of important concern, and pesticide manufacturers pay special attention to reduce nonintentional intoxications in field conditions. Pesticide authorization procedures now require running mortality surveys to ensure that doses encountered in the field remain below lethal levels for honey bees.

However, a growing body of evidence shows that sublethal doses—doses that do not entail direct mortality—still have the potential to induce a variety of behavioral difficulties in foraging honey bees, such as memory and learning dysfunctions and alteration of navigational skills (*9*). Neonicotinoid pesticides used to protect crops against aphids and other sap-sucking insects are especially liable to provoke such behavioral troubles. They are highly potent and selective agonists of nicotinic acetylcholine receptors, which are important excitatory neurotransmitter receptors in insects (*10*, *11*). Effects of sublethal neonicotinoid exposures in honey bees may include abnormal foraging activity (*12*–*14*), reduced olfactory memory and learning performance (*15*–*17*), and possibly impaired orientation skills (*18*). Yet, the consequences of such behavioral difficulties on the fate of free-ranging foragers and on colony dynamics are extremely difficult to assess in the field and remain poorly investigated.

In this study, we tested the hypothesis that a sublethal exposure to a neonicotinoid indirectly increases hive death rate through homing failure in foraging honey bees. We focused our attention on thiamethoxam, a recently marketed neonicotinoid substance (*19*) currently being authorized in an increasing number of countries worldwide for the protection of oilseed rape, maize, and other blooming crops foraged by honey bees. We proceeded in two steps. First, we assessed mortality induced by homing failure (m_{hf}) in exposed foragers. This was achieved by monitoring free-ranging honey bees with radio-frequency identification (RFID) tagging technology (*14*, *20*). Second, we assessed the extent to which m_{hf}, in combination with natural forager mortality, may upset colony dynamics. For that purpose, m_{hf} was introduced into a model of honey bee population dynamics (*21*).

We used a custom-made RFID device (*20*) to monitor the fate of 653 individual free-ranging foragers in the course of four separate treatment-versus-control homing experiments (*22*). The study was conducted in an intensive cereal farming system of western France, as a part of the ECOBEE monitoring facility (Zone Atelier Plaine et Val de Sèvre, Centre d'Études Biologiques de Chizé) and in a suburban area in Avignon, southern France. To simulate daily intoxication events, foragers received a field-realistic, sublethal dose of thiamethoxam (a real dose of 1.34 ng in a 20-μl sucrose solution) and were released away from their colony with a microchip glued on their thorax (Fig. 1A). RFID readers placed at the hive entrance (Fig. 1B) were set to detect on a continual basis tagged honey bees going through the entrance. Mortality due to postexposure homing failure, m_{hf}, was then derived from the proportion of nonreturning foragers. To further discriminate m_{hf} from other causes of homing failure in treated foragers—such as natural mortality, predation, or handling stress—we simultaneously released equal numbers of control foragers fed with an untreated sucrose solution. Hence, m_{hf} was calculated as the proportion of nonretuning treated foragers relative to expectations given by the proportion of returning control foragers. Depending on the experiment, tagged honey bees were released up to 1 km away from their respective colony, a distance usually covered by foragers during normal foraging flights (*23*). Experiments were conducted on individuals from three different colonies (*22*).

Our strategy was not to get an estimate of m_{hf} per se. Instead, we assessed its upper and lower bounds, depending on whether foragers were familiar or not with the foraging site in which they might get intoxicated. Indeed, one might expect that foragers familiar with the pathway back to the colony are less prone to homing failure than are unfamiliar foragers. Under field conditions, many foragers are probably familiar with the pathway back to the colony because they repeatedly forage on the same

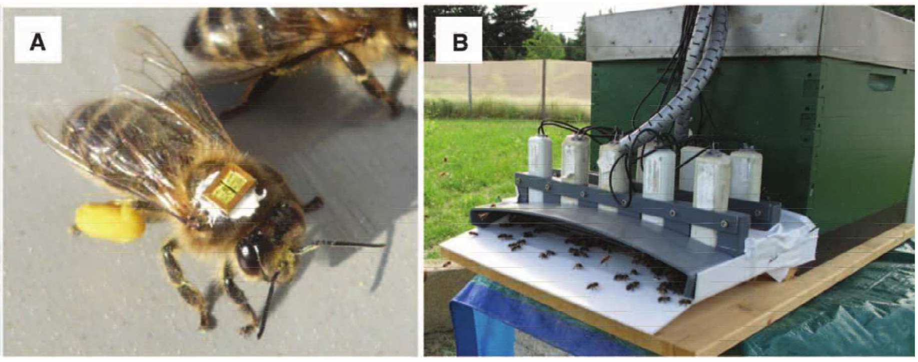

Fig. 1. Honey bee RFID monitoring equipment. (**A**) A pollen-forager honey bee fitted with a 3-mg RFID tag. (**B**) A hive entrance equipped with RFID readers for detecting returning marked foragers.

