

Estimation of pollinating insect populations in agro-ecosystems: comparing oilseed-rape fields and AES flower-strips

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Estimation of pollinating insect populations in agro- ecosystems: comparing oilseed-rape fields and AES flower-strips.

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*To Aurélie Casadella,
left too soon.*

« Ecosystem services make human life possible by, for example, providing nutritious food and clean water, regulating disease and climate, supporting the pollination of crops and soil formation, and providing recreational, cultural and spiritual benefits. Despite an estimated value of \$125 trillion [in 2014], these assets are not adequately accounted for in political and economic policy, which means there is insufficient investment in their protection and management. »

(FAO, 2017)

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Résumé

La tendance globale de déclin des populations d'insectes pollinisateurs est maintenant établie. Bien que plusieurs facteurs soient impliqués dans cette baisse d'effectifs et de diversité, la simplification des paysages est l'un des principaux facteurs du déclin des populations d'insectes. Au niveau agricole, de nombreuses cultures sont au moins partiellement dépendantes de la pollinisation par les insectes. La perte du service éco-systémique de pollinisation entomophile s'accompagne 1) d'une baisse de qualité et de quantités de nombreuses productions agricoles, et 2) de la disparition d'un certain nombre de cultures. Les agriculteurs, de plus en plus influencés par les politiques agricoles à grande échelle et les marchés internationaux, sont tout à la fois les premiers responsables, les premières victimes mais aussi les principaux acteurs pouvant contrer ce déclin inquiétant. La politique agricole commune européenne a créé et subventionne des Mesures Agro-Environnementales (MAE) destinées à compenser ou réduire les effets négatifs de l'agriculture intensive sur l'environnement. Cependant, tant l'intérêt biologique de la plupart de ces MAE, que le niveau exact de dépendance à la pollinisation par les insectes de nombreuses cultures, restent mal connus.

Dans ce contexte, au travers de cette thèse, un de nos objectifs a été d'établir le niveau de dépendance à la pollinisation entomophile de la principale grande culture, potentiellement la plus entomophile du sud de la Belgique, le colza, *Brassica napus*. Nous avons étudié la biologie de la reproduction pour six des variétés de colza actuellement les plus cultivées dans le Condroz. Nous avons estimé le déficit de rendement lié au défaut de pollinisation par les insectes pour ces variétés. L'estimation de ce déficit apportera des arguments montrant l'intérêt limité pour les cultures de colza de la mise en place et du soutien de mesures dédiées à l'amélioration du service de pollinisation entomophile en zones de grandes cultures.

Parallèlement, nous avons étudié deux types d'aménagements MAE favorables aux insectes pollinisateurs en zones de grandes cultures, dans deux régions biogéographiques du sud de la Belgique, la Hesbaye et le Condroz. Nous avons pour objectif d'estimer les ressources florales fournies (pollen et nectar) et la diversité des insectes utilisant ces ressources, pour évaluer le potentiel de ces espaces en termes de service écosystémique de pollinisation.

Nos résultats montrent que les rendements, des variétés actuellement cultivées de *Brassica napus* sont très peu affectées par la pollinisation par les insectes. L'autopollinisation spontanée est très efficace. De plus, les taux de visites des fleurs par des insectes est extrêmement faible, seul 1% des fleurs ont été visitées par un insecte. La production de colza dans cette région, qui présente les rendements parmi les plus élevés au monde, se fait donc indépendamment d'une pollinisation entomophile.

Les MAE étudiées, des « bandes aménagées », ont montré une production de nectar intéressante (7 mg sucres/m²/jour) mais une production de pollen

principalement de faible qualité nutritive, déficitaires en certains acides aminés essentiels. Les MAE étudiées ont majoritairement accueilli des insectes d'espèces très communes et largement répandues en Europe. Ces espèces sont reconnues comme de très bons pollinisateurs. Cependant, 74% des 85 groupes/espèces d'insectes observées présentent des effectifs extrêmement faibles. L'intérêt de ces MAE pour la conservation de la biodiversité semble limité.

Nous proposons plusieurs pistes d'amélioration. Dans un premier temps les bandes fleuries devraient être intégrées dans des plans de gestion à l'échelle du paysage, permettant de combiner plusieurs MAE complémentaires (notamment bandes fleuries et MAE favorisant des espèces ligneuses à floraison printanières). Dans un second temps la gestion des bandes fleuries et la composition des mélanges semées pourraient être améliorés pour répondre plus précisément aux exigences d'espèces d'insectes localement présentes.

Abstract

The global trend of pollinator populations decline is now established. Even if several factors are responsible for this decline, landscape simplification is one of the primary drivers of insect decline. Numerous crops are, at least partly, dependent on insect-mediated pollination. The loss of this ecosystem service of insect-mediated-pollination conduct 1) to a reduction of the quality and quantities of numerous agricultural produces, and 2) made their production impossible. The farmers, primarily influenced by large-scale agricultural policies and markets, are both the firsts responsible, the firsts victims but also the leading actors that could invert this worrying decline. The European Common Agricultural Policy created and subsidizes several Agri-Environment Schemes (AES) to mitigate adverse impacts of intensive farming. But the precise biological interest of most of these AES and the exact level of insect-mediated-pollination dependency of numerous crops are still poorly known.

In this context, with this thesis, we aimed to investigate the level of insect-mediated-pollination dependency of the main crop potentially the most dependent to insects in South Belgium, oilseed rape, *Brassica napus*. We studied the breeding requirements of six of the most cultivated *Brassica napus* cultivars in Condruz. We estimated the yield gap induced by the deficit of insect-mediated pollination on these cultivars. Such deficit value will provide figure showing the small interest, for oilseed rape, of the implementation of schemes dedicated to insect-pollination services improvement in intensively managed farmlands.

At the same time, we studied two types of AES favourable for pollinating insects in intensively farmed area, in two biogeographic regions of South Belgium, Hesbaye and Condruz. We aim to assess the flower resources (pollen and nectar) such schemes provided to insects. We also sought to explore the diversity of insects hosted by such areas, to evaluate their potential to support insect-mediated pollination services or insect diversity.

Our results show that yields of current *Brassica napus* cultivars are nearly not affected by insect-mediated-pollination. The spontaneous self-pollination is very effective. Moreover, the insect-visit rate was low, 1% of the flowers were visited. The oilseed rape yield, among the higher in the world, is reached independently of insect-mediated pollination.

The studied AES, some “flowered-managed-field-strips”, provided interesting nectar resources (7 mg sugar/m²/day) but mainly poor-nutritive-quality pollen (essential amino acid deficient). The studied AES hosted mainly species of very common polylectic insect. These species are known to provide valuable pollination services. But 74% of the 85 morphotypes of insect observed were scattered, the biodiversity conservation interest of these AES strips seems to be small.

Thus, we made recommendations to improve these AES. First of all, flower-strips AES should be included in large-scale landscape management plans, combining flower-strips with complementary AES (especially AES including

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spring flowering woody species). Then flower-strip management and sown plant mixes could be improved according to local insects needs.

Context of the study

Since the fifties and the green revolution, the European agriculture follows a trend of intensification. This intensification goes through an increase of pesticide uses and landscape simplification. In parallel of this intensification, we observed a global trend of pollinating-insect decline. The offer of insect-mediated pollination service is therefore dwindling while the demand is increasing. As a consequence, a deficit of insect-mediated pollination is increasingly observed and would imperil several crop productions.

Since 1992, the European Common Agriculture Policy (CAP) subsidises specific measures to mitigate adverse impacts of intensive farming on the environment. These measures, the Agri-Environmental Schemes (AES), represent a sizeable part of the CAP but the evaluation of several AES remains delicate and incomplete. Thus, we cannot appreciate the pertinence of such AES and there is no field-data based improvement.

In South Belgium, two variants of an AES are implemented since 2008 in favour to the small wild fauna. The pollinator-strips are sown with perennial flower mixes to provide flower feeding resources to insects and the fauna-strips mainly sown with annual plant mixes to provide seeds for birds. These AES set up with entomophilous plant species, could be especially interesting to support flower-feeding insects and pollination service. Nevertheless, they were not yet evaluated. To appreciate the relevance and the efficiency of such AES strips, three steps will be necessary.

First, even if in a global context the support to insect-mediated pollination service is undeniably needed, local farming practices can modify the demand of insect-mediated pollination service. For most of the entomophilous crops, the exact level of insect-mediated pollination dependency is not known with certainty and can strongly fluctuate according to cultivars and cropping conditions. Therefore, the local estimation of pollination deficit is a needed prelude to evaluate the local need of pollination service and thus relevance of the implementation of schemes dedicated to pollinator support.

Secondly, it is necessary to evaluate the food resources (pollen and nectar) provided by the AES to flower-feeding insects. What are the quantities of resources provided by the AES to flower-feeding insects? Are these resources of sufficient quality and adequately distribute along the insect activity season?

Thirdly, the supported insect communities will be to evaluate and the environmental-services provided have to be assessed according to local needs. Which insects do AES support? Are these insects able to provide insect-mediated pollination services to crops? Are AES supporting insect diversity?

Introduction

I. PLANT-INSECT RELATIONSHIPS: NEEDS OF BOTH SIDES

A. *The plant side: dependency on pollinators*

a) *Pollination principles: spontaneous or vector-mediated pollen transfers*

Pollination is a transfer of pollen, the male gametophyte, from the anthers to the stigmas. Pollination is a crucial preliminary step in sexual plant reproduction (Willmer, 2011). It can occur spontaneously within a flower or among nearby neighbouring flowers or may involve biotic or abiotic vectors. The two most important pollination vectors are the wind (abiotic pollination) and the animals (biotic pollination). The autonomous self-pollination is only linked to spontaneous plant movements or gravity. The wind is a common pollination vector, mainly in temperate tree species. The wind pollination is much less precise than biotic pollination and requires huge quantities of pollen (Fægri and van der Pijl, 1979). At the opposite, animal-mediated pollination is a less random process (Fægri and van der Pijl, 1979). Several attractive characteristics as rewards, odours, colours or shape, contribute to the fidelity of visitors to reduce pollen loss and increase pollination effectiveness. In Europe, animal-mediated pollination is carried out by insects and refers to entomophilous pollination.

Entomophilous pollination often involves mutualistic interactions among flowers and insects (Fægri and van der Pijl, 1979). Insects visit flowers for food resources, mainly pollen and nectar, and contribute to pollination. The close agreement that can exist between flower resources and insect preferences favours the insect fidelity to a plant species (Waser, 1986). This fidelity reduces the stigma clogging with pollen from another species and the loss of pollen on another plant species. Most of the plants are generalists, they attract a broad diversity of insects and can be pollinated by different insect species. More rarely, the pollination of a plant species is linked to a reduced number of pollinating insect species. These species are less generalist or more specialist species. Insects are also considered generalist (polylectic) when they visit different plant species, and specialist when they visit only few plant species.

• **Fidelity** is the attachment of single foraging individual to a specific plant species (or type) despite the wide range of flower species available to it. Fidelity is also known as the pollinator flower constancy or the 'crop attachment' in the agricultural literature (Sanderson and Wells, 2005).

A pollinating insect is a flower visitor insect that realises pollination. All the pollinating insects are flower visitors, but all the flower visitors are not necessarily pollinators. To be efficient pollinators, flower visitors must have the ability to passively or actively pick up and carry pollen. Nevertheless, pollen aggregates in pollen loads, is mixed with saliva or compacted it in a way that makes it unfit for pollination. Thus, only the free pollen, not aggregated in pollen load, can be transferred from anthers to stigmas of flowers from the same species. An insect that has access to the resources it

visits the flower for, without touching the stamen nor the stigma (i.e. a tiny insect in a large flower, or a nectar robber) cannot be a pollinator.

The presence of surface structures as hairs, fur or scales strongly improve the pollen pick up and carriage capacities of an insect and so its chances to be an effective pollinator (Willmer, 2011). Coleoptera are therefore morphologically not willing to be efficient pollinators. Moreover, behavioural factors and morphological correspondences among insects and flowers are crucial to make the flower visitor an effective mover of pollen and thus potential pollinator (Goulson, 1999; Toullec, 2008). An insect that has access to the resources it visits the flower for, without touching the stamen nor the stigma (i.e. a tiny insect in a large flower, or a nectar robber) cannot be a pollinator. Last but not least, the flower constancy (fidelity) is another criterion to appreciate the capacity of an insect to be an effective pollinator.

b) The dependency on pollinators: the bases of a mutualistic relationship

Insects play a fundamental role in the pollination service (Allen-Wardell et al., 1998). Around the world, 87% of flowering plant species need animals for their pollination (Ollerton et al., 2011). Among cropped food species, around 75% are recorded as at least partly dependent on insect pollination (Klein et al., 2007). Pollinator dependency ratio takes into account the real impact of insect pollinators on crop production. This dependence ratio enables the calculation of the production loss in case of a complete disappearance of pollinators. This ratio only concerns the production of plant parts directly consumed by humans (Gallai et al., 2009). Entirely dependent (pollinator dependency ratio = 1) signifies that without any insect pollination, the plant (as Melonseed, *Cucumis melo*) cannot produce any seed nor fruit. Other species have an only partial dependency on insect pollination ($0 < \text{pollinator dependency ratio} < 1$). As for eggplants, *Solanum melongena*, pollination can occur without insect visits. Nevertheless, insect pollination increases productivity. Seeds and fruits can be more numerous, of bigger size or have a slightly different biochemical composition, increasing market value of harvest. But accurate valuation of such partial pollinator dependency is complex and currently provided with large variations in the dependency ratio among studies (Gallai et al., 2009). Moreover, insect pollination dependency can vary according to cultivars and cropping conditions (Hein, 2009). Cropping conditions can create yields limiting factors (e.g. water stress) or reduce the insect pollination dependency, improving wind pollen dispersion or spontaneous pollination. It is therefore delicate to estimate the quantity of pollinating insects needed to ensure a high quality and abundant agricultural production.

Pollinator abundance and their diversity increase the pollination effectiveness (Greenleaf and Kremen, 2006; Klein et al., 2003). A reduced abundance of pollinating insects induces a reduced number of visited flowers and so a poor pollination rate. Therefore, the reduction of pollinator species richness and abundance reduces plant reproduction success (Thomann et al., 2013). Pollinator shortages can adversely affect crop production and commodity

markets (Kevan and Phillips, 2001). Pollination is even more important for the worldwide market since the 90's as the world demand for pollinator-dependent crops, notably legumes and energy crops, is increasing (Aizen et al., 2009; Breeze et al., 2014) and increases more quickly than the demand of pollinator-independent crops (x1.9 vs x1.3 from 1994 to 2010, Lautenbach et al., 2012). The part of the worldwide agricultural production linked to insect pollination have been estimated to be €153 billion a year (Gallai et al., 2009).

B. The insect side: nutritional needs and pollinator diversity

a) Diverse nutritional needs, fulfilled by plant diversity

Flower-feeding insects visit flowers to feed on, eating nectar and pollen. They mostly collect nectar for its sugar content. Sugar composition and concentration are the main quality criteria for insect nectar consumption. According to plant species, nectar contains different sugars at different concentrations that will suit different insect species (Baude et al., 2016; Hicks et al., 2016; Nicolson et al., 2007; Percival, 1961). Insects consume pollen as a source of essential amino-acids, polypeptides, sterols, vitamins and other micro-nutrients (Nicolson and Human, 2013). Pollen composition is even more critical as it is the largest source of essential amino acids and macronutrients for numerous larvae species (Liolios et al., 2016; Tasei and Aupinel, 2008). Among other micro-nutrients, the amino-acid pollen concentration and diversity vary according to plant species (Roulston and Cane, 2000; Weiner et al., 2010). Some pollens contain all the essential amino-acids for larvae development, whereas other pollens are deficient in some essential amino-acid and cannot be used alone to feed the larvae (Nicolson and Human, 2013; Somme et al., 2016). Nevertheless, insects can mix pollen sources to complete or mitigate low pollen quality (both nutritional value and available quantities) and get an equilibrated diet (Eckhardt et al., 2014). Social Hymenoptera species, as well as feeding on flowers, have to bring floral resources to feed larvae. The composition of the floral resources is of particular importance for the bee species demography as these resources directly impact the larvae development (Moerman et al., 2015). Food resource availability is an important factor of insect populations regulation (Roulston and Goodell, 2011). Nevertheless, plant species with poor-quality pollen can be suitable resources as long as the diversity of flowering plant species in the landscape allows insects to mix complementary pollen sources. Thus, a diversity of flowering plant species, which allows insects to collect different pollen to equilibrate their pollen diet, is of high importance for insect, especially bee, conservation (Müller et al., 2006).

b) Main groups of pollinating insects

In Europe, the main flower-visitor insects include Hymenoptera (47%), Diptera (26%), Coleoptera (15%) and Lepidoptera (10%) (Knuth, 1906; Willmer, 2011). The most numerous, effective and admit pollinating insects are Hymenoptera, mainly bees (Free, 1993; Kevan and Baker, 1983; Willmer, 2011). Even often omitted and poorly studied as pollinators, many flies species, mainly Syrphidae, are nevertheless effective pollinators (Inouye et

al., 2015; Ssymank et al., 2008). Lepidoptera, both butterflies and moths, are also recorded as pollinators (Crepet, 1984; Weiss, 1995; Willmer, 2011).

1. Hymenoptera

Among the Hymenoptera order, species belonging to the Apoidea superfamily ('bee' species) present morphological and behavioural elements suitable to provide a high-pollination efficiency (Kevan and Baker, 1983; Klein et al., 2007). The most particular Hymenoptera feature to carry pollen is the plumose hairs more or less all over the insect body (Willmer, 2011). The pollen is easily fixed on these plumose hairs and stays available to be deposited on the next visited flower.

Apis mellifera, the honeybee, was the only managed pollinating species for centuries in Europe and has been seen as the main plant pollinator. But more recently the other bee species, from the same Apidae family or not, are also recognised as essential pollinators (Ollerton et al., 2012). Moreover, a moderate bee competition among different bee species increases the number of insect visits per flower, increasing the quantity of pollen deposited per stigma, and the bee movements among plant individuals. Indeed, the bee diversity is needed to reach pollination of high quality (Garibaldi et al., 2013; Greenleaf and Kremen, 2006; Mallinger and Gratton, 2015; Rader et al., 2013). Insect diversity is also needed to pollinate entomophilous plant species that honeybee cannot pollinate (as an example some Ericaceae species that require buzz pollination (Javorek et al., 2002)).

i) *Honeybee, Apis mellifera, the domesticated Hymenoptera*

Honeybee, *Apis mellifera* (Apidae), is the commonest and the best known Hymenoptera species. *Apis mellifera* is regarded as the primary pollinating insect, a very efficient pollinator for many plant species, including some crops (Forup et al., 2008; Free, 1993; Knuth, 1909, 1908, 1906; McGregor, 2009). Thanks to a medium size proboscis (6 mm long, Knuth, 1906) this species visits a large diversity of plant species; it is a generalist, polylectic insect species.