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site (*24*). However, many others are unfamiliar, too. Those include young honey bees at the onset of foraging, scouting honey bees that look for new food sources, and foragers newly recruited by scouting bees on the basis of the dance information (*25*). Most importantly, systemic pesticides such as thiamethoxam are readily present in the nectar and pollen when flowering starts and receive the first visits of honey bees, hitherto unfamiliar with this newly available food source.

To account for individuals' past foraging experience, we conducted two distinct homing experiments. Experiment 1 simulated intoxication at a familiar foraging site, and experiment 2 at a random site regarding past foraging experience. These experiments were assumed to return the lower and upper bounds of m_{hf}, respectively. In experiment 1, we referred to as "familiar" foragers those foragers for which we could make sure they covered at least once the pathway from the release site back to the colony. For that purpose, we selectively captured foragers returning to the colony with pollen loads from a known location and subsequently released them at that location. To ascertain pollen origin, we sowed beforehand a 1-ha field with scorpion weed *Phacelia tanacetifolia*, a highly attractive floral resource with bright blue pollen that is easily recognizable (*26*). Given that no other phacelia fields occurred in the area, we could ensure that phacelia-carrying honey bees came back from our experimental field. The colony was specifically placed 1 km away from the field for subsequent forager release (Fig. 2). In experiment 2, we used the non-phacelia pollen foragers. They were released in equal groups at six sites equally spaced on a 1-km circle around the colony (Fig. 2). Following that design, release sites were considered as random locations regarding the past experience of foragers.

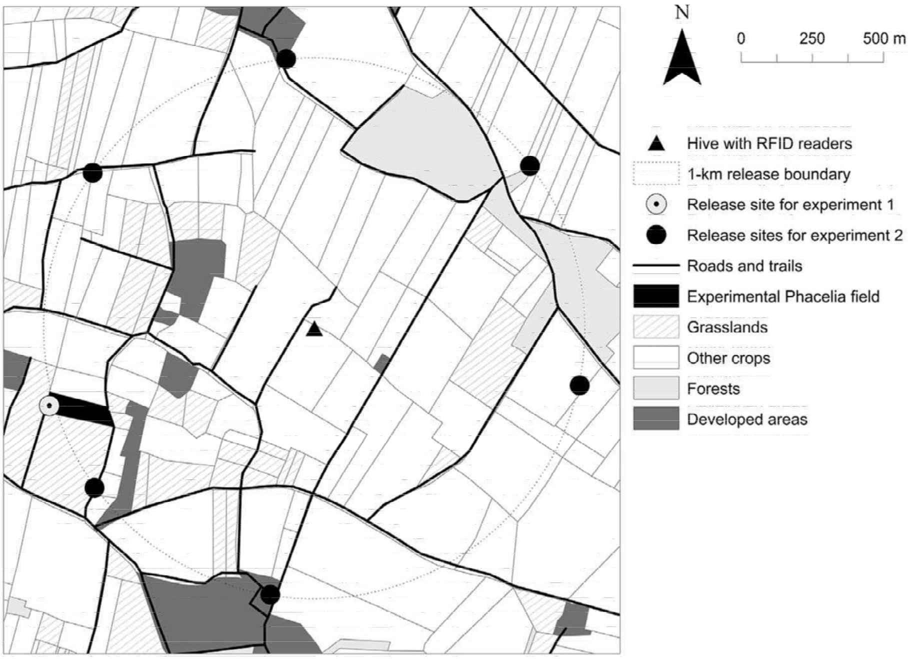

Fig. 2. Study area and location of honey bee release sites relative to the colony hive in experiments 1 and 2.

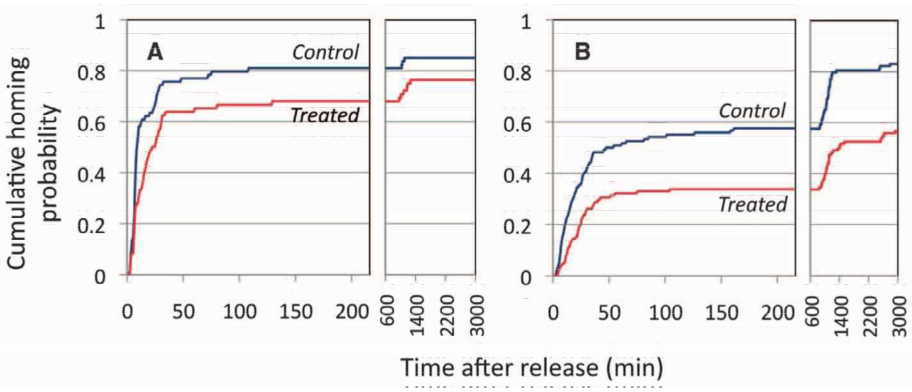

Fig. 3. Cumulative homing probability of foragers released 1 km away from the hive. Temporal gaps denote the nighttime between the first and second days of release. (**A**) Homing experiment 1 was carried out with foragers familiar with the release site, and (**B**) experiment 2 with foragers released at random sites regarding their past experience. In both cases, treated honey bees that received a nonlethal dose of thiamethoxam returned to the hive in significantly lower proportions than did control honey bees (table S1).

Both experiments 1 and 2 evidenced substantial mortality due to postexposure homing failure, m_{hf}, with the proportion of treated foragers returning to the colony being significantly lower than that of control foragers (exact binomial tests, $P = 0.033$ and $P < 0.001$, respectively) (Fig. 3 and table S1). Additionally, m_{hf} was greater in treated foragers that tended to be unfamiliar with the foraging site, as indicated by their significantly lower homing proportions as compared with familiar foragers (exact binomial tests, $P < 0.001$). Experiments 1 and 2 returned m_{hf} estimates of 0.102 and 0.316, respectively, potentially setting the lower and upper bounds for real m_{hf} values. In other words, 10.2 to 31.6% of exposed honey bees would fail to return to their colony when foraging in treated crops on a daily basis. For the sake of comparison, foragers live ~6.5 days and therefore die at an average rate of 1/6.5 = 0.154 individual day^{-1} (*27*). Therefore, the probability that a forager would die because of homing failure during a day spent foraging on treated crops (up to 0.316) may attain twice the probability this same forager has to die naturally that day (~0.154).

Such an additional mortality might represent a heavy burden to bear for colonies exposed to treated crops in their environment. When implementing m_{hf} into a honey bee population dynamics model (*21*), all the tested scenarios predicted a major deviation from the expected dynamic (Fig. 4). In our simulations, we considered the evolution of a typical colony during the first 3 months of a beekeeping season, encompassing the oilseed rape blooming period, which was April to May in our study area (*22*). At this time of the year, colonies emerge from the wintering period. Population size is rather low (<20,000 individuals) and gradually expands in order to rapidly increase food storage and ensure colony sustainability. The daily egg-laying rate of the queen is a critical parameter in this colony dynamic because it determines the daily egg-hatching rate and in turn the rate at which honey bees working in the hive will be replaced as they become themselves foragers. We simulated three scenarios with realistic levels of egg-laying rate (*28*), namely a rate allowing for a normal colony development (Fig. 4A), a rate ensuring equilibrium population (Fig. 4B), and a slightly deficient rate forcing the population to stabilize at a lower size (Fig. 4C). In each case, we also computed the expected trends if most foragers (90%) were exposed to nectar of treated oilseed rape each day and therefore had a natural mortality increased by a homing failure probability m_{hf}. Regardless of the queens' egg-laying rate, populations from colonies exposed to the treated nectar would follow a marked decline during the