Human widely kept *Apis mellifera* for honey production and pollination services for several thousands of years. This highly social species lives in large colonies (10 to 60 000 individuals) (Toullec, 2008). Within each colony, the social organisation is developed, with communication skills (among other used to communicate flower patches location) and worker specialisation (reducing the time necessary per flower to collect pollen and nectar) (von Frisch, 1951), increasing the efficiency of flower resources collection. *Apis mellifera* is the only social bee species in Europe of which queens have a life expectancy up to eight years (Haddad et al., 2007) and thousands of individuals from the same colony survive the winter and emerge in early spring. Thus, they have to store large quantities of resources (nectar (in honey form) and pollen) to survive during winter months without any flower available. Due to this huge need for resources and the skills provided by the social

organisation of colonies, *Apis mellifera* is very competitive for floral resource collection regarding to wild species (Herbertsson et al., 2016).

Nowadays we consider that, in Europe, all *Apis mellifera* individuals come from managed hives (Jaffé et al., 2010; Moritz et al., 2007). Therefore, *Apis mellifera* no longer suffers from nesting site losses but strongly suffers from pesticide uses, losses of floral resources at the landscape scale (Decourtye et al., 2010; Potts et al., 2010), pest and disease infestations.

ii) Bumblebees, *Bombus* species

The *Bombus* (Apidae) genus groups both rare to very common, widely spread species. In Belgium, 30 species are present (Pauly and Rasmont, 2010). *Bombus* species are polylectic, but their proboscis is longer than those of *Apis mellifera*, from eight to 21 mm (Knuth, 1906). Therefore, *Bombus* species can visit flowers with deeper corolla than honeybees. *Bombus* are solid-build, very hairy, species. Thus, they can carry a large amount of pollen available for pollination. Some *Bombus* species, as *Bombus terrestris*, are bred and used to pollinate some crops as tomatoes, *Solanum lycopersicum*.

Except for kleptoparasite (cuckoo) species, *Bombus* live in medium-sized annual colonies up to 200 individuals (Prys-Jones and Corbet, 2011). Only new queens survive the winter. Therefore, colonies just have small food stocks, to buffer short (few days) gaps in foraging activities, and to continue to daily feed the larvae even during raining periods, unsuitable to flight. Young queens emerge alone from early to late spring according to species (Benton, 2006; Falk, 2015), without any food stock. The flying season of queens, males and workers extend up to mid-autumn. The available flower resources at emergence are crucial and condition the new colony size and strength (Mommott et al., 2007; Persson et al., 2015).

iii) Other wild bees

Although the non-Apidae wild bee are less abundant than *Apis mellifera* and *Bombus* genus, non-Apidae wild bees represent most of bee diversity. At European scale, 1965 bee species are described, and 300 were observed in Belgium (Rasmont et al., 2005). Several of these species are reputed very efficient pollinators, and they often provides a significant part of pollination service (Kremen et al., 2002) or at least increases the quality of the pollination service provided by *Apis mellifera* (Blaauw and Isaacs, 2014; Garibaldi et al., 2014, 2013; Greenleaf and Kremen, 2006; Mallinger and Gratton, 2015).

Most of non-Apidae wild bee species are solitary bees. Solitary bee species lay eggs in individual close cells, with a sufficient amount of food (mix of pollen and nectar) to feed the larvae up to the imago stage. They have no food stock and are directly dependent on the flower resources immediately available. The continuity of floral resources' availability through the season and concordance of emerging periods with blooming time are crucial. Flying periods range from 15 to 157 days (Evans, 1984) and emerging periods extend from mid-March to end of September (Scheper et al., 2014). The ecological requirements of solitary bee species are very diverse. Lengths of proboscis vary from short

(one mm) to long (21 mm) and most of these species are more specialised, visiting a lower number of flower species, than *Bombus* species and *Apis mellifera*. Most of the wild bee species have specific nesting site requirement as soil, hollow stems or old snail shells (Falk, 2015). Solitary bee species are more sensitive to landscape changes than *Apis mellifera* or *Bombus terrestris* and can disappear following a host plant species or a habitat loss (Kennedy et al., 2013; Scheper et al., 2014).

2. Flies and hoverflies, the Diptera

Among the Diptera order the Syrphidae family (also named flower flies or hoverflies) groups most of the flower-feeding Diptera species (Kevan and Baker, 1983). In Belgium, Speight et al. (2015) recorded 310 Syrphidae species. A large part of the Syrphidae species are bee-mimics and densely hairy, but with simple hairs, less efficient as Hymenoptera plumose hairs to fix pollen grains. Among the vast diversity of non-Syrphidae Diptera, some are flower-feeding species, but these non-Syrphidae Diptera are often less hairy than Syrphidae and poorly appropriate to carry pollen. Therefore, they are often recognised as only accessory pollinators for most of the European plant species (Willmer, 2011). Thus, these Diptera species could be of low importance for the pollination services and are often not considered in pollinator centred studies.

For Syrphidae, even when imagoes are flower-feeding, flower resources are not enough to complete the life circle, and other food resources are needed during the larval stages. Contrary to Apoidea, Diptera have maggot-like larvae, which feed independently to the imagoes. Females lay eggs directly in or on a host or a suitable environment adapted to host larvae. According to the Syrph-the-Net database (Speight et al., 2015), among the 926 Syrphidae species recorded in Europe, 37% of the species have predatory larvae. Larvae of the other species feed on plants (35%) or on micro-organisms (36%, some species could use more than one food resource). More than 7% of these species have larvae commensal or quasi-commensal within the nest of some Aculeate Hymenoptera species. Beyond this diversity of diets, Syrphidae larvae use different habitats. Nevertheless, none of the larvae was recorded as dependent on the available flower resources for its development.

Flying periods extend from early March to end of November (Stubbs and Falk, 2002). Some species can have an adult stage as short as three days (Conn, 1976) when some other species live a long enough time to be migratory species (Heal, 1982; Stubbs and Falk, 2002). The different Syrphidae species present various types of mouthparts, with tongue up to 8 mm (Gilbert, 1981). Some are adapted to collect resources from deep corolla flowers while others are only suitable for flowers with easily accessible nectaries (Stubbs and Falk, 2002). The collected nectar varies from liquid (low sugar concentration <45% W/W) to very viscous (>80% W/W).

3. Butterflies, the Lepidoptera

The Lepidoptera group together butterflies (diurnal) and moths (mostly nocturnal) species. A total of 483 species are recorded in Europe, 115 in Wallonia (Dopagne, 2015). Some species with a very fleeting imago stage (one day) could survive without collecting flower resources, whereas other species with long imago period are frequent flower visitors and efficient pollinators (Willmer, 2011). At adult stage, Lepidoptera have a long coiled and elastic proboscis from one to 80 mm long in Europe (Knuth, 1906). This particular proboscis make Lepidoptera obligate liquid feeders. Lepidoptera preferentially collect low concentration nectar (sugar content <45% W/W). Lepidoptera mouthparts can carry large loads of pollen and actively participate in pollination (Courtney et al., 1982).

The Lepidoptera larval stages are herbivorous caterpillars that feed on plant leaves (occasionally on wood material or flowers), independently from the imagoes. The plant species used by larval stages are often different from the species whose flowers are visited by imagoes (Lafranchis, 2014).

c) Pollinator decline: consequences of excessive landscape simplifications

The global trend of pollinator populations decline is now established. It started since the late 50's for both managed and wild insect species (Kluser and Peduzzi, 2007; Kluser et al., 2010; Potts et al., 2009). Despite the scarcity of data for most of the wild flower-visiting insect species (Fox, 2013; IUCN, 2016), we know that lots of species are extinct or threatened (IUCN, 2016; Nieto et al., 2014; Swaay et al., 2010). These diversity and abundance declines of flower-visiting insects are multifactorial. Factors as global warming (Mommott et al., 2007), pesticide use (Godfray et al., 2015; Goulson et al., 2015), and landscape simplification (MacDonald and Johnson, 2000) are involved, alone or combined together (Vanbergen & The Insect Pollinators Initiative, 2013). For the current study, we focus on landscape simplification which is a homogenization of the landscape induced by a reduction of the landscape diversity and complexity, and the loss of landscape elements, especially the natural or semi-natural areas. Landscape simplification was mainly induced by increasing urbanisation (Fox, 2013) and agricultural intensification (Klein et al., 2007; Stanley and Stout, 2013).

With agricultural intensification, average field area increases, hedgerows and semi-natural field margins are remove (MacDonald and Johnson, 2000) and weeds are chemically exterminated (Bretagnolle and Gaba, 2015). Moreover, extensively managed hay meadows and Fabaceae crops (as *Medicago sativa*, *Pisum sativum* or *Trifolium* spp.) are decreasing, which participates to the global landscape impoverishment (Bommarco et al., 2012a; Goulson et al., 2005; Grixti et al., 2009). This impoverishment conducts to the reduction of food availability and diversity, i.a. both qualitative diversity and temporal diversity, essential to numerous insect species (Carvell et al., 2006; Deguines et al., 2014; Franzén and Nilsson, 2008; Keller et al., 2005; Kennedy et al., 2013; Le Féon et al., 2010; Winfree et al., 2011). Numerous woody species of the semi-natural areas, as willows, have an early blooming period that

provides flower resources to insects earlier than most of the cropped or wild herbaceous species (Aupinel et al., 2001; Eppink et al., 2012). The presence of woody and herbaceous species in a close vicinity extends the period of flower resources availability for insects. The diversity of species in the landscape is thus an important parameter to provide flower resources from early spring to late autumn.

Most of the flower-visiting insects are sensitive to the composition and quantities of floral resources (Pernal and Currie, 2001). The reduction of flower richness induced by landscape simplification often goes hand in hand with a reduction of flower number. These two combined decreases induce a reduced quantity of flower resources available or the loss of particular flower resources needed to feed flower-visiting insects. The reduction of plant diversity and density endanger numerous insect species (Dempster and Pollard, 1981; Sutter et al., 2017).

With landscape simplification and the decline of semi-natural areas, the availability of appropriate nesting sites is more and more problematic. This scarcity of nesting sites impacts the population strength of the following year directly (Osborne et al., 1991; Potts et al., 2005). These landscape perturbations imperil the survival of insect communities.

Even if the exact biology, repartition or population strength of more than 57% of the European wild bee species are not well known, 10% of species are recorded as threatened on the European scale (Nieto et al., 2014). For the hoverflies, Speight and Castella (2016) recorded 11% of European species as threatened. For Lepidoptera, the IUCN recorded 19% of the European species as vulnerable to regionally extinct (Swaay et al., 2010). In Belgium, the situation is not better, 25% of the 330 bee species are decreasing (Rasmont et al., 2005), 41% of the 310 hoverfly species are threatened (Speight et al., 2015), and 18 of the 115 Lepidoptera species are already extinct (Dopagne, 2015). Numerous insect species have an urgent need for support to not disappear and preserve a pollinator diversity useful to various plant species.

The flower-visiting insect decline seems to create severe mismatches between pollination requirement and available pollination services that might be compromising the production of pollinator-dependent crops in some areas (Allen-Wardell et al., 1998; Breeze et al., 2014; Garibaldi et al., 2009). As an example, several of the new high-genetic-potential cultivars (e.g. *Brassica napus*) cannot express their full yield potential in fields, and a pollination deficit is often pushed forward to explain this yield gap (Allen-Wardell et al., 1998; Rodrigo and Herrero, 1996). The FAO defined the 'crop pollination deficit', as the yield deficit that "refers to inadequate pollen receipt that limits agricultural output" (Vaissière et al., 2011).

The management of insect pollination, providing honeybee hives (*Apis mellifera*) or other insects as bumblebees colonies (*Bombus terrestris*), or populations as mason bees (*Osmia lignaria*) or leaf-cutting bees (*Megachile rotundata*), is expensive (around 350€/ha/year, Allsopp et al., 2008).

According to the cost and the needed logistics, it seems not currently realistic to conduct pollination management on all the cropped areas, especially in poor countries where farmers cannot afford commercial pollination services (Aizen and Harder, 2009). And even less for the numerous wild plant species that also need insects for their pollination. Moreover, reducing the pollination service offer to only a few managed insect species might jeopardise this service. That could create a high risk of vulnerability making it less resilient and more sensitive to the variation of the strength of these few species (Yachi and Loreau, 1999). As an example, since 2006, the Colony Collapse Disorder (CCD) syndrome, induced large-scale, unexplained massive losses (up to 30%) of managed *Apis mellifera* (Ellis et al., 2010; vanEngelsdorp et al., 2009; Williams et al., 2010). These CCD induced losses reduced to the same extent the pollination services offer. Also, even if the beekeeping is a popular activity (both as a professional business and as a hobby), the density of European (managed) honeybee colonies is similar to the density of wild honeybee colonies in adverse climatic conditions in Africa (Jaffé et al., 2010). The current level of beekeeping activity is far away to compensate for the loss of wild colonies, and so do the pollination ecosystem service following on. It is clear that pollinating insects, even the most famous kept species, need support.

Such CCD or insufficient colony number could imperil managed pollination services and highlight the risk of predominantly using a single species as a managed pollinator. It is therefore of particular importance to conserve a sufficient insect diversity, with redundant species, to secure the worldwide insect-pollination service and thus the food production (Goulson, 2003; Tscharrntke et al., 2012).

C. Flower-insect interaction networks: the condensed representation of complex interactions

a) Networks principles, all connected!

A food web is an interaction network, a kind of map that represents which species interact with which other species, often in a prey/predatory relationship. An interaction network helps picture how a community is put together and how it works (Pimm et al., 1991). It describes 'nodes' which are connected by 'links'. Most of the ecological networks drawn so far comprise species as nodes and particular kind of interactions between two species as links (Blüthgen, 2010). Food webs can have a vertical (linear) organisation, centred on a single species or species aggregate, and mapping the linked prey and the organism they eat and so on. Food webs can also be built with a horizontal organisation, picking a group of species in a habitat (regardless of their interactions) and mapping their prey or predators. This second type of food webs is community food webs. Food webs can be qualitative, based on a presence/absence interaction matrix, or quantitative when the number of interactions is known.

The bipartite networks are community networks with only two levels of communities, i.e. flower visitors (predatory) and the flowers they visit (prey).

The flower visitors and visited flowers bipartite networks are particular in the way that both partners can take advantages of the interaction. Such networks are named bipartite mutualistic networks (Fig. 1).

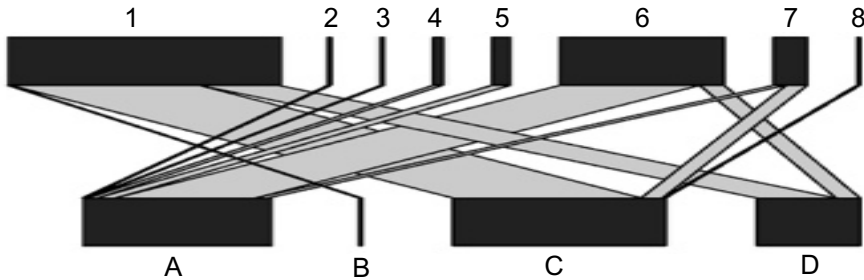


Figure 1. A bipartite mutualistic quantitative network among eight insect species (1 to 8, on the top) and four plant species (A to D, on the bottom). Each black rectangle corresponds to a species. The size of each segment is proportional to the number of recorded interactions (flower visit, in grey). Links are drawn in grey between two species.

To interpret the networks, to analyse and quantify their structure, lots of numerical indices have been built. These metrics provide information on networks structure and organisation, like stability, the level of connectance, the species specialisation or the robustness of the network to perturbation (Memmott, 2009). Nevertheless, mutualistic quantitative networks are more complex in their analyses of the observed interactions than 'simple' qualitative webs. In mutualistic quantitative networks, both partners take advantages of the interaction (Ings et al., 2009; Thébault and Fontaine, 2010).

For quantitative bipartite mutualistic networks metrics are a kind of one dimension projection value of multi-dimensional models. Thus, each value can correspond to several situations, and their analyse is therefore not absolute. A metric alone is not indicative, but comparing metrics of the same network related to a perturbation can help understanding the way the network evolves (Blüthgen, 2010). These metrics can thus be especially powerful to analyse restoration projects when networks of paired sites (before/after restoration) can be done. These analyses are also powerful to study punctual community perturbation as the introduction of alien species.

Numerous classical metrics focus on the specialisation of species, to explain if interactions occur randomly or not. Three of the most common metrics are compartments, connectance and nestedness. In a network, the compartments define groups of species that interact together but not with the species of another compartment of the same networks. Compartments are likely to correspond to habitat boundary (Montoya et al., 2006). The network connectance is the realised (observed) proportion of possible links (Dunne et al., 2002). A high connectance value reflects a high generalisation of the species in the network. But include all the present species in the interaction

matrix or only the species with interactions, highly modified the connectance value.

$$\begin{aligned} \text{connectance} &= \frac{\text{sum of observed links}}{\text{number of possible links}} \\ &= \frac{\text{sum of observed links}}{\text{number of plant species} * \text{number of insect species}} \end{aligned}$$

Connectance is based on a presence/absence matrix, the information about observation frequency is lost, but many zeros could reflect a low frequency rather than a high specialisation. Nestedness, referred as the network temperature, is the expression of symmetric interactions among plant species and pollinators. A nestedness of 100 reflects a maximally 'un-nested' matrix (so a perfectly random repartition of insect species among plant species), and a cold nestedness (=0) reflects a perfectly nested matrix.