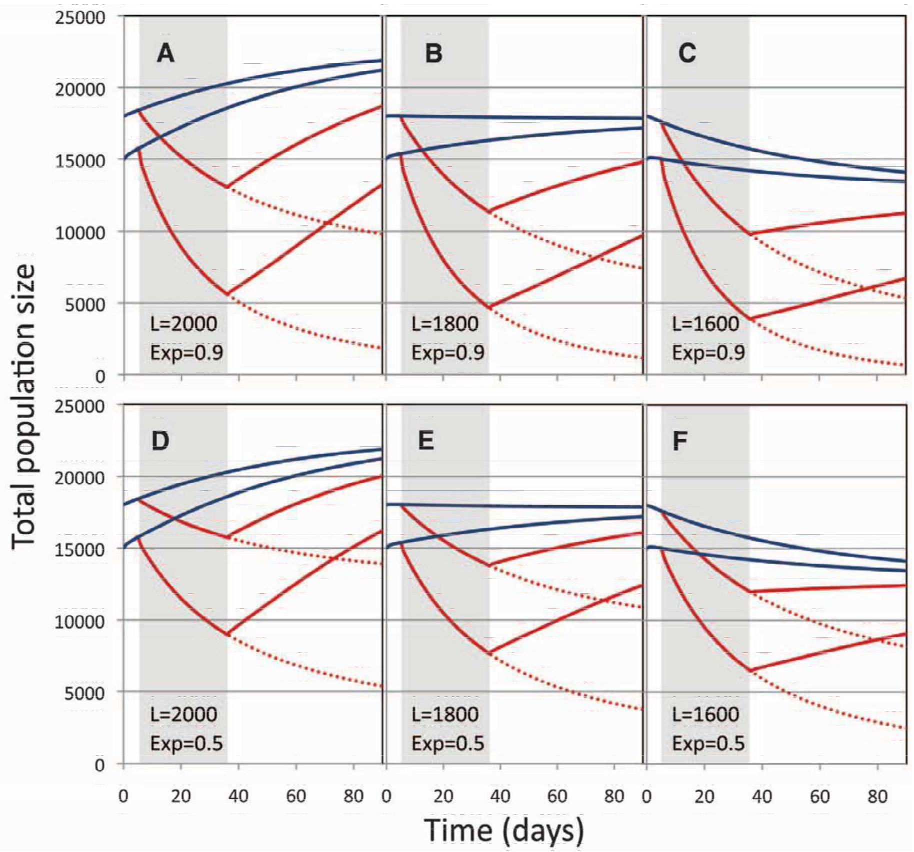

Fig. 4. Comparison of honey bee population dynamics between simulated colonies exposed to thiamethoxam (red lines) or not exposed (blue lines), following six demographic scenarios. *L* is the queen's daily laying rate (eggs per day). "Exp" is the proportion of foragers exposed to treated crops during the day. The nonexposed colony follows either (**A** and **D**) a normal development trajectory (at *L* = 2000), (**B** and **E**) an equilibrium dynamic (*L* = 1800), or (**C** and **F**) a slightly declining trajectory (*L* = 1600). Shaded areas delineate the exposure period (for example, oilseed rape). Pairs of trajectories in exposed colonies were obtained with the lower and upper bounds of homing failure mortality (0.102 and 0.316) in order to delineate the best and worst estimates for population dynamics, respectively. Dotted lines extend the declining trajectory expected for a sustained exposure. [Simulations derive from demographic models in (*21*)]

blooming period and would hardly recover afterward (Fig. 4, A to C). When combined with natural forager mortality, m_{hf} raised total forager death rate up to a point that could hardly be compensated for by the rate at which new foragers are recruited. In the worse scenarios, populations would fall down to 5000 individuals, which is the lowest level one can usually observe in current beekeeping practices. With an exposure rate reduced to 50% of foragers exposed to treated nectar each day (Fig. 4, D to F), the model still predicts a major deviation from normal conditions.

In an attempt to verify the applicability of these results to other contexts, we repeated two additional experiments with two different colonies (fig. S2 and table S1). In experiment 3, we tested whether m_{hf} was still significant when exposure occurred in the least challenging situation: in the direct vicinity of the colony and with honey bees familiar with the foraging site. We repeated experiment 1 with phacelia foragers captured from a beehive placed at the phacelia field margin and released from inside the phacelia field, only 70 m away. Homing failure (m_{hf} = 0.061) (fig. S2A and table S1) was much reduced as compared with that of experiment 1 (m_{hf} = 0.102) but was still significant (exact binomial test, P = 0.003). In experiment 4, we transposed experiment 2 into a different landscape. A beehive was placed in a suburban area in southern France, including a mosaic of mixed farming fields and orchards of moderate size. Foragers were released 1 km away at six equidistant sites. Homing failure (m_{hf} = 0.098) (fig. S2B and table S1) was significant as well (exact binomial test, P = 0.029) but much smaller than in experiment 2 (m_{hf} = 0.316).