Nevertheless, most of these classical metrics, as connectance metrics or the number of compartments are highly dependent on the total observation frequencies of each species and the sampling effort (Dormann et al., 2009; Goldwasser and Roughgarden, 1997; Ings et al., 2009; Martinez et al., 1999). Blüthgen (2010) explained that "variations in total observations frequencies may alone explain networks patterns that have often been interpreted as 'specialisation asymmetries' (nestedness, dependence asymmetries)". Moreover, he showed "analytically that 'dependent asymmetries' between two species (or guilds) only reflect variation in their total observation frequencies". Thus, the species that appear the most endangered or specialised are simply those with one or few observations (Blüthgen, 2010). And yet, in bipartite mutualistic networks, numerous species are observed with a low frequency (Blüthgen, 2010). The calculated metrics are thus wrong to characterise a species, but they are relevant for impact-based hypotheses where relative frequencies are part of the interpretation (Blüthgen, 2010). Nevertheless, it is essential to keep in mind that such metrics are frequency impacted metrics. Comparisons among networks could so be conducted with circumspection (Dormann et al., 2009).

Some weighted metrics were developed to correct the bias due to total observation frequencies. One of the most classical weighted indexes is H_2' that describes niche properties of a network. H_2' is a weighted complementarity specialisation index at the network level (each species is weighted by its observation totals). H_2' is not systematically biased by the total observations frequencies. Nevertheless, a truly specialised but rarely observed species cannot be detected as a specialist with H_2' thus an important part of rarely observed species distorts the H_2' value.

• **Mutualistic network:** a network where both interacting protagonists obtain reciprocal benefits. Flower-insect interaction networks are classical mutualistic food webs as insects benefit from food resources from flowers (pollen and nectar), and the plant could benefit from pollination service provides by the insects.

- **Connectance:** is the realised (observed) proportion of possible links (Dunne et al., 2002).
- **Compartments:** define groups of species that interact together but not with the species of another compartment of the same networks.
- **Nestedness:** in flower-pollinator food webs, the nestedness temperature is the expression of most random use of plant species by pollinators.
- **H₂'**: is a weighted complementarity specialisation index that describes the level of species specialisation at the network level (each species is weighted by its observation totals).

b) Restoring networks, a necessity to keep functioning ecosystems

The landscape simplification and the induced plant and insect losses, modify the plant-insect networks species compositions. Thus, interactions among plants and insects are perturbed and so the ecosystem functioning and the induced ecosystem services. Both sides of the networks (plant and insect species) need to be supported. Several measures can be implemented to recreate the necessary plant-insect network elements for ecosystem functioning.

1. A diversity of solutions, more or less complete.

To maintain the plant-insect interactions several management solutions exist and can be implemented (Kearns et al., 1998).

- **Domestication of wild bees and other pollinators**

The provision of pollination services by insect populations managed by humans could be a way to maintain plant-pollinator interactions in the benefit of plant reproduction (Klatt et al., 2013). It can make sense for the agricultural production, but it is not obvious that enough insects could be provided to efficiently pollinate the crops (Breeze et al., 2014; Osborne et al., 1991), nor that enough species could be domesticated to provide all the needed specificities of wild plant-insect interactions (Garibaldi et al., 2011). Moreover, such solution is only a half solution as it does not take into account the conservation of wild insect diversity.

- **Removal of alien pollinators**

In many parts of the world insect species were introduced to provide pollination services to crops (like *Apis mellifera* and *Megachile rotundata* in America, *Apis mellifera* and *Bombus terrestris* in New Zealand). These alien species can be very competitive for floral resources and negatively impact local species populations (with different intensities according to the species), strongly modifying the plant-insect interaction patterns (Dafni et al., 2010; Dohzono and Yokoyama, 2010; Stout and Morales, 2009). Nevertheless, removing alien species can be very long and expensive, if possible without destroying native species at the same time. Promoting the

preservation and use of native species to provide eco-system services could prevent the introduction of new exotic species.

- **Legal protection**

Both plant and insect species can benefit from legal protection. The International Union for Conservation of Nature and Natural Resources (IUCN) lists the species with a degree of vulnerability (from least concerned to extinct, see IUCN (2016) or Nieto et al. (2014) and Swaay et al. (2010) for the red list of bees and butterflies at the European scale). Authorities often use such listing to protect the most vulnerable species and delay their extinction legally. The legal protection can be beneficial, but it often arrives too late to preserve a species at a large community level and only on few hotspots of protected biodiversity (Doremus, 1991).

- **Reintroduction of plants and pollinators**

Reintroduction of species can be a nice way to recreate plant-insect interactions. Nevertheless, reintroduction alone, without removing the environmental or management elements that locally conducted to the species extinction is bound to fail. The success of reintroduction is conditioned to the existence of an intact functioning ecosystem (Kleiman et al., 1994). Reintroduction cannot be conducted alone and have to be integrated into a full ecosystem management process.

- **Public education** is a long-term process but a solution to a real mass consciousness of the importance of biodiversity and its preservation, but it is a long process. Public education is a necessary part of a conservation project, that must go with any political decision and concrete actions on the field (Mabelisa and Maksymiukb, 2009; Novacek, 2008).

- **Changed agricultural practices and uses of pesticides and herbicides**

Agriculture, and especially agriculture intensification, is among the first activities conducting to landscape simplification and subsequent plant and pollinator losses (Godfray et al., 2015; Goulson et al., 2015; Klein et al., 2007; MacDonald and Johnson, 2000; Poláková et al., 2011; Stanley and Stout, 2013). Changing the agricultural practices to more sustainable ones could undeniably reduce the negative impacts on pollinator populations (Aviron et al., 2009; Holzschuh et al., 2008).

- **Conservation of habitats and pollinators** is the most global and completed aim. To reach such purpose, several of the previous propositions will have to be implemented together. The flower-strips are an example of a scheme that combines several actions: species reintroduction, modification of management practices and public education, both in urban and farmland areas.

2. Flower-strips, an all-purpose scheme.

The flower-strips are areas where ideally native wild plants are re-introduced or re-used to create suitable habitat to host insects, birds or plants. When implemented in farmland the flower-strips are coupled with a necessary farmer education and sensitisation to wild plant and pollinator losses and more globally the biodiversity conservation purposes (Soini and Aakkula, 2007). The flower-strips implementation implies at least local modification of farming practices.

In farmland, flower-strips, sometimes as field margins (Marshall and Moonen, 2002), are a common measure to promote biodiversity support, for both plants and animals (Haaland and Gyllin, 2011; Haaland et al., 2011; Pywell et al., 2011; Tschumi et al., 2016, 2015; Westphal et al., 2015). Most of the flower-strips are implemented within the context of specific agro-environment subsidised measures, and management is imposed. The sown species have to present a low risk of crop invasion, and the non-toxicity of plants can be criteria to permit the farmers to produce hay from flower-strips (Le Roi et al., 2010). The exact strip purposes can vary from native wild plant conservation (Jacot et al., 2007; Le Roi et al., 2010; Westbury et al., 2008), bird conservation (Marshall and Moonen, 2002; Vickery et al., 2009), or insect conservation, often flower-feeding insects (Balzan et al., 2014; Campbell et al., 2017; Feltham et al., 2015; Haaland and Gyllin, 2011, 2010; Haaland et al., 2011; Haenke et al., 2009; Marshall et al., 2006; Sutter et al., 2017; Wood et al., 2015). Such facilities aim to increase diversity providing food and refuge area to insects. In farmland, flower-strips are often implemented with the aim to support pollination or pest management services to surrounding fields (Balzan et al., 2016, 2014; Campbell et al., 2017; Feltham et al., 2015; Tschumi et al., 2016, 2015; Westphal et al., 2015). Other purposes as cultural services and landscape improvement can also be included in the strips specifications (Westphal et al., 2015; Wratten et al., 2012). The management of flower-strips or field margins is often limited to the annual sowing or one or two mowing a year. Spontaneous species are usually welcome (except some competitive perennial weeds as *Cirsium* or *Rumex* species) and can increase the value of the sown mix (Campbell et al., 2017; Carvell et al., 2006; Memmott et al., 2010; Westbury et al., 2008). In some cases, flower-strips are only composed of spontaneous species (Bäckman and Tiainen, 2002; Carvell et al., 2004; Kells et al., 2001). But the interest of such flower-strips can be questioned as they are only small areas in large intensively farmed landscapes (Kovács-Hostyánszki et al., 2016).

Numerous specific 'flower-strips' commercial seed mixes are now available on the market (Hicks et al., 2016). But, to meet the highly aesthetic plant species association requested by gardeners, most of these mixes include exotic ornamental species, even if their interest to native insect species can be reduced (Hitchmough, 2000; Memmott and Waser, 2002). In farmland, even if some exotic species can be used (i.e. *Phacelia. tanacetifolia*), the flower-strips can vary from spontaneous species strips (Carvell et al., 2004; Holzschuh et al., 2008; Sutherland et al., 2001), pure wild-flower-strips (Carreck and Williams, 2002; Carvell et al., 2007; Gathmann and Tscharnkte,

2002) or a mix of Poaceae and wild-flower sown species (Gathmann and Tschardtke, 2002; Junge et al., 2015; Wood et al., 2015). Several species as *Trifolium pratense*, *Trifolium hybridum*, *Centaurea nigra*, *Lotus corniculatus*, *Phacelia tanacetifolia* are often used in European sown mixes (Hickman and Wratten, 1996; Junge et al., 2009; Tschumi et al., 2015; Wood et al., 2015).

II. ENTOMOPHILOUS CROPS: THE OILSEED RAPE CASE

A. Most of the Belgian crops are not dependent on insect for pollination

According to the Direction générale des Statistiques (2016), in Belgium, >90% of the cropped areas (1,338,566 ha in 2013) are used for crops not dependent on insect pollination (Fig 2).

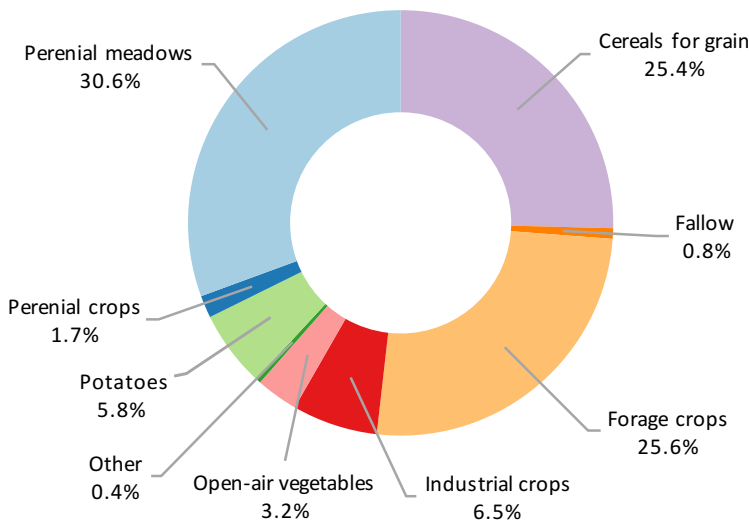


Figure 2. Main Belgian crops in 2015. Values are percentages of the total cropped area. Adapted from Direction générale des Statistiques, (2016). Forage crops are mainly maize (*Zea mays*) and temporary meadows; industrial crops are mainly sugar beetroots (*Beta vulgaris*) and oilseed rape (*Brassica napus*).

Yields of meadows, cereals, maize (*Zea mays*), potatoes (*Solanum tuberosum*) or beetroots (*Beta vulgaris*) are not linked to insect pollination. But oilseed rape (*B. napus*, 12,200 ha in Belgium in 2014, FAOSTAT, 2016) is frequently cited in the literature as one of the emblematic crops that will suffer from pollinator decline (Carrington, 2013; Corbet et al., 1992; European Commission, 2015a; Garibaldi et al., 2016, 2009; Potts et al., 2016, 2014). *Brassica napus* is indeed often recorded as largely visited by insects, especially by honeybees (Delaplane and Mayer, 2000; Delbrassinne and Rasmont, 1988; Halinski et al., 2015; Kirk and Howes, 2012; Westphal et al.,

2003; Witter et al., 2014), that Abrol (2007) described as “marvellously coevolved pollen transferring devices” for that species. Moreover, oilseed rape is considered as the most important honey crop (the crop that provides the most of flower resources to honey bees) since the Second World War (Calder, 1986; Delaplane and Mayer, 2000; Kirk and Howes, 2012; Westcott and Nelson, 2001). Oilseed rape is, the largest Belgian crop that could benefit from insect pollination services. Indeed, oilseed rape is beneficial for honeybees that produce oilseed rape honey, but the reciprocal benefit is uncertain.

B. The pollinator dependency of Brassica napus is nevertheless largely questioned

Within the FAO estimation of world crops insect dependency (Gallai, 2008), *B. napus* was referred as only moderately dependent on insect pollination (pollinator dependence ratio of 0.25), but with a large variability across references (from 0.1 to 0.4). Some studies concluded to a null effect of insect pollination on rape yields (Langridge and Goodman, 1982; Mesquida et al., 1988) whereas other studies showed a high insect pollination effect that increases yields by five times (Munawar et al., 2009). In consequence, it is unclear how the insect pollination could increase the yield of *B. napus*. This potential increase is especially questioning as “in spite of the entomophilous structure of the rape flower, which is very attractive to nectar- and pollen-gathering bees, the crop gave good yields without insect pollination” (Free and Nuttall, 1968). Indeed, since the 70’s, the role of insect pollinators in *B. napus* yields is under debate (Free, 1993; Langridge and Goodman, 1982; McGregor, 2009; Mesquida and Renard, 1981; Williams and Free, 1978). Until these days, we do not know with certainty the exact degree of benefit obtained from insect pollination compared to wind pollination, especially for the most recent cultivars (Hayter and Cresswell, 2006; Jauker and Wolters, 2008; Klein et al., 2003; Morandin and Winston, 2005; Pierre and Renard, 2010). There is still no consensus on the exact impact of insect pollination on *B. napus* yield (See Appendix 1).

C. The choice of locally used current cultivars for a crop that genetically evolves quickly

The *B. napus* cultivars have considerably evolved during the last 40 years thanks to the genetic selection. In 2015 the European Agricultural species catalogue contented 1,331 current cultivars, and 953 cultivars that were not used anymore (European Commission, 2015b). Moreover, numerous new cultivars are created each year. Only in France, around 30 new rape cultivars are registered each year (Pierre and Renard, 2010). The very vast majority (86%) of cropped oilseed rape cultivars were winter oilseed rape cultivars; the spring oilseed rape was less cultivated (only 187 current cultivars among the 1,331 European ones, European Commission, 2015b).

Across the studies on oilseed rape pollination, a large diversity of cultivars was studied according to the location and the year of the study. No single pair

of cultivars would show the same biological characteristics (Blochtein et al., 2014) nor attractiveness and response to pollinators (Marini et al., 2015). These differences were mainly due to the quality and the quantity of nectar produced (Blažytė-Čereškienė et al., 2010; Kevan et al., 1991; Pierre et al., 1999) and the cultivar earliness. The nectar volume available had a direct impact on the pollinator behaviour (Hayter and Cresswell, 2006; von Frisch, 1951) and varied significantly among cultivars, from 0.2 to 6 μL /flower per day (Mesquida et al., 1991; Pierre et al., 1999). The differences in nectar total sugar concentration were huge among cultivars as it ranged from 8.25 to 66.56 g/100 g of nectar. Moreover, a variable genotype effect on the nectar composition (sucrose/glucose/fructose) appeared across the season. Finally, the pollination characteristics, i.e. the quantity of pollen production (Hayter and Cresswell, 2006), self-incompatibility, and predispositions to self- and cross-fertilisation (from 20 to 42%) also varied sharply among cultivars (Olsson, 1960). Thus, for our study, we decided to use several winter cultivars, the locally most cropped ones, to check their pollination biology before to evaluate the importance of insect pollination. In this way, we obtained data reflecting the current local situation, with cultivars as a random parameter.

- **Self-incompatibility:** genetic and physiological mechanisms that prevent self-fertilisation. To produce seeds, the plant needs to be pollinated with pollen from another plant with a different genotype (another cultivar).
- **Self-fertilisation:** or autogamy is the capacity to produce seeds after the fusion of two gametes from a single individual. In our case self-fertility occurs with self-pollination within the same flower and geitonogamous pollination among the flowers of conspecific plants (with the same genotype, i.e. same cultivar).
- **Cross-fertilisation:** or outcrossing is the fertilisation with gametes from two individuals genetically different. In our case cross-fertilisation occurs when a flower is pollinated with pollen from a plant of a different genetic (of another cultivar).

D. Choosing the appropriate method to evaluate pollinator dependency

Most of the studies to investigate the pollinator dependency of *B. napus* were based on exclusion inclusion observations. Mesh bags or cages were used to both, keep insects out from a control plot and in an experimental plot. Meshes varied from 0.7 to 2.5 mm (Bommarco et al., 2012b; Durán et al., 2010; Jauker and Wolters, 2008; Jauker et al., 2012; Langridge and Goodman, 1982; Lerin and Rivault, 1982; Marini et al., 2015; Mesquida and Renard, 1982, 1981; Mesquida et al., 1988; Pierre et al., 2009, 2002; Stanley et al., 2013; Steffan-Dewenter, 2003). The size of the cages varied from four to 12.5 m² and the number of enclosed insects from one to around 40,000 (Adegas and Nogueira Couto, 1992; Free and Nuttall, 1968; Jauker and Wolters, 2008; Jauker et al., 2012; Langridge and Goodman, 1982; Lerin and Rivault, 1982; Mesquida and Renard, 1981; Mesquida et al., 1988; Munawar et al., 2009; Pierre et al., 2002, 2009; Steffan-Dewenter, 2003; Williams et al., 1987). This mesh bag or cage

method was very convenient to manage insects, but it can create local perturbations of micro-climatic conditions, as reducing sunlight and wind flow, or increasing temperature and hygrometry (Durán et al., 2010; Kearns and Inouye, 1993; Langridge and Goodman, 1982; Lerin and Rivault, 1982; Marini et al., 2015; Mesquida and Renard, 1981; Mesquida et al., 1988; Pierre et al., 2009, 2002). The use of cages could so modify the insect visits, but also the oilseed rape fitness, the pollen viability, and the wind effect. These exclusion inclusion observations seem more adapted to answer the question 'Are insects able to provide a pollination service to oilseed rape?' than 'Is oilseed rape dependant on insect pollination in field conditions?'.