Our study clearly demonstrates that exposure of foragers to nonlethal but commonly encountered doses of thiamethoxam can affect forager survival, with potential contributions to collapse risk. Furthermore, the extent to which exposures affect forager survival appears dependent on the landscape context and the prior knowledge of foragers about this landscape. Higher risks are observed when the homing task is more challenging. As a consequence, impact studies are likely to severely underestimate sublethal pesticide effects when they are conducted on honey bee colonies placed in the immediate proximity of treated crops. This study raises important issues concerning exposed solitary bee species, whose population dynamics are probably less resilient to forager disappearance than are honey bee colonies.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1215039/DC1
Materials and Methods
Figs. S1 and S2
Table S1
Reference (*29*)
Database S1

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Published online 29 March 2012;
10.1126/science.1215039

Vol de retour des butineuse après intoxication, suite

Paper published in Science, (2012) 337, 1453.

Authors: Henry, M., Beguin, M., Requier, F., Rollin, O., Odoux, J. F., Aupinel, P., Aptel, J., Tchamitchian, S. & Decourtye, A.

Title: Response to Comment on "A Common Pesticide Decreases Foraging Success and Survival in Honey Bees".

Response to Comment on "A Common Pesticide Decreases Foraging Success and Survival in Honey Bees"

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Cresswell and Thompson have suggested an elegant way to improve honey bee colony simulations when forecasting the fate of colonies exposed to pesticides. Following their recommendations, we rescaled the model on a sound empirical data set. The adjusted forecast is bleaker than their tentative scenario.

Henry *et al.* (*1*) reported that sublethal doses of thiamethoxam, a neonicotinoid pesticide used on some common flowering crops, reduce the ability of exposed foraging honey bees to find the way back to their colony. The daily mortality probability due to homing failure, termed m_{hf}, was estimated to lie somewhere between 0.102 and 0.316 for foragers exposed and released 1 km away from their colony. Honey bee population models (*2*) predict that this abnormal mortality level causes a major deviation from the expected demographic trajectory. Models were run with 1-month exposure durations, with the underlying idea to simulate colonies exposed to treated oilseed rape.

Cresswell and Thompson (*3*) have proposed an adjustment of the population model and found no population change over this duration, at least with the least pessimistic exposure scenario ($m_{\mathrm{hf}} = 0.102$). Specifically, they have suggested an elegant way to assess w, the only model parameter that could not be calculated from empirical data. Parameter w is a negative feedback constant that moderates the production rate of new workers as the colony matures. Cresswell and Thompson cleverly used empirical colony growth data to infer w. They assumed that a colony of 18,000 individuals may grow by 40% in a month during oilseed rape blooming period (*4*), which is reached with $w = 16{,}000$. When this analytical solution is transposed to the exposure scenario, no population change is detected [figure 1 in (*3*)]. Cresswell and Thompson noted that our parameterization ($w = 27{,}000$) assumed an 11% growth only in the absence of pesticides and therefore predicts an excessive decrease for exposed colonies (–30% with $m_{\mathrm{hf}} = 0.102$).

The technical comment by Cresswell and Thompson is a sound cautionary note about simulation-based risk assessment of nonintentional pesticide effects. However, we would like to rectify an inaccurate statement in their comment. We did not claim that our simulation outcome had predicted colony collapse due to homing failure. Instead, we concluded that the levels of homing failure we measured are high enough to cause "a major deviation from the expected dynamic" (*1*). This conclusion is not ruled out by Cresswell and Thompson's model adjustment, as is merely illustrated by the virtually constant colony size gap (a ~45% difference) between exposed and nonexposed scenarios, regardless of the chosen w value [figure 1 in (*3*)]. Furthermore, we believe that the tentative value of 40% for colony growth on which Cresswell and Thompson have based their reasoning is not robust. It seems that it was obtained from three monitored colonies only, and no indication is given on the use of oilseed rape in the vicinity (*4*). Given the tremendous variability one usually observes among colonies, any attempt to derive model parameters from empirical data deserves stronger support. Here, we followed Cresswell and Thompson's valuable suggestion to solve the calculation of w, using a strong empirical data set.

We reanalyzed the ECOBEE (Ecological Honeybee Colony Monitoring) data set used in our original study to set a range of realistic starting values for colony size (*1*). ECOBEE is managed by our research groups with the objective to provide ecologists with detailed honey bee colony dynamic data under real beekeeping management conditions. Colony monitoring data, including adult population size, are collected biweekly within a network of about 50 colonies per year. Over the 2008 to 2011 beekeeping seasons— i.e., before thiamethoxam was marketed for oilseed rape protection in our study area— a total of 208 colonies have been monitored. They were allocated into 40 apiaries, evenly distributed over the 450-km^2 study area in order to cover a wide range of landscape contexts. As explained in our original study, this territory is an intensive cereal farming system where oilseed rape accounts for 8 to 10% of total land cover.

We computed an empirical colony size model to derive real colony growth data and to recompute exposure simulations accordingly. Colony size was modeled using generalized additive mixed models (GAMM). This modeling technique allows adjusting a temporal spline based on maximum likelihood, while giving the possibility to account for repeated measurements on the same colonies within a given year. The temporal axis was scaled on the Julian date since the beginning of oilseed rape blooming. Blooming dates are available from local long-term phenological surveys (*5*). The temporal spline predicts a steep population growth encompassing the blooming period, and a gradual decline thereafter, as colonies expend less effort into reproduction and

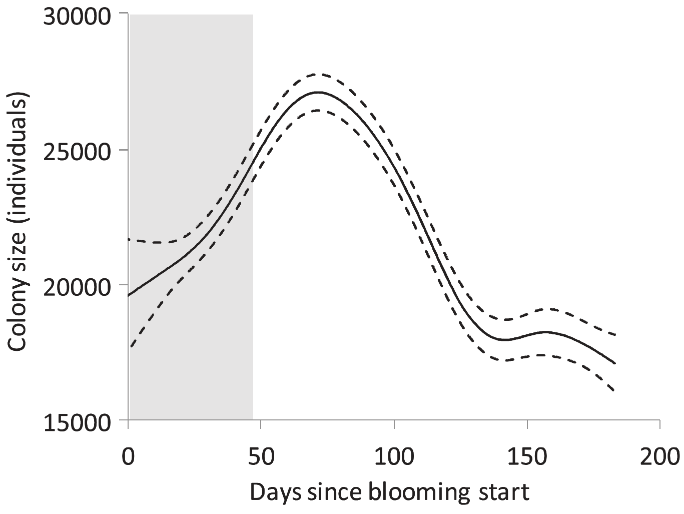

Fig. 1. Empirical honey bee colony size data (±1 SE) used to recalculate population models. Colony size is measured biweekly by weighting all hive elements (comb, frames, and supers) with and without bees, as a part of the ECOBEE monitoring facility. Seasonal changes in colony size are modeled by a temporal spline (GAMM). The shaded area denotes oilseed rape blooming period.

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more into food storage (Fig. 1). Although the oilseed rape blooming period hardly lasts more than 1 month in a particular field, there is a substantial temporal lag among fields and phenotypes, so that blooming period covered on average 48 days at the territory scale during the 2009 to 2011 ECOBEE monitoring program. To pinpoint the most plausible range of values for w, we sought for the analytical solutions that matched the average colony size values observed during different 30-day periods: (i) the initial blooming period (days 0 to 30 on the temporal axis, 11.7% growth), (ii) the full blooming period (days 10 to 40, 15.0%), and (iii) the late blooming period encompassing the steepest population growth (days 18 to 48, 18.7%). Analytical solutions for w were 24,932, 22,880, and 20,886, respectively, and also returned a close correspondence between observed and theoretical average age of onset of foraging (observed = 17.7 to 19.4 days, model = 18.2 days) and overall adult life span (observed = 22.3 to 22.8, model = 24.6). When homing failure was set to $m_{hf} = 0.102$, predicted population changes were –28.6%, –25.5%, and –22.1%, respectively.