Thus, we decided to study only free plants in open fields (no any bags or cages). We observed the seed production of free plants, without any treatment and recorded the number of flowers visited by insects to estimate the current importance of insect pollination (both honeybees and wild insects) on the seed production. At the same time, we estimated the deficit of pollination services comparing the seed production of free plants with the seed production of hand supplemental pollinated plants. To make our observations more reliable, we decided to follow the design recommended by the FAO to detect and assess pollination deficits in crops (Vaissière et al., 2011), with several observation plots per field, at three given distances from the field edge.

• **Hand supplemental pollination:** a classical technique used to mimic pollination or to supplementing natural pollination to ensure a maximal pollen transfer with known pollen. A large quantity of fresh pollen, directly collected from anthers of another flower, is manually carefully dropped on a receptive stigma with a fin brush or a blade-ended dissection needle (Kearns and Inouye, 1993).

The honeybee was the most studied pollinating insect on *B. napus* (Adegas and Nogueira Couto, 1992; Free and Nuttall, 1968; Jauker et al., 2012; Langridge and Goodman, 1982; Mesquida and Renard, 1981; Mesquida et al., 1988; Munawar et al., 2009; Pierre et al., 2009, 2002; Steffan-Dewenter, 2003; Williams et al., 1987). During its active foraging period (from March to October in Belgium), a bee colony from a standardised beehive, collects resources from one to four million flowers (van der Steen, 2015). In Belgium, 32,000 hives were registered (FAOSTAT, 2016), which represent a yearly need of 32 to 128 billion flowers. One hectare of *B. napus* daily provides from 25 to 91 million of flowers (P.O. and Rosana Halinsk respectively, pers. obs., 2016) during the 20 to 82 days of flowering (Bommarco et al., 2012b; Delbrassinne and Rasmont, 1988; EMBRAPA, 2016; Free and Ferguson, 1980; Lerin and Rivault, 1982; Manning and Boland, 2000; Manning and Wallis, 2005; McGregor, 2009; Morandin and Winston, 2005; Stanley et al., 2013; Tomm et al., 2009; Williams, 1984; Williams et al., 1987). In Belgium, in 2014 12,200 ha of oilseed rape were cropped (FAOSTAT, 2016) that represent a daily production of 312 to 1,138 billion flowers. Each day during the blooming period, the Belgian oilseed rape fields produced enough flowers to cover 2.4 to 35.5 times the yearly needs of the national apiary. The number of flowers produced by oilseed rape is so huge that it is poorly probable that

honeybees will visit them all. But other wild insects, like *Osmia rufa*, *Episyrphus balteatus* and *Eristalis tenax*, were also recorded as oilseed rape pollinators (Jauker and Wolters, 2008; Jauker et al., 2012; Steffan-Dewenter, 2003). It is so legitimate to question the actual local dependency of *B. napus* on pollination by insects and investigate the relative importance of *Apis mellifera* and wild insects in its pollination.

E. Studied area and fields

In Belgium, 55% of the oilseed rape production is concentrated in one region, the Condroz, South Belgium (APPO, 2015). In Condroz oilseed rape fields represent 5% of the cultivated areas (Castiau et al., 2011). This region is very favourable to oilseed rape production, as the yields are very high. The yield averages 2 tons/ha worldwide, 3.5 tons/ha in Western Europe, and 4.0 tons/ha in Belgium (FAOSTAT, 2016; FEDIOL, 2016).

In 2014, organic farming represented only 8.4% of the Walloon farmland areas (Annet and Beaudelot, 2016) but only 3.8% of the cereal and industrial crops (Direction Générale Statistique, 2017), we so focussed on conventionally cropped oilseed rape fields. Nevertheless, to prevent any direct effect on insects and toxicity during field sessions, no chemical was spread during the blooming period. The studied fields reflected the local field size diversity, from 2 to 25 ha (mean 11 ± 6.6 ha). To avoid strong differences among field surroundings, we excluded fields with neighbouring apiaries, orchards, forests or villages. The surrounding areas were open agricultural landscapes.

III. AGRO-ENVIRONMENT SCHEMES: THE MANAGED STRIPS

A. The Common Agricultural Policy (CAP) as an actor of environment-friendly management

In the European Union (EU), since 1962, the Common Agricultural Policy (CAP) is a powerful tool giving the main trends of European agriculture. The CAP was created to improve the European agricultural production to face the post second World War food demand. The first objective was to put a strong emphasis on high productivity. Even if the CAP budget switched from 66% of the EU budget in 1962 to 38% today, it still accounts for a huge amount of money (€362.8 trillion from 2014 to 2020) (European Commission, 2012).

In the 90's, the fact that increasing and excessive agricultural intensification cannot be sustainable (Bommarco et al., 2013), started to be integrated within the CAP considerations. Gradual CAP revisions (1992, 2003 and 2013) made it greener, including more and more incentive measures to environment-friendly management (Hart and Baldock, 2011). The Agro-Environment Schemes (AES) were introduced in the 1992 CAP reform. This reform introduced the principle of sustainable development, launched by the 1992 Rio Earth Summit (European Commission, 2017). The farmers were encouraged to be more environmentally friendly to try to mitigate the adverse impacts of the high level of agricultural intensification that imperils the ecosystem services (Scheper, 2015). Measures like crop diversity, income

reduction, semi-natural elements conservation and soil preservation were encouraged.

The AES are now tools to provide direct payments to farmers who subscribe to environmental commitments related to the protection of the environment and maintaining the countryside to support and restore biodiversity in farmland (European Commission, 2012). Based on a voluntary five-year engagement, these AES give to the farmers the opportunity to be paid (or at least compensated) for specific practices beneficial to local biodiversity and environment (Batáry et al., 2011; Kleijn and Sutherland, 2003). These practices are as diverse as low-income and extensive farming, or landscape management as hedges preserving, flower-strips, or pond preserving. They aim to reduce pollution from farming activities and preserve the ecosystem services provided by landscape elements.

With the AES, the ecosystem services are promoted. The ecosystem services are defined as all the services provided by ecosystems that will contribute to the human well-being. Thanks to the implementation of adequate AES the farmers are most compelling actors of the environment and ecosystem services conservation and restoration (Millennium Ecosystem Assessment, 2005; Power, 2010).

B. AES implementation, Belgian AES and “flower-strips”

AES are implemented according to strict specifications. The definition of AES specifications and the control of their implementation are delegated to the member states that could transfer this to a regional administration. That makes AES more flexible and adaptable to local farming systems and specific environmental conditions, increasing the needed links with local specificities (Batáry et al., 2011). Within the EU a significant number of AES exist and coexist, often only on a small territory (smaller than a country). This diversity makes hard the establishment of a global overview of the AES efficiency at the EU scale. Because of many AES are implemented with vague objectives not sufficiently quantifiable as “encouraging farmers to protect and enhance the environment”, it is not possible to measure their degree of achievement or to establish cost-effectiveness analyses (Uthes and Matzdorf, 2013).

The implementation of these AES is strictly checked, to validate the subsidies payment. But these controls are often more administrative than biological, based on the implementation specifications and not on the results. Thus, these evaluations provide few amounts of information on the real biological, ecological or agronomical interests or effectiveness of such agro-environment measures. Even if several studies already exist on the environmental impacts of some AES (Uthes and Matzdorf, 2013), numerous of these AES or AES issues are not well studied at large scale and on actual farmland, outside of experimental plots. Their effectiveness was even questioned (Kleijn and van Langevelde, 2006; Scheper, 2015). Nevertheless, evaluating the biological effectiveness and relevance of such schemes is essential to assess pertinence and identify improvements in composition and management (Batáry et al., 2015; Dicks et al., 2013).

In Belgium, AES are implemented by province. In Wallonia (South Belgium), 11 AES are proposed to the farmers. These AES included in the Rural Development Walloon Program aim to promote sustainable farmland practices to maintain the semi-natural areas and protect natural resources and biodiversity. These AES are declined under four categories, one dedicated to the conservation of ecological networks elements, one specific to meadows, one for livestock, one dedicated to cropped areas and the last one devoted to a global approach at the farm scale (Table 1 and Supplemental table S1 for subsidies levels, Natagriwal, 2016).

Table 1. 2014-2020 Walloon AES, according to the Walloon rural development plan.

Category	Agri Environment Scheme	Code
Ecological network	Hedgerows and line trees	MB1
	Trees, bushes and groves	MB1
	Ponds	MB1
Meadows	Natural meadows	MB2
	Liable to flooding meadows	MC3
	High biological value meadows	MC4
Livestock	Threatened local breeds	MB11
Crops	Grassy turning areas	MB5
	Environment-friendly crops	MB6
	Managed plots	MC7
	Managed strips	MC8
Global approach at the farm scale	Fodder autonomy	MB9
	Agro-environmental plan of action	MC10

For a maximum interaction with crops, we chose a measure implemented in cropped fields, the managed strips. The managed strip AES (MC8) are implemented according to six variants: one grass strip (namely erosion strip) and five flower-strips (namely fauna, pollinator, landscape, meadow flower and field flower-strips). This MC8 measure is the only AES including flower-strip type variants (fauna- and pollinator-strips) dedicated to wild land-fauna and existing since the 2005 CAP revision.

The linear shape and location of these strips in fields seem to be of particular interest to recreate links among landscape elements and reinsert biodiversity in intensively managed farmland (Albrecht et al., 2007; Cole et al., 2015; Kallioniemi et al., 2017). The different types of Belgian flower-strips include species as diverse as annual corn fields species (*Cyanus segetum*, *Glebionis segetum* and *Papaver rhoeas*), exotic species (*Phacelia tanacetifolia*), cropped species (*Daucus carota*, *Fagopyrum esculentum*, *Helianthus annuus*, *Lotus corniculatus*, *Medicago sativa*, *Onobrychis* spp. and *Raphanus sativus*), or wild flowers species (*Achillea millefolium*, *Centaurea jacea*, *Leucanthemum vulgare*, *Medicago lupulina*, *Silene x hampeana*), grasses species (*Agrostis capillaris*, *Dactylis glomerata*, *Festuca rubra*, *Poa* spp. and *Schedonorus arundinaceus*) and cereals (*Triticum aestivum*).

Several studies provided clues about the interest of such flower-strips schemes, but in different contexts and with different seed mixes and management. Some butterfly surveys were conducted on the pollinator-strips (Dopagne, 2015), but not flower resources nor a global study of the hosted flower-visitor insect diversity were conducted. Potential interest of bird-strips to flower-visitor insects was not investigated yet. Although these MC8 AES strips are among the most subsidised, €1250/ha/year, land managers have no idea of the real pertinence, interest or efficiency of the schemes they implemented.

C. The two types of studied AES flower-strips: one shape, different purposes and plant mixes

The fauna and pollinator MC8 AES variants are 6 to 21 m wide strips (from 20 m to more than one km long) sown with particular plant mixes along the border or across fields (Le Roi et al., 2010). The plant species used on these AES were chosen to be altogether affordable (not expensive), locally produced seeds, easy to sow and with little risk of neighbouring crop invasion. For the pollinator type, species must provide flower resources for insects, while for the fauna type, species must provide shelter and seeds for birds. Nevertheless, both of these AES could provide attractive resources for pollinating insects (Carvell et al., 2007).

These strips provide both a perennial uncut refuge section and combinations of grasses and flowering entomophilous plant species. Nevertheless, fauna- and pollinator-strips AES have different management and floral composition.

The bird-strips are primarily designed for granivorous bird conservation. They consist of three parallel sections. An uncut perennial-grass refuge section sown with *Dactylis glomerata* (50% of the seed mix weight), *Schedonorus arundinaceus* (49%) and *Trigonella* sp. (1%) (sown density 20 kg/ha). An annually set up feeding section containing one cereal, *Triticum aestivum* (65%) and three entomophilous species *Fagopyrum esculentum* (25%), *Phacelia tanacetifolia* (3%) and *Raphanus sativus* (7%), all dedicated to providing seeds for birds during winter (sown density 40 kg/ha) (Le Roi et al., 2010). *Raphanus sativus* was specially chosen to provide seeds up to late in the winter. The third section is an optional un-sown annually harrowed strip (Fig. 3).

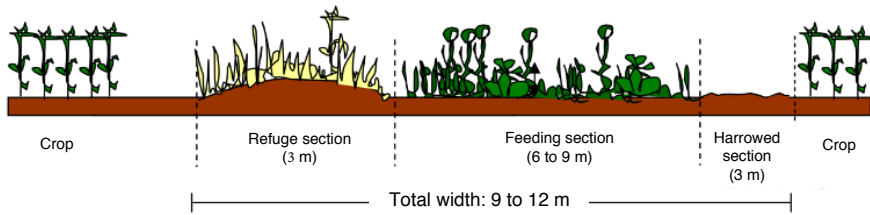


Figure 3. The bird-strip organisation with the tall grasses refuge section, the feeding section and the optional harrowed section. Adapted from Le Roi et al. (2010).

The pollinator strips are perennial areas sown with a single mix of plant species (30 kg/ha). This mix consisted of three native species of Poaceae (85% of the seed mix weight, *Agrostis capillaris*, *Festuca rubra*, *Poa* spp.) and ten entomophilous wild-flowers; mainly belonging to Asteraceae (e.g. *Achillea millefolium*, *Centaurea jacea*, *Leucanthemum vulgare*) and Fabaceae (e.g. *Medicago lupulina*, *Medicago sativa*, *Lotus corniculatus*, and *Trifolium pratense*). The additional sown flower species were *Daucus carota*, *Malva moschata* and *Silene x hampeana*. Pollinator strips are sown with a single seed mix but also presented three longitudinal sections, set up with differential mowing schemes, with no mowing (uncut refuge section), one or two mowing a year (Le Roi et al., 2010). These different mowing regimes should elongate the flowering period of some species that regrow after mowing and have a second blooming cycle (Fig. 4). The large amount of Poaceae is therefore attractive for farmers to enhance the value of AES mowing management, producing hay on these AES areas. The Poaceae made the AES strips closer to hay-meadows and provide soil cover all over the year. That would increase the durability of the plant mix year after year and prevent any excessive spontaneous species development.

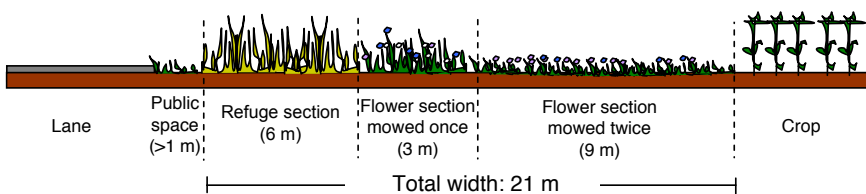


Figure 4. The pollinator-strip organisation with the un-mowed refuge section, the tall flowering section mowed once a year, and the low flowering section mowed twice a year. Adapted from Le Roi et al. (2010).

D. Plant species observation methods, inventories, flower densities and flower resources

To assess flower resources three elements are needed, the diversity and density of plant species and the quantities of resources provided per species.

a) Observed area, observing the same quadrats all along the year

In pollinator diversity studies, the records of flower diversity were frequently coupled with the estimation of flower resources or flower densities. Often the flowering plants were observed on the same transect as used for insect observations. It is important to observe each time precisely at the same location to study the evolution of flower densities and diversities among field sessions, independently to local plant communities' variations. Permanent transects could, therefore, be defined and materialised on fields with wood ranging-poles (Antonini et al., 2006; Artz and Waddington, 2006; Baldock et al., 2015; Bartomeus et al., 2008; Hicks et al., 2016; Lopezaraiza-Mikel et al., 2007). This design is more sensitive to changes than random sampling and allows to detect more precisely differences among sampling runs (Elzinga et al., 1998).

In other cases, the transect is subsampled with one to 20 quadrats from 0.25 to 10m² with a fixed or random position along the transect (Baldock et al., 2015; Hicks et al., 2016; Kallioniemi et al., 2017; Lebeau et al., 2015). Small quadrats were very sensitive to local flora variation, and large quadrats were too big to be entirely observed when flower density was high. Large quadrats could thus be subsampled, but this lead to the variation of sampling effort among records of the study (Kallioniemi et al., 2017). Hicks et al. (2016) studied the optimisation of sampling design to subsample a flowered-strip transect with quadrats. They found that conducting flower observations on one 1-m² quadrat every five meters along a one metre wide, 100 m long transect is the best compromise between sampling effort and low variance flower unit estimations. We followed their recommendations and worked with the same fixed transect and sampling design.

b) Flower density, take open flowers only into account

In several studies dedicated to flower-visiting insects, the botanical diversity is reduced to an open flower diversity. Poaceae and plant species without open flowers during observation sessions were not recorded. All the other plants with open flowers were identified up to the species (Lambinon and Verloove, 2012; Rameau et al., 2012; Rose, 2004; Tison and de Foucault, 2014).

The flower density is often estimated on the field using the concept of flower units, to optimise human resources and time devoted to field observations. A flower unit is one or multiple flowers that an insect can visit without flying (Dicks et al., 2002). Nevertheless, the application of this definition varies among studies and species. In Kallioniemi et al. (2017) "The registered flower units differed depending on the type of flower, but the same flower unit was consistently recorded within species. Flower units included individual flowers

(e.g. Convolvulaceae), flower clusters [secondary umbels] (e.g. Apiaceae), flower heads (e.g. Asteraceae and clovers), racemes (e.g. other Fabaceae), and flower stalks (e.g. Campanulaceae and Ranunculaceae).” In other studies, the flower unit were secondary umbels for Apiaceae, flower heads for *Trifolium*, capitulum for Asteraceae or branches (e.g. Ericaceae) (Baldock et al., 2015; Carvell et al., 2007; Hicks et al., 2016).

A flower unit is recorded as ‘open’ when at least one of the single flower is open (Baldock et al., 2015; Carvell et al., 2007; Hicks et al., 2016). The number of flower units is not an exact count of open flowers, but an estimation. The flower unit estimation could result from an exhaustive count on the field or from an estimation using abundance ranges (Carvell et al., 2007). Combining large flower units with abundance ranges create a substantial uncertainty that seems un-adapted to flower resources estimation. Moreover, the use of flower units could make complicated the comparison of flower densities among studies and even among plant species of a study.

Therefore, we chose to work on fixed standardised transect of 1x100 m and to count flower units on fixed 1-m² transect every five meters to obtain low variance flower unit estimation, sensitive to seasonal changes. We also chose to record the number of single flowers for as many species as possible and to use the flower units for as few species as possible (only Asteraceae), to increase the possible comparisons or data sharing among studies.

c) Flower resources, nectar and pollen productions

1. Nectar production

The nectar standing crop is a measure of the nectar available from a randomly sampled unprotected flower patch at an instant point. It is a measure of the nectar immediately available. But as an instant point measure, it is odd to extrapolate or to use to calculate a global quantity of available resources and underestimates the nectar quantities that an insect, that not randomly visits flowers, can collect from flowers (Kearns and Inouye, 1993).

Some authors measured the nectar secretion rate by emptying flowers at regular intervals (Dósa, 2003; Schmidt et al., 2015; Williams, 1997; Wist and Davis, 2006). As for 24h measures, flowers were protected from insects by mesh bags. The data obtained are complex to extrapolate to flower communities as nectar secretion is not a continuous phenomenon (Petanidou et al., 1999). The frequent nectar sampling along the day on the same flower could mimic the visits of insects. But the total production of nectar will be dependent on the sampling time and frequency (Wist and Davis, 2006). Therefore, such measures are not well adapted to estimate the potential nectar production of a flowered area.

Thus, the nectar production has usually been studied in the field measuring the 24h nectar production. Nectar sampling is realised with glass microcapillaries from a flower isolated from insect visits with mesh bag during 24h (Baude et al., 2016; Harder and Cruzan, 2012; Hicks et al., 2016; Petanidou et al., 1999; Pierre et al., 1999; Zającz et al., 2006). This measure

of cumulative nectar production is therefore independent of the time of sampling and represent the balance between rates of secretion and depletion (Williams, 1997). The nectar production is nearly always reduced to the quantity of sugar produced by a flower during 24h. This value is therefore easy to extrapolate to large areas when the number of flowers is known. Moreover, the sugar quantities data facilitate the comparisons among species, as both differences of nectar volume and concentration are included.

The 24h measure has the double advantage to include all the daily variations that could impact the nectar secretions and to be easily extrapolated to field data to obtain daily standing production or monthly quantities produced.

Nevertheless, nectar secretion is known to be dependent of several parameters as time in the season, flower age, flower location on the plant and weather, soil and environment conditions (Petanidou et al., 1999). Thus, sampled flowers should be representative of the diversity of conditions of the flower population studied.

Bags used for insect exclusion can modify the micro-climatic conditions of the flower and thus nectar secretion. Standard 1-mm mesh bags, the most used method, were recorded as having a minimal effect on nectar secretion (Corbet, 2003).

Two recent studies (Baude et al., 2016; Hicks et al., 2016) provided databases of 24h nectar and pollen productions of 171 wild species. They observed lower variations among UK sites than among individuals. Therefore, the combination of flower resource production from different locations and studies should be reliable, as far as the climatic conditions and protocol used are similar.

In the light of the recent and large databases provided by Baude et al. (2016) and Hicks et al. (2016), we measured 24h nectar production following the same protocols as presented in these two studies. Under climatic conditions close to the UK ones, nectar production has only been measured for the species not already present in these databases.

2. Pollen production

Pollen production is often based on the counting of pollen grains present in anthers (Kearns and Inouye, 1993). According to anther size, one to several anthers are used, and pollen grain number was extrapolated to flower level. Pollen grains are either extracted by stamen crushing or ultra-sonication (Hicks et al., 2016; Vear and Pham-Delegue, 1990) from freshly dehiscent stamens, kept in the lab to be protected from any wind or insect visits that could remove some pollen grains. Pollen grains can be exhaustively counted for a given number of anthers, or put in suspension then counted in a known volume. Counts are realised either using a haemocytometer (Hicks et al., 2016; Szabo and Najda, 1985) or a particle counter (Vear and Pham-Delegue, 1990).

Due to the very small amount (weight) of pollen per flower and the hard work needed to extract pollen, very few studies weight pollen to estimate its production per flower (Kearns and Inouye, 1993). To make pollen counts comparable among species, pollen grain number can be converted to pollen volume, using a volume estimation of pollen grains measuring their two main axes (Buchmann and O'Rourke, 1991; Hicks et al., 2016).

A study (Hicks et al., 2016), proposed a formula to estimate pollen production according to anthers size and number. Such estimation calculation could be useful, but it is maybe necessary to test it under other conditions to validate the calculation for the studied species.

As for nectar secretions, we used the databases provided by Hicks et al. (63 species, 2016) as working base to be completed.

E. Flower-feeding insect observation methods

Several techniques exist to record insect density and diversity. Nevertheless, few are adapted to flower-feeding insects, providing both a large range of sampling and allow to collect both density and diversity data. As an example, the sticky traps alter too much the specimens to use them for precise morphological observations and are not specific to flower visitors. The pheromone traps are too specific, designed to catch one or few species (Kearns and Inouye, 1993). Moreover, most of these techniques failed to provide information on visited flowers, except when pollen loads can be collected on caught specimens.

a) Direct observations, a data-rich but time-consuming method

Insect nets, expressly designed for insect catching are by far the best way to collect them. The method permits to select the caught insect and keep it in a perfect condition for morphological identification (Kearns and Inouye, 1993). Coupling with flower visit observation, aerial net catching allows to couple each caught insect with a visited flower species and so to obtain a maximum of observations about the flower visitors on the observed areas. Nevertheless, this technique is time-limited, and observations can only be conducted for a short period during the day. A part of the diversity could be missed, especially insects that could have particular flying periods, as nocturnal species or the small insects that could be under-caught regarding large specimens (Prado et al., 2017).

On mixed plant communities, transect observations are more reliable than timed observations and minimise the risk of unique-interaction observations (Gibson et al., 2011). To reduce the impact of observations on insect populations, it is possible to lessen as possible the catching, releasing insects (Prado et al., 2017) but numerous specimens need lab identification. For common insect species as *Aglais urticae*, *Inachis io*, *Apis mellifera* and *Eristalis tenax*, direct identification on the field are possible (Evans, 1984). For the common *Bombus* genus, on-field identification can easily be done using the Operational Taxonomic Units (OTU) described by Terzo and Rasmont (2007), based on a coat colour key (Fig. 5). On the field, *Bombus* can be

recorded up to the OTU and subsampled for precise lab identification up to the species. Species diversity in a given ecosystem is wide, and their exact identification of all the species can be tricky. Therefore, in many networks, some species, often of secondary importance, are aggregated by taxonomic affinities (Pimm et al., 1991).

We conducted insect observations on the 100-m² standardised transects used for plant observations (Dicks et al., 2002). We used aerial net only to catch insects we observed visiting a flower. Common species were identified on the field and released; only a few specimens were caught for confirmation of identification. We caught any other flower visitors for lab identification up to the species. We identified Hymenoptera (Archer, 2014; Falk, 2015; Goulet and Huber, 1993; Patiny and Terzo, 2010; Terzo and Rasmont, 2010a, 2010b; Wright, 1990), Lepidoptera (Skinner and Wilson, 2009; Tolman and Lewington, 2009), Coleoptera (Auber, 1960; Unwin, 1984), and Syrphidae flies (Speight and Sarthou, 2016; Stubbs and Falk, 2002; Verlinden, 1994) to species. Non-Apidae wild bees were identified by a taxonomist of the Royal Institute of Natural Sciences of Brussels. We identified non-Syrphidae Diptera to family level (Oosterbroek, 2006; Stubbs and Falk, 2002). We gathered non-Syrphidae Diptera in a unique insect group. Each *Bombus* OTU constituted an insect group. For all the other insects, a group corresponded to a single species.

OTU	Coats	Associated species
Campestris		<i>Bombus campestris</i>
Hortorum		<i>Bombus barbutellus</i> *, <i>B. hortorum</i> , <i>B. jonellus</i> *
Hypnorum		<i>Bombus hypnorum</i>
Lapidarius		<i>Bombus. cullumanus</i> *, <i>B. lapidarius</i> , <i>B. ruderarius</i> *, <i>B. rupestris</i> , <i>B. soroeensis</i> *, <i>B. wurfleinii</i> *
Masquée		<i>Bombus. sylvarum</i> , <i>B. veteranus</i>
Pascuorum		<i>Bombus humilis</i> *, <i>B. pascuorum</i>
Pratorum		<i>Bombus pratorum</i>
Terrestris		<i>Bombus cryptarum</i> , <i>B. lucorum</i> , <i>B. magnus</i> *, <i>B. terrestris</i>
Vestalis		<i>Bombus bohemicus</i> , <i>B. norvegicus</i> *, <i>B. sylvestris</i> , <i>B. vestalis</i>

Figure 5. Colour coats of the Operational Taxonomic Units (OTU) of *Bombus* and the associated species in Belgium. * indicate rare species. Adapted from Brédat (2010) et Terzo and Rasmont (2007).

• **OTU:** Operational Taxonomic Units are groups of species corresponding to distinct coats, easily distinctive on the field. In our study, we use the OTU only for *Bombus* species, as presented in Terzo and Rasmont (2007).

b) Pan traps, a complementary trapping method

Direct observations are useful to catch insect diversity when there are enough flowers to observe. But when no or few flowers are present, flower-insect interaction observation provides poor data. A complementary method could thus be used to catch insect diversity even when no or few flowers are present.

Pan traps are classical simple passive traps to record flower-visitor diversity. The presence of flowers is not needed as pan traps mimic the colour of flowering plants to attract the insects (Prado et al., 2017). This passive insect trapping could moreover compensate some bias of direct observation and netting. There is no observant effect, even small insects often under-sampled with nets, are caught, and insects behaviours are not modified by human presence (Roulston et al., 2007). Moreover, a 24h or 48h exposure under good weather conditions caught both diurnal and nocturnal insects all along the day and night without interruption. Direct field observation sessions could not provide such records.

As colour is an important attractant for many flower-visiting insects, the diversity of insect sampled could be increased using a combination of pans of different colours (Vrdoljak and Samways, 2012). The combination of three high reflectance colours, blue, white and yellow, provide the most representative flower visitor sampling as possible (Westphal et al., 2008).

The first bias of pan traps is their attractiveness relative to the surroundings. There is a contrast effect, the less flowered the surrounding, the more attractive the pan traps (Prado et al., 2017; Saunders and Luck, 2013). Moreover, the caught insects stay several hours in water and can thus be altered, which made harder the identification. Pan trapping is not restricted to flower-visitor insect species. An important sorting is necessary to keep only insects recognised as obligate flower visitors.

We identified Hymenoptera, except parasitoid ones, Lepidoptera, and Syrphidae Diptera. Regarding the numerous flies caught by pan traps, the precise identification of all Diptera would be too time-consuming relating to the scope of our study. Moreover, non-Syrphidae flower-feeding Diptera species are not easily recognisable from non-flower-feeding Diptera before a full identification up to species level. Thus, we decided not to investigate non-Syrphidae Diptera caught by pan traps.

IV. QUESTIONS AND THESIS ORGANISATION

The following research is organised around three questions (Fig. 6).

Q1: What are the pollination dependency of the main crops? Evaluation of reproductive biology and insect dependency of Belgian oilseed rape cultivars.

We first analysed the reproductive biology of oilseed rape cultivars to evaluate the importance of self-pollination, cross-pollination and spontaneous self-pollination. Then we analysed insect diversity and abundance in 12 oilseed rape fields in Condroz (seven cultivars). We recorded flower visit rate by insects and the pollen flux carried by the wind. We questioned the relative importance of insects vs the wind as pollinator vector. We analysed seed production of free and hand supplemented pollinated plants and calculated pollination deficit of oilseed rape in field conditions.

Q2: What are the abilities of flower-strip AES to provide pollen and nectar to flower-feeding insects along the year?

We monthly recorded flower diversity and density on ten flower-strips of two types in Condroz and Hesbaye. We distinguished sown and spontaneous species, and measured the provision of flower-resources: pollen and 24h sugar nectar productions. We first investigated pollinator flower-strips during two years, then bird flower-strips during two years.

Q3: What is the flower-feeding insect diversity using the resources provided by flower-strip AES?

On the studied flower-strips, in addition to flower-resources, we analyse the density and diversity of flower-visitor insects. We recorded flower-insect interactions and studied the pollen diet of *Bombus*. We completed our observations by pan-trapping to compare local insect diversity to flower-visitor diversity.

Estimation of pollinating insect populations in agro-ecosystems: comparing oilseed-rape fields and AES flower-strips.		
Q1: What are the pollination dependency of the main crops? Evaluation of reproductive biology and insect dependency of Belgian oilseed rape cultivars.		
• pollination biology of oilseed rape cultivars • self-pollination, cross-pollination and spontaneous self-pollination	• pollen limitation index in the field condition • insect and wind as pollinator vectors in the field condition	Chapter 1: Breeding system and pollination biology of Belgian oilseed rape cultivars (<i>Brassica napus</i> L.)
Q2: What are the abilities of flower-strip AES to provide pollen and nectar to flower-feeding insects along the year?	Q3: What is the flower-feeding insect diversity using the resources provided by flower-strip AES?	
• flower diversity, flower density and flower resources of the pollinator flower-strip AES	• flower-visitor diversity and density on the pollinator flower-strip AES	Chapter 2: Flower-strip agri-environment schemes provide diverse and valuable flower resources for pollinating insects
• flower diversity, flower density and flower resources of the bird flower-strip AES	• flower-visitor diversity and density on the bird flower-strip AES	Chapter 3: Agri-environment schemes targeted at farmland bird populations benefit pollinating insects
Chapter 4: Landscape structure is more determinant for flower-visiting insects than the type of managed strip agri-environment schemes		

Figure 6. Research questions and thesis organisation.

V. SUPPLEMENTARY MATERIAL

Supplemental table S1. 2014-2020 Walloon AES, according to the Wallon rural development plan.

Title	Code	Annual subsidy
Ecological network		
Hedgerows and line trees	MB1	25€/200m
Trees, bushes and groves	MB1	25€/20 elements
Ponds	MB1	100€/ pond > 25m ²
Meadows		
Natural meadows	MB2	200€/ha
Liable to flooding meadows	MC3	200€/ha
High biological value meadows	MC4	450€/ha
Livestock		
Threatened local breeds	MB11	120€/ cow 200€/ horse 30€/ sheep
Crops		
Grassy turning areas	MB5	900€/ha
Environment-friendly crops	MB6	200€/ha
Managed plots	MC7	600€/ha
Managed strips	MC8	1250€/ha
Global approach at the farm scale		
Fodder autonomy	MB9	From 50 to 100€/ha
Agro-environmental plan of action	MC10	Up to 3500€ according to the other engaged AES

Results

**Part 1: Breeding
system and
pollination biology
of Belgian oilseed
rape cultivars
(*Brassica napus* L.)**

Breeding system and pollination biology of Belgian oilseed rape cultivars (*Brassica napus*).

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I. ABSTRACT

Oilseed rape (*Brassica napus*) is considered an insect-pollinated species, but the degree of insect-dependent pollination differs between cultivars and the contribution of wind pollination remains a matter of debate. In addition, the breeding system has not been assessed for several new cultivars and spontaneous self-pollination has rarely been addressed. This study aimed to clarify the current situation in Belgium by analyzing the breeding system and pollination biology of the most cropped Belgian cultivars. We performed hand pollinations under controlled conditions to analyze the breeding system and conducted field observations to identify the main pollen vectors. The results show that the studied cultivars were highly self-compatible (SCI=1). Nevertheless, pollen vectors were necessary to produce high fruit and seed set because spontaneous self-pollination occurred at a low rate under greenhouse. All cultivars produced abundant nectar (1.2 μ l per flower per 24 h) with a high sugar content (48%). Surprisingly, very few insects were observed; only 1.1% of the flowers received insect visits during their life span. Moreover, wind pollination was only responsible for a small portion (2.5%) of the pollen deposition on stigmas. Despite the lack of pollen vectors, approximately 300 pollen grains were present per stigma. Indirect effects of insects and wind, which favor contact within and between flowers, ensure high fruit and seed sets in the main Belgian oilseed rape cultivars.

II. ABBREVIATIONS

OTU: Operational Taxonomic Unit

PL: Pollen-Limitation index

SCI: Self-Compatibility index

SFI: Self-Fertility index

III. INTRODUCTION

Oilseed rape, *Brassica napus* L. ssp. *napus*, is an important crop worldwide that was cultivated on 35.7 million hectares in 2014 (FAOSTAT, 2016). Oilseed rape is Europe's largest oilseed crop and the second largest protein crop (FAOSTAT, 2016). The yield averages 2 tons/ha worldwide, 3.5 tons/ha in Western Europe, and 4.0 tons/ha in Belgium (FAOSTAT, 2016; FEDIOL, 2016) and depends on the number and the weight of the seeds. Pollination and fertilization are therefore crucial to optimize yield. Winter oilseed rape cultivars are the most widely cultivated in the world, including in Belgium. The flowering of these winter oilseed rape cultivars last from 20 to 69 days from early April to June (Delbrassinne and Rasmont 1988; Manning and Boland 2000). In the undetermined inflorescences, the most productive flowers are in the lower half of the main raceme (Clarke, 1979; Diepenbrock, 2000; Lerin and Rivault, 1982; Tayo and Morgan, 1975; Williams et al., 1986). This mass-flowering crop is considered an insect-pollinated species because it produces nectar in such quantities bees can use it for an abundant production of honey (Calder, 1986; Delaplane and Mayer, 2000; Kirk and Howes, 2012; Westcott and Nelson, 2001). However, the contributions of wind and spontaneous pollination remain under debate (Adegas and Nogueira Couto, 1992; Durán et al., 2010; Free and Nuttall, 1968; Gallai, 2008; Jauker et al., 2012; Knuth, 1908; Langridge and Goodman, 1982; Munawar et al., 2009; Steffan-Dewenter, 2003), and there is still no consensus about the need to include pollination vectors to increase the yield of oilseed rape (Mussury and Fernandes, 2000; Stanley et al., 2013). Spontaneous pollination refers to pollen transfer from anthers to stigmas within a single flower or between flowers without the aid of an external pollen vector, such as wind or an animal. Several studies have shown that the relative contributions of these different types of pollination vary between cultivars and in different regions (Free, 1993; Marini et al., 2015; Oad et al., 2001). To determine the pollination mechanisms within a defined region, studies on locally grown cultivars are needed.

Our study focuses on the main hybrid winter oilseed rape cultivars in Belgium: DK Exsor, Dynastie, DK Excellium, DK Explicit, DK Exquisite, DK Exstorm. All cultivars were developed by the seed company DEKALB. Our aim was to identify the main pollination contributors to Belgian oilseed rape cultivars in the field.