These empirical-based scenarios are more pessimistic than the steady colony state predicted by Cresswell and Thompson. However, we agree that substantial improvement is needed before one could use honey bee colony modeling in its current form for risk assessment. We initially used modeling as a tool to get estimates of what observations made at the individual level would imply for the colony as a whole. Sound model adjustments have been proposed (*3*), but further issues remain to be documented to gain accuracy. Among others, homing failure should be reevaluated with regard to (i) doses matching in-hive exposures of conspecifics and larvae by contaminated pollen and honey (*6*) and (ii) acute versus chronic exposure regimes at the foragers' life scale. The latter aspect, in particular, is still an unsolved debate. It is currently unclear whether acute experimental exposures overestimate sublethal effects compared with chronic regimes (*7*). Likewise, population modelers should consider trying different values of the egg-laying rate, which appears to follow a sharp decline after oilseed rape blooming, as well as the post-exposure homing distance foraging honey bees need to cover. Those two parameters are expected to be largely influential in the procedure.

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Valorisations de thèse

PUBLICATIONS

PEER-REWIEVED SCIENTIFIC PUBLICATIONS:

In prep.

Requier, F., Odoux, J. F., Decourtye, A. & Bretagnolle, V. (in prep.) A review of honey bee ecology in agricultural habitats: floral resources have been overlooked as a possible cause of honey bee colony loss in intensive cereal farming systems.

Requier, F., Odoux, J. F., Henry, M. & Bretagnolle, V. (in prep.) Carry-over effects of spring pollen food-shortage on honey-bee colony winter survival in farmland habitats.

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OTHER PRESENTATIONS:

Requier, F., Odoux, J. F.*, Tamic, T., Henry, M., Decourtye, A. & Bretagnolle, V. (2013) Que mange l'abeille domestique en paysage agricole intensif ? *AG Syndicat d'Apiculture de l'Eure*. Conches, France.

Requier, F., Odoux, J. F. & Bretagnolle, V. (2013) Dynamique spatio-temporelle des ressources et écologie de l'abeille domestique en paysage agricole. *A.G.* "*Association Les Ailes de la Vie - Protection et Découverte des Abeilles*" Andilly, France.

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MEMBERSHIP IN SCIENTIFIC ORGANIZATIONS:

Membership in the international congress « Ecology & Behaviour », Chizé, France, 2012.

TEACHING & STUDIENT SUPERVISION

STUDENT SUPERVISION

2013

Lindy Mary Corredores Hurtado, Master 2, Cartographie Bio-production des Écosystèmes, Université de Nantes, 2013, « Distribution des Messicoles en Plaine Agricole et Utilisation par l'Abeille domestique (*Apis mellifera L.*) » (co-supervision with Jean-François Odoux and Vincent Bretagnolle)

Yoanna Marescot, Master 1, Ingénierie en écologie et en gestion de la biodiversité, Université of Montpellier 2, 2013 : « Rôle des adventices dans l'écologie de l'abeille en plaine agricole » (co-supervision with Jean-François Odoux and Vincent Bretagnolle)

2012

Flora Guillier, Master 2, Ecology, Biodiversity and Evolution, University of Paris 6, Pierre et Marie Curie (France), 2012 : « Etude des compromis de sélection alimentaire chez l'abeille domestique (*Apis Mellifera, L.*) pour des ressources polliniques variables dans l'espace et dans le temps » (co-supervision with Vincent Bretagnolle)

Mathilde Roger, Diplôme d'Ingénieur, Agronomiques, Agroalimentaires, Horticoles et du Paysage Institut Supérieur des Sciences, University of Angers (France), 2012 : « Santé de l'abeille domestique en paysage agricole » (co-supervision with Jean-François Odoux and Vincent Bretagnolle)

Laura Miot, Licence 3, PARTAGE, University of Rennes 1 (France), 2012 : « Ressources florales et influence de la composition paysagère sur l'abeille domestique (Apis mellifera) en système agricole céréalier » (co-supervision with Jean-François Odoux)

2011

Johanna Brenner, Master 2, Biodiversity, Ecology and Evolution, University of Göttingen (Germany), 2011 : « *Spatiotemporal variation in honey reserves within agricultural landscapes* » (co-supervision with Jean-François Odoux and Vincent Bretagnolle)

TEACHING

2012

Université Populaire Sud Deux-Sèvres, France, « Aperçu sur la biologie et l'écologie de l'abeille domestique *Apis mellifera* L. », 4h.

2011

Lycée agricole Georges Desclaude de Saintes, Charente-Maritime, France, « L'écologie alimentaire de l'abeille domestique *Apis mellifera* L. en paysage agricole : quels sont les ressources florales », 10h.