We first investigated whether spontaneous pollination can occur and whether cultivars are self-compatible and do not suffer from inbreeding depression. We also assessed attractiveness of the studied cultivars to insects by analyzing the quantity and quality of the produced nectar, and studying the diversity and abundance of the insect visitors in the fields. Finally, we estimated the pollination quality by assessing whether the amount of pollen deposited on stigmas was sufficient to ensure fruit and seed set.

To answer the first question, we investigated the DK Exquisite cultivar (with DK Excellium as parent donor) under greenhouse conditions. Fruit and seed production were compared after spontaneous self-pollination, and hand self-

and cross- pollinations to assess the effectiveness of cross-pollination and pollen vectors. We further studied natural pollination under field conditions in the main six cultivars grown in Belgium. Both insect and wind pollination were estimated and pollen transfer limitation was studied.

Despite a very low impact of direct insect- and wind-pollination of oilseed rape in Belgium, the high self-compatibility of tested cultivars combined with the indirect wind pollination resulted in high fruit and seed sets in fields.

IV. MATERIALS AND METHODS

A. Plant material

Six winter oilseed rape cultivars produced by DEKALB, which is the breeder of the majority of the oilseed rape cultivars grown in Belgium, were used (DK Exsor, Dynastie, DK Excellium, DK Explicit, DK Exquisite, and DK Exstorm). Due to the high degree of genetic similarity, only two cultivars were studied under greenhouse conditions to determine the breeding system of these cultivars.

B. Breeding system

Experiments were performed in the greenhouse of the Université catholique de Louvain (Louvain-la-Neuve, Belgium: 50°39'N; 4°37'E). Additional light (red (650 nm) and blue (440 nm)) was provided using LED lamps (LUMIGROW, PRO-325, Emeryville, California, USA). The mean daily temperature averaged $22.5 \pm 2.97^{\circ}\text{C}$ (close to the optimum pollen germination temperature; Young et al. 2004) and the mean daily relative humidity averaged $93 \pm 3.2\%$.

To assess the breeding system, 12 DK Exquisite plants were used as the maternal parents, and 10 DK Excellium plants were used as pollen donors for hand-pollination. Plants between the BBCH stage 53 and 55 (flower buds raising the youngest leaves, visible but still closed; Federal Biological Research Centre for Agriculture and Forestry 2001) were chosen in mid-April 2015 in two conventional fields. The plants were removed from the field, individually potted into 8-L pots filled with soil from the field, and transferred to the greenhouse. Plants were daily watered with rainwater. To avoid undesirable pollination, plants were placed at least 80 cm apart. The absence of apomixis was checked by emasculating flowers at the bud stage and leaving them unmanipulated. The absence of apomixis was confirmed by the lack of fruit and seed development in the emasculated flowers.

To assess the breeding system, a total of 480 flowers from the base of the main raceme of the 12 maternal plants (DK Exquisite) were chosen in sets of ten consecutive flowers (four sets per plant) and allocated to one of the three pollination treatments.

To quantify spontaneous self-pollination, twelve sets of flowers were left unmanipulated (Treatment 1). The other sets of flowers were hand-pollinated with pollen from another flower of the same plant (twelve sets, Treatment 2) to test for self-compatibility, or cross-pollinated with pollen from a flower of the other cultivar, DK Excellium (twelve sets, Treatment 3), to assess inbreeding depression.

Fruits (siliques) matured within 51 days. Fruit set, number, and weight of seeds per silique were recorded. Only fruits with at least one well-developed seed were recorded. The weight of the seeds from freshly harvested fruits was measured using a scale with a sensitivity of 0.01 g (PG503 DeltaRange Balance, Painesville, Ohio, USA).

C. Pollination biology

a) Study area and plant material

In 2014 and 2015, we selected 12 conventionally managed winter oilseed rape fields, located in the Condroz, South Belgium (50°22'N, 5°13'E, 250 to 300 m a.s.l.). Eight fields were studied in 2014 (cultivars Dynastie, DK Excellium, DK Explicit, DK Exquisite, DK Exstorm) and 4 fields were studied in 2015 (DK Exsor, DK Excellium).

The field sizes ranged from 2 to 25 ha (mean 11 ± 6.6 ha) and the fields were at least 3 km apart, to avoid spatial pseudoreplication. The surrounding area was open agricultural landscape dominated by pastures and medium-sized fields where mainly cereals, corn, oilseed rape, and sugar beet were grown. Fields with neighboring apiaries, orchards, forests or villages were excluded. No insecticides were applied during the period of oilseed rape flowering (from April 2nd to May 13th in 2014 and, from April 20th to May 26th in 2015).

As recommended by the FAO (Vaissière et al., 2011) three sampling plots (of six plants at the same developmental stage) were chosen per field, to estimate the influence of increasing distance from the edge of the field. Due to differences in field size and shape, sampling distances were 30, 150, and 280 m from the field edge in 2014, and 3, 30, and 90 m from the field edge in 2015.

b) Pollen viability

B. napus flowers have an average lifespan of three days (Rosa et al., 2010). Pollen viability of one-, two-, and three-day-old flowers was investigated. A total of 20 plants from 4 cultivars (DK Excellium, DK Explicit, DK Exquisite, DK Exsor) were transplanted from five conventional fields (see the section on breeding system for details). Potted plants were placed outside the lab and watered daily with rainwater. Pollen viability of 50 flowers per flower age was estimated with fluorescein diacetate solution (Heslop-Harrison and Heslop-Harrison, 1970). The reagent was prepared by adding a few drops of fluorescein diacetate dissolved in acetone (2 mg/mL) into a freshly prepared 5 M sucrose solution. Freshly collected stamens were individually crushed in a drop of the reagent, incubated for 10 min and mounted on microscope

slides. Observations were conducted using a fluorescence microscope (Nikon Eclipse E400, Gotenba, Japan, excitation filter 420–440 nm, emission filter 480 nm, G= 100X). To determine pollen viability, 500 pollen grains were observed per stamen. Viable grains had fluorescent protoplasm and the percentage of viable pollen was calculated.

c) Nectar production

To estimate nectar production in 2014, 240 flowers from the 6 studied cultivars were bagged 24 hours before sampling to exclude visitors (Delnet Pollination, Middletown, USA). Nectar was collected with 1- μ L glass capillary tubes (Hirschmann Laborgerate, Eberstadt, Germany), and the nectar volume was estimated by measuring the length of the nectar column in the capillary tube. The sugar concentration was measured with a low-volume hand refractometer (Eclipse Hand held Refractometer, Bellingham & Stanley Ltd, Tunbridge Wells, UK) and expressed as the percentage of sucrose (w/w). The nectar sugar content per flower (s, mg) was calculated from the equation $s = 10 \cdot d \cdot v \cdot C$, where v is the volume calculated as above (mL), and d is the density of a sucrose solution at a concentration C (g sucrose/100 g nectar) as read from the refractometer. The density was calculated following the method described by Prys-Jones and Corbet (2011).

d) Insect visitors

Insect visitors were recorded in the 12 fields, during eight days in 2014 and four days in 2015. Observations were carried out under conditions suitable for insect visits (Baldock et al., 2015; Willmer and Stone, 2004) between 09:00 am and 05:00 pm under sunny conditions with little wind (1.8 ± 0.9 Beauforts in 2014 and 1.8 ± 0.5 in 2015) and warm temperatures ($>10^{\circ}\text{C}$: from 10 to 18°C in 2014 and 13 to 18°C in 2015), and nebulosity $<50\%$. The visitation rates (number of flowers visited / number of open flowers) were monitored during 20-minute intervals in 85 plots of 0.5 m^2 . Visitors were identified in the field, to the species level for *Apis mellifera* and to Operational Taxonomic Unit (OTU) for *Bombus* spp., (Terzo and Rasmont, 2007).

e) Airborne pollen flow

In 2015, during the peak flowering period, pollen traps were used to determine the presence of airborne pollen and to estimate the relative contribution of wind pollination. Glass microscope slides coated with petroleum jelly (Vaseline) were placed vertically at three observation distances in three fields, 30 cm above the top flower level, in three directions, for 24 hours. On each of the 27 slides, pollen grains were counted on 4 cm^2 per slide and expressed in a number of pollen grains per cm^2 per 24 h (Mesquida and Renard, 1982). Mean values were calculated per set of three slides to estimate the airborne pollen quantity per sampling plot.

f) Pollination quality

To assess the pollen deposition under field conditions, we developed a new method in which deposited pollen on stigmas were double-stained with acid fuchsin and aniline blue and examined under a fluorescence microscope.

The staining with acid fuchsin reveals pollen grains on stigmas whereas staining with aniline blue reveals pollen tube initiation on the same slide.

In each field plot, five pistils (three to five days after the end of anthesis) were sampled and fixed using FAA (40% formaldehyde; acetic acid; 95% alcohol; 1:1:8). Before observation, the pistils were rinsed with distilled water, stained for 10 min in 0.1% aniline blue solution in KH_2PO_4 (0.1 M), rinsed with distilled water and stained a second time for 10 min in 0.005% acid fuchsin solution. The pistils were subsequently rinsed with distilled water. Stigmas were cut transversally and mounted in water on microscope slides. The samples were observed using a fluorescence microscope (Nikon Eclipse E400, Gotenba, Japan, excitation filter 420–440 nm, emission filter 480 nm, G= 100X).

g) Pollen transfer limitation

To estimate pollen transfer limitations under field conditions, 324 plants were allocated to one of two pollination treatments. The first treatment was carried out in the presence of pollinators (T1; free exposure for control or open pollination). Flowers allocated to the second treatment (T2; supplemental hand-pollination) were cross-pollinated by hand. Because stigmas are receptive for three days, flowers were hand-pollinated every three days during the flowering period with pollen from freshly opened flowers of nearby plants. The numbers of fruits and seeds from the first 30 flowers of the main racemes were analyzed in July.

V. DATA ANALYSES

The nectar production, fruit set, and seed number per fruit from field plants were compared by Kruskal-Wallis rank sum test (R package 'asbio') and when needed, a *post hoc* Nemenyi test was performed. Greenhouse data were Box-Cox transformed and analyzed with ANOVA, using the Nemenyi *post hoc* test (R package 'stats'). Pollen viability and pollen deposition data were compared using ANOVA in combination with the Tukey *post hoc* test (R package 'stats'). All analyses were conducted with the R software, version 3.2.4 (R Core Team, 2016). The mean values are given with their standard deviations.

The self-fertility index (SFI) and self-compatibility index (SCI) were calculated according to the method of Lloyd and Schoen (1992). The SFI estimates the capacity of a plant to produce fruits ($\text{SFI}_{\text{fruit}}$) and seeds (SFI_{seed}) without any pollen vector. It is calculated as the fruit set (or seed set) as the result of spontaneous self-pollination relative to results of cross-pollination by hand (Kouonon et al., 2009; Lloyd and Schoen, 1992). The SCI determines the capacity of a plant to produce zygotes following self-pollination relative to the production of zygotes following outcrossing. It is calculated as the ratio between the fruit set ($\text{SCI}_{\text{fruit}}$) or seed set (SCI_{seed}) produced after hand self-pollination and the fruit set (or seed set) produced after hand cross-pollination (Lloyd and Schoen, 1992). SCI or SFI values higher than 0.2 indicate self-compatibility or spontaneous selfing respectively (Lloyd and Schoen, 1992).

The levels of inbreeding depression of different yield parameters (fruit set, seed number, and seed weight) were determined as the ratio between relative performance of self-pollinated (w_s) and cross-pollinated flowers (w_c) ($\delta = 1 - (w_s/w_c)$; Charlesworth and Charlesworth 1987).

The pollen limitation index (PL) was calculated for fruit (PL_{fruit}) and seed (PL_{seed}) production. PL was determined as the ratio between the number of open-pollinated fruits or seeds (P_o) and hand-pollinated fruits or seeds (P_s) (PL = $1 - (P_o/P_s)$; Larson and Barrett 2000).

VI. RESULTS

A. Breeding system (greenhouse experiments)

Our results show that self-fertilization occurs (SFI_{fruit}=0.7 and SFI_{seed} = 0.8), although fruit set and the number of seeds per fruit were significantly higher after hand self-pollination (26 and 36% higher on average, respectively) when compared with spontaneous self-pollination ($F=7.615$, $p=0.011$; $F=16.890$ $p<0.001$, respectively, Table 2).

Table 2. Fruit set, seed number per fruit and seed weight of ten consecutive flowers of oilseed rape (cultivar DK Exquisite) according to pollination treatment. Treatments were performed under controlled conditions in greenhouses. Different letters indicate significant differences.

Treatment	Fruit set (%)	Seed number per fruit	Total seed weight (g)
Spontaneous-self pollinated	78 ± 24.8 ^b	19 ± 8.4 ^b	0.55 ± 0.35 ^b
Hand-self pollinated	98 ± 3.9 ^a	26 ± 8.1 ^a	0.89 ± 0.30 ^a
Hand-cross pollinated	98 ± 4.5 ^a	26 ± 5.7 ^a	0.89 ± 0.23 ^a
F	7.081	3.595	5.076
P	0.003	0.039	0.012

Values are mean ± standard deviation, n=12 per treatment

No significant differences in fruit set ($F=0.234$, $p=0.633$), the number of seeds per fruit ($F=0.063$, $p=0.805$) or the seed weight ($F=0.234$, $p=0.998$, Table 1) were detected between cross- and self- pollinations by hand. Cultivar DK Exquisite is therefore fully self-compatible (SCI=1.0) and does not suffer from inbreeding depression ($\delta_{seed}= 0.027$, $\delta_{fruit} = 0$).

B. Pollination biology (field observations)

a) Pollen viability and nectar production

No significant difference in pollen viability was observed among the studied cultivars ($W=9.41$, $df=4$, $p=0.052$). For all cultivars, pollen viability was

significantly higher on the first day of anthesis ($56 \pm 30\%$) when compared with subsequent days ($F=15.59$, $df=2$, $p < 0.001$, Fig. 8), but the viability did not significantly differ between the second and the third day of anthesis ($40 \pm 2\%$ and $34 \pm 2\%$ respectively, $p=0.420$, Fig. 8).

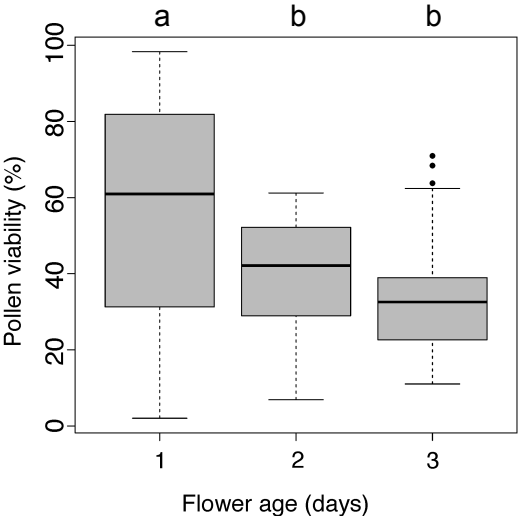


Figure 8. Oilseed rape pollen viability after flower opening (anthesis). 95% interval, 2nd and 3th quartiles, median line; N=50 per age (cultivars DK Exquisite (n=20), DK Excellium (n=10), DK Exsor (n=10), DK Explicit (n=10)). Different letters indicate significant differences.

Table 3. Oilseed rape nectar volume and sugar production per flower per 24 h in different cultivars (n=30 flowers per field). Different letters indicate significant differences according to the Nemenyi test.

Cultivar	DK Exquisite	DK Excellium	DK Exsor	Dynastie	DK Explicit	DK Exstorm	All	Kruskal-Wallis test
Number of fields	2	2	1	1	1	1	8	
Nectar volume per flower (μL)	0.73 ± 0.65 ^{bc}	0.80 ± 0.87 ^c	0.96 ± 0.59 ^{bc}	1.69 ± 1.16 ^{abc}	2.39 ± 1.58 ^a	1.78 ± 1.51 ^{ab}	1.24 ± 1.2	$W=46.62$, $p<0.001$
Nectar sugar concentration (%)	57.92 ± 9.61 ^a	60.19 ± 16.14 ^a	58.05 ± 16.04 ^a	13.08 ± 7.27 ^b	23.05 ± 11.52 ^b	59.57 ± 6.28 ^a	47.76 ± 22.1	$W=114.18$, $p<0.001$
Sugar production per flower (μg)	557 ± 499 ^{cd}	668 ± 741 ^{cd}	693 ± 530 ^{ac}	280 ± 261 ^d	612 ± 530 ^{bcd}	1342 ± 1136 ^{ab}	645 ± 692	$W=26.49$, $p<0.001$

Values are mean $\pm$ standard deviation.

The nectar production varied according to the cultivars ($W=46.62$, $df=5$, $p<0.001$, Table 3) and the mean nectar production reached $1.2 \pm 1.2 \mu\text{L}$ per flower per 24 h. The sugar concentration was high ($47.8 \pm 22.1 \mu\text{g}$ of sugar / $100 \mu\text{g}$), whatever the cultivar but was lower for *Dynastie*, *DK Explicit*, and *DK Exstorm* compared to the other cultivars ($W=114.18$, $df=5$, $p<0.001$, Table 2), resulting in high mean values for the daily sugar quantity ($645 \pm 692 \mu\text{g}$) produced per flower. The sugar content per flower varied in different cultivars ($W=26.49$, $df=5$, $p<0.001$, Table 3) and the highest sugar content was observed in flowers from cultivar *DK Exstorm*.

b) External pollen vectors

The airborne pollen flow averaged 472 ± 198 pollen grains per cm^2 of slide per 24 h (Table 4). The quantity of available airborne pollen was not significantly different within a field at different distances from the edge ($W=1.31$, $df=1$, $p=0.519$), or between different cultivars ($W=0.10$, $df=1$, $p=0.757$).

Insect visits were very low during the two years of observations and during the 85 observation periods of 20 minutes with a total of 1,700 min. of observation. During the entire observation time, only 11 honeybees (*Apis mellifera*, Hymenoptera) and one *Bombus terrestris* OTU were observed visiting oilseed rape flowers. As a consequence, only 38 of the 98,550 observed flowers were visited by an insect and the resulting visitation rate was approximately 0.0004 (Table 5). Based on this observed flower visitation rate and the assumption of nine hours of pollinator activity per day during the three days of anthesis, we can estimate that insects visited less than 1.1% of the oilseed rape flowers under the field conditions in this study.

Table 4. Oilseed rape airborne pollen grains collected per cm^2 of vertical slide per 24 h in different cultivars and at different distances from the field edge (3 slides exposed in 3 directions on each site). No significant difference was observed.

Distance from edge	DK Excellium	DK Exsor
3 m	187 ± 157^a	633 ± 507^a
30 m	604 ± 769^a	673 ± 473^a
90 m	302 ± 226^a	436 ± 597^a

Values are mean $\pm$ standard deviation

c) pollination quality

The total number of pollen grains present on stigmas (300 ± 204) and the pollen grain germination rate on stigmas ($29 \pm 17\%$), did not significantly differ between cultivars ($W=8.75$, $df=4$, $p=0.068$ and $W=4.91$, $df=4$, $p=0.296$, respectively) or between the different sampling distances ($W=1.25$, $df=2$, $p=0.742$ and $W=2.92$, $df=2$, $p=0.404$, respectively) in open pollinated flowers (Table 6).

Table 5. Flower and insect observations in 12 oilseed rape fields with different cultivars in Belgium. 85 observations were performed on 0.5 m² during 20-min. time periods.

	2014					2015	
	DK Excellium	DK Exquisite	DK Exsor	DK Exstorm	Dynastie	DK Excellium	DK Exsor
Number of 20-min. periods	23	9	7	7	9	15	15
Insects observed	1	0	1	2	0	2	6
Flower visited	2	0	1	14	0	16	6
Flowers observed	29 000	4 500	10 500	9 500	4 950	18 500	21 600

Values are sums of observations.

Table 6. Total number of pollen grains present and pollen grain germination rates on oilseed rape stigmas under conditions of open pollination in 6 fields in 2014, in different cultivars and at different distances from the field edge. No significant differences between cultivars and distances were observed.

	Distance (m)	DK Exquisite (2 fields)	DK Excellium	DK Exsor	Dynastie	DK Explicit
	<i>n</i>	81	35	45	46	45
Pollen grains per stigma	30	362.5 ± 251.2 ^a	275.0 ± 202.2 ^a	287.9 ± 178.8 ^a	254.8 ± 158.6 ^a	300.0 ± 181.7 ^a
	150	324.3 ± 252.6 ^a	186.4 ± 182.1 ^a	412.9 ± 222.2 ^a	234.4 ± 156.2 ^a	291.7 ± 165.9 ^a
	280	415.2 ± 208.1 ^a	239.4 ± 247.8 ^a	302.9 ± 201.7 ^a	232.4 ± 110.5 ^a	194.0 ± 102.8 ^a
% germination	30	25.1 ± 14.1 ^a	28.1 ± 11.3 _a	34.3 ± 24.2 ^a	33.5 ± 23.6 ^a	25.9 ± 14.2 ^a
	150	35.7 ± 15.7 ^a	32.2 ± 15.5 _a	27.9 ± 18.7 ^a	27.6 ± 18.7 ^a	38.4 ± 17.5 ^a
	280	25.8 ± 15.7 ^a	30.3 ± 16.7 _a	29.6 ± 23.4 ^a	17.1 ± 13.1 ^a	32.9 ± 11.6 ^a

Values are mean ± standard deviation, N=252

d) Pollen transfer limitation

Globally, supplemental hand pollination significantly increased fruit set (81 ± 20% versus 70 ± 26% respectively, $W=12$, $df=1$, $p < 0.001$, Table 6) and the number of seeds per fruit (19 ± 7 versus 15 ± 7 respectively, $W=15$, $df=1$, $p < 0.001$, Table 7) when compared with open pollinated flowers. However, such

differences were not significant for all cultivars (Table 7). The respective mean fruit- and seed- pollen limitation index were $PL_{\text{fruit}} = 0.14$ and $PL_{\text{seed}} = 0.21$.

Table 7. Fruit set, seed production (under conditions of open vs. hand-pollination) and pollen transfer limitation (PL) in different cultivars, in oilseed rape fields, based on the first 30 flowers of the main raceme. Different letters (for a same parameter and cultivar) indicate significant differences.

Cultivar	Open pollination			Hand-pollination			PL_{fruit}	PL_{seed}
	n plants	Fruit set (%)	Seed per fruit	n plants	Fruit set (%)	Seed per fruit		
DK Excellium	16	76 ± 22 ^a	15.25 ± 5.36 ^A	18	80 ± 20 ^a	18.61 ± 3.75 ^A	0.05	0.18
DK Explicite	41	71 ± 24 ^a	15.35 ± 5.88 ^A	44	80 ± 18 ^b	17.64 ± 5.64 ^A	0.11	0.13
DK Exquisite	9	89 ± 15 ^a	21.45 ± 8.12 ^A	13	77 ± 25 ^a	23.57 ± 6.6 ^A	-	0.09
DK Exsor	12	83 ± 11 ^a	19.29 ± 6.02 ^A	12	92 ± 8 ^b	27.41 ± 5.07 ^A	0.09	0.30
DK Exstorm	17	57 ± 29 ^a	13.74 ± 7.54 ^A	18	85 ± 13 ^a	17.87 ± 4.78 ^A	0.33	0.23
Dynastie	18	53 ± 28 ^a	11.14 ± 5.58 ^A	16	72 ± 27 ^a	14.24 ± 6.54 ^A	0.26	0.22
All	113	70 ± 26 ^a	15.33 ± 6.72 ^A	121	81 ± 20 ^b	18.97 ± 6.48 ^B	0.14	0.19

Values are mean ± standard deviation

VII. DISCUSSION

A. Oilseed rape is self-compatible but requires pollen vectors to ensure high fruit and seed set

The expected self-compatibility of cultivar DK Exquisite was confirmed ($SCI=1$) and levels of inbreeding depression were very low in this hybrid cultivar. Our results show that cross-pollination is not obligate for this cultivar and, to the best of our knowledge, it is not obligate for other closely related Belgian cultivars either. Our results are in agreement with several previous observations in oilseed rape hybrid cultivars (Delaplane and Mayer, 2000; Soengas et al., 2013) as well as in other oilseed rape lines (Olsson 1955, 1960; Rives 1957; Williams 1978; Williams et al. 1986). Such self-compatibility facilitates cultivation of only one cultivar per field.

Moreover, under greenhouse conditions in the absence of external pollen vectors, spontaneous self-pollination did occur even though hand pollination increased both fruit and seed set. The observed self-fertility ($SFI=0.7-0.8$) in this study was high when compared with other studied cultivars (Williams 1978, Eisikowitch 1981; Williams et al. 1986). The DK Exquisite cultivar has, therefore, self-fertility potential even though external pollen vectors do

increase seed set. Some authors have demonstrated that, under controlled conditions in the absence of external pollen vectors, shaking the plants to increase contact between flowers results in increased pollination success (Williams 1978; Williams et al. 1986).

B. Insect and wind pollination were low in the Belgian fields

The daily nectar production was high (645 ± 692 μg of sugars per flower per 24 h). Such high nectar production is consistent with previous studies and suggests a high attractiveness of the plants for foraging insects (550 μg of sugar per flower per 24 h; Pierre et al. 1999). Oilseed rape, therefore, is a valuable honey resource in the landscape because one hectare produces on average 14.5 L of nectar per day during peak flowering.

Surprisingly, our numerous field observations showed a very low insect visitation rate, despite the favorable weather conditions in different field situations; very few insects were observed in the fields and foraging insects visited a very small portion (1.1%) of flowers. Because we selected fields without nearby bee hives (at a distance less than 2 km), our visitation rates were lower than those reported by several previous studies that were performed in the presence of honeybee hives (Free and Nuttall, 1968; Langridge and Goodman, 1982; Manning and Boland, 2000; Manning and Wallis, 2005; Mesquida and Renard, 1981). However, low visitation rates have been reported before in other oilseed rape pollination studies with only 1.2 to 3.6 insect visitors observed per m^2 per hour, a rate that is insufficient to provide effective pollination services (Bartomeus et al., 2014; Blažytė-Čereškienė et al., 2010; Woodcock et al., 2013). The absence of sufficient insects results in a high pollination demand that is not met by the available pollination service, and this imbalance has been demonstrated in several locations across Europe, especially in Belgium (Schulp et al., 2014). Such low insect visitation rates in mass flowering crops are generally explained by the large quantities of flowers that are open simultaneously (Holzschuh et al., 2011). The crop is a valuable food resource for insects, but insects were not present in adequate numbers to pollinate all flowers.

The wind may play an additional role in oilseed rape pollination (Abrol, 2012, 2007; Gallai, 2008; Knuth, 1908; McGregor, 2009; Williams, 1984). The airborne pollen flow observed under our field conditions was high (472 ± 198 pollen grains/ cm^2 stigma per 24 h); approximately ten times higher than the airborne pollen flow observed by Mesquida and Renard (1982). Nevertheless, with a stigma surface of approximately 0.5 mm^2 , the observed high airborne pollen flow could only be responsible for a potential average deposition of 8 pollen grains per stigma. Because we observed an average of 300 pollen grains per stigma, wind deposition would only contribute 2.5% of all pollen deposition. We therefore conclude that airborne pollen flow and wind pollination contributed little to pollination in oilseed rape under the field conditions in this study. Indirect effects of the wind such as shaking the plants to increase flower contact, could explain the high pollen deposition on stigmas (Eisikowitch, 1981; Williams, 1978; Williams et al., 1987, 1986).

Despite the high observed pollen deposition, some of the studied cultivars suffered from pollen limitation ($PL_{\text{fruit}} = 0.14$ and $PL_{\text{seed}} = 0.21$) under the field conditions in this study. A similar level of pollen limitation was previously recorded in several other cultivars (Morandin and Winston, 2005) but not all cultivars suffered from pollen limitation (Stanley et al., 2013). Our supplemental hand-pollination tests were performed on plant sections and not on entire plants. Strong physiological compensation mechanisms may occur at the plant level and result in a high final yield, independent of the quality of insect or wind pollination (Clarke and Simpson, 1978; Diepenbrock, 2000; Lerin and Rivault, 1982; Mesquida et al., 1988; Mesquida and Renard, 1981; Thurling, 1974; Williams and Free, 1979). Because breeders mainly select new oilseed rape cultivars based on the effectiveness of their compensation mechanisms, current yields appear to be only slightly sensitive to insect or wind pollination (Pywell et al., 2015).

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IX. CONFLICT OF INTEREST

The authors declare having no conflicts of interest.

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Part 2: AES estimation of flowering resources and insect visitors

Chapter 1: Flower-strip agri-environment schemes provide diverse and valuable summer flower resources for pollinating insects

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Running title: Flowered strips support pollination services

I. ABSTRACT

1. The diversity and abundance of insect pollinators are declining. This decline reduces the potential ecosystem services of pollination for wild and cultivated plants. Specific agri-environment schemes (AES) are subsidised to support and conserve biodiversity in farmlands. In Belgium, the pollinator flower-strips AES, strips of flower-rich hay meadows, has been promoted as a potential scheme to increase pollinator abundance and diversity, even if their effectiveness has not been locally evaluated.

2. The main objective of this research is to assess the capacity of pollinator-strip AES to provide flower-resources to diverse pollinators. During two years, we monthly measured the availability of flower resources (pollen and nectar) produced on four flower-strips surrounded by intensive farming in Belgium. We counted and identified insects that visited these flowers, and we constructed the plant-insect interactions networks.

3. The pollinator-strip AES presented a mix of both sown and spontaneous plant species. The ten sown plant species were all present, even after eight years of strip settings. Three of them, *Centaurea jacea*, *Lotus corniculatus*, and *Daucus carota* were mainly visited for nectar collection, and a spontaneous non-sown species, *Trifolium repens*, had a key role in providing high-quality pollen to insects. Most of the observed flower-visiting insects belonged to common species of Hymenoptera and Diptera. All are considered highly efficient pollinators.

4. *Synthesis:* The Belgian pollinator flower-strips are effective AES that provide flower resources to pollinators, mainly during summer and support pollination services. Nevertheless, spring and autumn flower resources

remain poor and could reduce the strips' effectiveness for supporting long-term insect diversity.

II. KEYWORDS

AES; *Centaurea jacea*; Nectar; Pollen; Pollination networks; Pollinators

III. INTRODUCTION

Pollinator decline and the resulting pollination crisis endanger the sexual reproduction of numerous plant species, wild and cultivated (Allen-Wardell et al. 1998; Cane and Tepedino 2001; Ghazoul 2005; Biesmeijer et al. 2006; Potts et al. 2010). Approximately 78% of temperate plants depend on insects for pollination (Ollerton et al. 2011). Several agricultural practices, mainly linked to agricultural intensification, are considered as the major causes of this crisis. These practices include pesticide use (Godfray et al. 2015; Goulson et al. 2015) and landscape modifications like land consolidation and hedgerows destruction leading to insect habitat and resources loss (Kennedy et al. 2013; Deguines et al. 2014; Goulson et al. 2015). In the European Union, since 1992, incentive measures to environment-friendly farmland management are promoted by the Common Agricultural Policy (CAP). These measures, named agri-environment schemes (AES), are subsidised by the CAP to compensate farmers that implement AES to compensate for landscape homogeneity, excessive intensive farming and by encouraging biodiversity in farmlands (Goulson et al. 2015; Wood et al. 2017). Several regional pollinator initiatives propose specific pollinator-friendly managements. But lot of them are devoted to honeybee and not to the pollinator diversity (Decourtye et al. 2010). In 2005, the Walloon government introduced 11 AES, among which the AES 'managed-strips'. In 2013, 1275 km of these managed-strips were implemented (Natagriwal 2016). The aim of one of these managed-strip AES, the pollinator flower-strip AES, is to support wild pollinating insects (Le Roi et al. 2010; Natagriwal 2014). The Belgian pollinator flower-strips are within-field strips of flower-rich hay meadows. They are sown with a mix of four native grasses and ten entomophilous wildflowers, mainly belonging to Asteraceae (e.g. *Leucanthemum vulgare*) and Fabaceae (e.g. *Medicago lupulina*, *Medicago sativa*, *Lotus corniculatus*, and *Trifolium pratense*). Pollinating insects forage on flowers to collect sugars, proteins, and lipids from nectar and pollen (Müller et al. 2006; Michener 2007). Insect development and population growth depend on the quality and diversity of the available flower resources (Pernal and Currie 2001; Tasei and Aupinel 2008; Eckhardt et al. 2014; Vanderplanck et al. 2014). Some insects, especially *Bombus*, can develop flower fidelity based on the resources quality (Leonhardt and Blüthgen 2012; Ruedenauer et al. 2016). Studying this fidelity can help to understand which plants are the best pollen providers. Natural regeneration of plants and spontaneous species settlement are unpredictable but can strongly modify the composition of the sown flower-strips, changing their interest for insects (Carvell et al. 2004; Pywell et al. 2005). Evaluating the biological effectiveness of such flower-strips under local constraints (e.g.

landscape, seed bank, insect diversity, farming practices) is essential to assess pertinence and identify improvements to composition and management (Dicks et al. 2013; Batáry et al. 2015). AES optimisation has been identified as a key policy-relevant question (Merckx et al. 2009), but such evaluation has never been performed in old (6 to 8 years old) farmer-implemented pollinator flower-strip AES. Moreover, most of the previous studies on flower-strips focussed on one or few insect groups such as *Bombus* (Carvell et al. 2007; Pywell et al. 2011), other wild bees (Scheper 2015; Wood et al. 2017), butterflies (Haaland and Gyllin 2010; Haaland and Bersier 2010; Pywell et al. 2011) or moths (Merckx et al. 2009) but rarely on the entire guild of flower-visiting insects.

The objective of this paper is to answer the following questions:

- 1) What is the entomophilous plant diversity, the flower resources (pollen and nectar) they provide and the relative importance of sown and spontaneous plant species?
- 2) What are the diversity and abundance of insects foraging on flower-strips?
- 3) What are the plant species visited for their pollen and do insects use pollen sources from outside of the flower-strips?
- 4) Is the flower resources provision by flower-strips adapted to insect phenology?

IV. MATERIALS AND METHODS

A. *Studied sites*

Monthly, from April to September, in 2014 and 2015, we studied four eight-year-old pollinator flower-strips located in Condroz, South Belgium (50°22'N, 5°13'E, 250 to 300 m a.s.l., Table 8). The Condroz is characterised by an east-west succession of calcareous sandstone ridges and fertile wind loess soils (Castiau et al. 2011). Meadows (47%), cereal (37%), oilseed rape (5%), sugar beet (4%) and potato (4%) crops dominate the Condroz agricultural landscape (Castiau et al. 2011).

Sites with neighbouring apiaries, orchards, forests, hedges, or villages were omitted to avoid high competition by honeybees, *Apis mellifera* hives or influence from non-agricultural habitats. Honeybees can be very competitive for flower resources, reducing the availability of flower resources for wild bee population in a radius up to one km around the apiary (Pyke and Balzer 1985; Paini 2004; Goulson and Sparrow 2009; Herbertsson et al. 2016). Sites were at least 3 km from apiaries during the flowering period. All the strips were at least 3 km apart to avoid pseudo-replication.

The four studied strips were 12 to 21 m wide, and 560 to 1000 m long (Table 8). They were sown in 2008 with a seed mix (30kg/ha) of perennial native grasses (85% Poaceae: *Agrostis capillaris*, *Festuca rubra*, *Poa* spp.), and wildflowers (4% Fabaceae: *Medicago lupulina*, *Medicago sativa*, *Lotus corniculatus*, and *Trifolium pratense*, and 11% other entomophilous species:

Achillea millefolium, *Centaurea jacea*, *Daucus carota*, *Leucanthemum vulgare*, *Malva moschata*, and *Silene x hampeana*, Le Roi et al. 2010). We studied three transects of 100 x 1 m per strip.

It is known that insects abundances, diversity and the plant they use vary among years (Bascompte and Jordano 2014). Therefore, to have a global overview of the flower-insect interactions occurring on the perennial pollinator flower-strip AES we pooled together sites and years.

Table 8. Location of the four studied pollinator flowered strips (all sown in 2008) in Belgian agricultural landscapes in 2014 and 2015.

	Locality	Location Lat. Long.	Length (m)	Width (m)
Strip 1	Ciney	50°18'25"N 5°08'43"E	1000	12
Strip 2	Ciney	50°16'54"N 5°07'54"E	560	12
Strip 3	Havelange	50°21'42"N 5°17'20"E	773	21
Strip 4	Ciney	50°17'38"N 5°00'13"E	1000	20

B. Observations

a) Flower observations

To assess the flower resources, we recorded the diversity, the density and the resource productions of the flowering entomophilous species.

For all species, we recorded flower unit densities every 5 m along each transect using 1-m² quadrats. We conducted counting once a month (around the 15th, according to weather conditions) from April to September. For Asteraceae, a flower unit corresponds to a capitulum while for all the other species the flower unit corresponds to a single flower.

For the flower resources already evaluated, we used values provided by Hicks *et al.* (2016), or Baude *et al.* (2016). For not still evaluated species, we sampled pollen volumes (μL) and 24h nectar sugar secretion (μg) per flower unit, according to Hicks *et al.* (2016) and Baude *et al.* (2016). Supplemental Table S2 presents data of the 12 species we measured.

We standardised all continuous variables to 1 m² ((sum of data)/(sum of observed areas)) or to 100 m² ((sum of data)/(sum of observed areas)*100).

b) Insect observations

We recorded the insect diversity foraging on the strips and the flowers they visited.

From April to September 2014 and 2015, we monthly recorded insect-flower interactions along two consecutive walks (100 m, 10 to 15 min) at least 10 min apart, in the same way, along each transect. We recorded only insects visiting an open flower unit. We conducted observations from 9:00 am to 6:00 pm on sunny and warm days (Willmer and Stone 2004; Baldock et al. 2015). Order

and time of observation varied among sampling runs to avoid confounding of time and transects. We performed a total of 11 field sessions (two walks each time) per strip.

We focused on insects considered to be effective pollinators, *i.e.* bees (Hymenoptera), butterflies (Lepidoptera), and hoverflies (Syrphidae, Diptera) (Biesmeijer et al. 2006; Potts et al. 2010). When practicable, we identified insects to species level in the field (*i.e.* *Apis mellifera*, *Eristalis tenax*, etc.). Due to morphological similarities, we identified bumblebees in the field up to their Operational Taxonomic Unit (OTU, Terzo & Rasmont 2007). To estimate the within-*Bombus* OTU diversity we collected one of every 30 bumblebees for lab identification. We individually caught other insects for lab identification. *Bombus* (Terzo and Rasmont 2010), Coleoptera (Auber 1960; Unwin 1984), Lepidoptera (Skinner and Wilson 2009; Tolman and Lewington 2009), and Syrphidae flies (Verlinden 1994; Stubbs and Falk 2002; Speight and Sarthou 2016) were identified up to the species by the authors. The non-Apidae wild bees were identified by a taxonomist of the Royal Institute of Natural Sciences of Brussels. The non-Syrphidae Diptera were identified to family level (Stubbs and Falk 2002; Oosterbroek 2006), and the number of morphotypes was recorded. For the current paper, we grouped parasitoid Hymenoptera and non-Syrphidae Diptera all together (one type of insects each) as they are considered poor efficient pollinators. For the other insects, a 'type' corresponds to a single species or an OTU for *Bombus*.

To appreciate the attractiveness of flower species according to the flower unit density, we calculated flower unit visitation rates as the total number of visits on a given species divided by the total number of available flower units of this species in the sampled transects.

c) Pollen load observations

To identify which flower species were visited by insects for pollen collection, and to assess the proportion of pollen collected from outside the managed strips, pollen loads were sampled all along the sampling walks. We focused pollen load observations on bumblebees as other bees were infrequent.

We removed (with a toothpick) one corbicular pollen load on every *Bombus* individual observed with pollen loads during the insect observations in 2014 and 2015. We analysed a total of 49 pollen loads, from *Bombus lapidarius* OTU (34), *Bombus terrestris* OTU (8), and *Bombus pascuorum* OTU (7). Pollen loads were acetolysed (Erdtman 1954, 1960; Hesse and Waha 1989) for identification under light microscopy (Leitz Wetzlar). Approximately 500 pollen grains per pollen load were identified using the lab collection of reference pollen slides and Reille's published pollen collections (Reille 1992, 1995). Species making up less than 2.0% of a pollen load content were not included in the quantitative analyses and were classified as 'Undetermined' since they could have arisen from contamination (Free 1970; Westrich and Schmidt 1986).

C. Statistical analyses

We performed all statistical analyses using the software R (version 3.2.4 GUI 1.67). Insect–flower interaction networks were visualised with the ‘bipartite’ R-package. We considered insect species as higher trophic level and plant species as lower trophic level.

To compare the correlation among insect diversity and density, flower resources quantities and flower unit densities, we calculated Pearson (when no ‘na’ values) or Spearman (when some ‘na’ value) correlation factors using the ‘rcorr’ function of the ‘Hmisc’ R-package.

To visualise distribution of insect–flower interactions and flower-resources through the season, we generated grey-coloured heat maps using the ‘heatmap.2’ function of the ‘gplot’ R- package.

V. RESULTS

A. Flowers

We identified 54 flower species and counted a total of 227,081 flower units. The observed flowers were distributed among ten sown, and 44 spontaneous species belonging to 18 different families (Fig. 9). The sown species were all observed in both years. All the spontaneous species were observed in 2015, but only 25 were recorded in 2014. The mean flower diversity (the number of flowering species per strip) reached 28.8 ± 9.5 species per strip over the two field seasons. Sown species represented 68.4% of the flower units. The Fabaceae provided the highest number of flower units (60.4%) with ten species, followed by Apiaceae (17.2%) with two species and Asteraceae (16.6%) with 18 species (Figs 9-10).

Four species, *Lotus corniculatus*, *Silene x hampeana*, *Centaurea jacea*, and *Trifolium repens* had the best continuity in flower resources provision as they flowered during eight to nine months over the 11 months of observation. Spontaneous species were the only blooming species in April (Fig. 9, Table 9). The main flowering peaks occurred from June to August: 27.5% of the total flower units were observed in June, 23.2% in July and 27.5% in August (Figs 1-2).

The global flower unit density over the two years was 81.6 ± 130.7 flower units/m². Most flower species (57%) showed a very low flower unit density (<1 flower unit/m²) (Fig. 9). However, the highest floral densities were reached during summer months with *T. repens* up to 192 flower units/m² in June, *Achillea millefolium* up to 187 flower units/m² in July and *Lotus corniculatus* up to 162 flower units/m² in August.

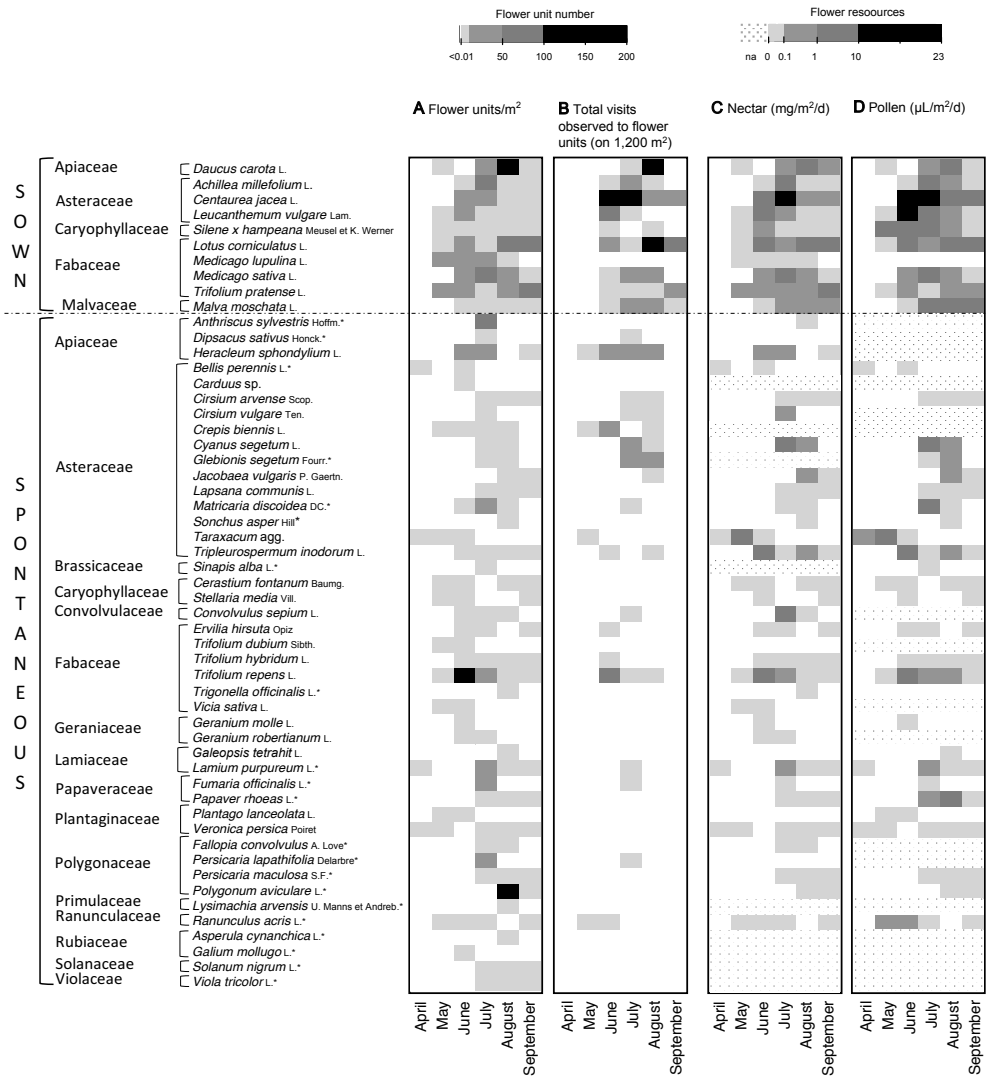


Figure 9. Flower unit density, flower unit visits numbers and flower resources densities across seasons in the four pollinator flowered strips in both 2014 and 2015. Species are named according to the APG III classification (Tison and de Foucault 2014). **A.** Mean number of flower units observed per m². **B.** Total number of flower unit visited per species ($n=3,097$, observed area=1,200 m²). **C.** Nectar sugar production per species, mg per m² per 24h. **D.** Pollen production per species, μ L per m² per 24h. * indicates species that flowered in 2015 only.

B. Flower resources

Flower resources were mainly available during June and July with 401 and 204 $\mu\text{L/day/m}^2$ of pollen and 51 and 91 mg/day/m^2 of nectar sugar. The sown species provided 84.7% of the pollen and 39.6% of the nectar resources (Table 9). Four families provided most of the pollen resources: Asteraceae (82.0%), Malvaceae (7.1%), Fabaceae (5.5%), and Papaveraceae (3.2%). Asteraceae flowers were the major nectar resources, providing 38.3% of all the nectar sugar in the strips, followed by Convolvulaceae (36.3%), Fabaceae (13.5%), and Malvaceae (4.4%) (Table 10). *Leucanthemum vulgare* and *Papaver rhoeas* were the primary pollen producers, supplying 15.9 and 13.3 μL of pollen per flower unit per day. *Cirsium vulgare* and *Taraxacum* agg. produced the most nectar, providing 2.6 and 2.4 mg of sugars per flower unit per day.

Table 9. Monthly pollen and nectar production per m^2 per 24h and contribution of sown species in the observed pollinator flowered strips in both 2014 and 2015. Flower unit density are means $\pm$ SD, resources values are calculation over the four strips altogether.

		April	May	June	July	August	September	Total
Sown species	Number of flowering species	0	6	9	10	10	9	10
	Mean flower unit density	0.00	13.76	34.81	110.76	103.12	27.66	48.35
	(/m ²)	$\pm$ 0.00	$\pm$ 30.77	$\pm$ 42.07	$\pm$ 192.03	$\pm$ 59.96	$\pm$ 55.31	$\pm$ 93.63
Spontaneous species	Number of flowering species	4	9	17	26	26	18	43
	Mean flower unit density	0.00	2.34	100.31	23.57	12.36	2.00	23.43
	(/m ²)	$\pm$ 0.03	$\pm$ 1.99	$\pm$ 201.19	$\pm$ 43.01	$\pm$ 25.26	$\pm$ 3.16	$\pm$ 87.61
Total flower unit visited		0	15	836	1106	805	121	3097
Contribution of sown species (%)		-	0	87	84	90	100	86
Pollen	$\mu\text{L/m}^2/\text{day}$	0.40	8.64	400.77	203.68	70.45	20.33	
	global %	0.06	1.23	56.90	28.92	10.00	2.89	100.00
	Monthly contribution of sown species (%)	0.00	39.53	93.33	84.92	38.82	92.32	84.70
Nectar	$\text{mg sugar/m}^2/\text{day}$	0.30	2.81	50.74	90.74	29.22	9.63	
	global %	0.16	1.53	27.66	49.47	15.93	5.25	100.00
	Monthly contribution of sown species (%)	0.00	35.37	63.31	23.36	35.00	84.39	39.62

C. Insect–flower interactions

Insects visited 28 of the 54 flowering species, primarily Asteraceae (65.1% of the total number of interactions), Fabaceae (18.4%) and Apiaceae (14.4%, Table 10). The sown species represented 86.6% of the insect visits (Table 9). *Medicago lupulina* was the unique sown species with no recorded visits (Table 3). The main visited species were among the most abundant sown flowering species: *Centaurea jacea* ($53.3 \pm 18.8\%$ of total visits), *Daucus carota* ($12.4 \pm 16.5\%$), and *Lotus corniculatus* ($10.7 \pm 21.1\%$, Table 10). These three species combined represented 33% of all flower units, 65% of the pollen, 66% of the nectar sugar production and hosted 80% of all insect visits (Table 3). All together, the three most-visited spontaneous species (*Cyanus segetum*, *Crepis biennis*, and *Glebionis segetum*) received 6.3% of all insect visits (Table 10). Pearson correlation factor between the number of insect visits and the number of open flower units per species was $r = 0.28$, $p = 0.0419$. Combining pollen or nectar quantities per flower unit and the flower unit density, we obtain the flower resource densities. These values had high Spearman correlation factor with insect visits for both pollen ($r = 0.93$, $p < 0.001$) and nectar sugar ($r = 0.98$, $p < 0.001$).

Six Asteraceae species had a high number of insect visits per flower unit: *Cirsium vulgare* (1.58 visits per flower unit), *Glebionis segetum* (0.29), *Cirsium arvense* (0.11), *Crepis biennis* (0.07), *Centaurea jacea* (0.03), and *Cyanus segetum* (0.03).

We recorded a total of 3097 insect-flower interactions, summarised by a global bipartite network (Fig. 10). Of the 64 observed insect types, 60 were observed on sown species and 29 on spontaneous plant species. The sown *Centaurea jacea* attracted the largest diversity of insects (60.9% of observed insect diversity). Three other sown species were visited by a large proportion of the observed insect diversity: *Daucus carota* (27.5% of insect diversity), *Leucanthemum vulgare* (24.6%) and *Lotus corniculatus* (21.7%, Table 10)

We recorded a total of 65.7% of non-Apidae wild bees on spontaneous species: 5.7% on *Cyanus segetum*, 51.4% on *Glebionis segetum*, 2.9% on *Lamium purpureum* and 5.7% on *Persicaria lapathifolia*. Non-Apidae wild bee species visited four sown species: *Achillea millefolium* (2.9% of the non-Apidae wild bee visits), *Centaurea jacea* (25.7%), *Daucus carota* (2.9%) and *Malva moschata* (2.9%) (Fig. 10). Oppositely, we observed 88.2% of the Apidae (*Apis mellifera* and *Bombus*) on sown species.

We observed the non-Syrphidae Diptera (41 morphotypes), in 60.7% of visited plant species, *Bombus lapidarius* OTU in 57.1% of visited plant species and *Eristalis tenax* in 46.4%. We observed *Apis mellifera*, *Bombus terrestris* OTU, and *Sphaerophoria scripta* each in 35.7% of visited plant species (Fig. 10).

Table 10. Plant species contribution (%) to global flower unit number, insect visits, flower resources (pollen and nectar) production and for each species, the number of visits observed for 100 flower units, over both 2014 and 2015 and the four studied strips. Plants are named according to the APG III classification (Tison and de Foucault 2014). The total number of insect types observed was 64. 'na' indicates the absence of flower resource production data.

	Family	Species	Flower units % (n= 227,081)	Visits % (n= 3097)	Contribution to pollen production (%)	Contribution to nectar production (%)	Number of insect types hosted	Number of visits for 100 flower units
SOWN	Apiaceae	<i>Daucus carota</i>	16.71	11.59	0.60	4.19	19	0.19
		<i>Achillea millefolium</i>	8.33	1.03	0.90	2.17	9	0.03
	Asteraceae	<i>Centaurea jacea</i>	4.60	53.02	61.71	54.39	39	3.14
		<i>Leucanthemum vulgare</i>	1.62	3.16	17.36	4.30	16	0.53
	Caryophyllaceae	<i>Silene x hampeana</i>	0.61	0.10	0.99	0.37	3	0.04
		<i>Lotus corniculatus</i>	11.67	12.53	3.20	7.17	15	0.29
	Fabaceae	<i>Medicago lupulina</i>	4.59	0.00	na	0.08	0	0.00
		<i>Medicago sativa</i>	11.36	2.58	1.44	4.79	14	0.06
	Malvaceae	<i>Trifolium pratense</i>	7.26	1.26	0.27	3.18	7	0.05
		<i>Malva moschata</i>	0.73	1.10	7.18	5.58	7	0.41
SPONTANEOUS	Apiaceae	<i>Anthriscus sylvestris</i>	0.82	0.00	na	0.08	0	0.00
		<i>Dipsacus sativus</i>	0.09	0.10	na	na	1	0.31
		<i>Heracleum sphondylium</i>	0.73	2.71	na	0.75	13	1.02
		<i>Bellis perennis</i>	<0.01	0.00	0.00	0.00	0	0.00
		<i>Carduus</i> sp.	<0.01	0.00	na	na	0	0.00
		<i>Cirsium arvense</i>	<0.01	0.16	0.00	0.10	3	10.66
		<i>Cirsium vulgare</i>	<0.01	0.61	na	0.19	4	182.20
	Asteraceae	<i>Crepis biennis</i>	0.07	1.55	na	na	7	6.48
		<i>Cyanus segetum</i>	0.23	2.97	1.62	3.34	6	3.59
		<i>Glebionus segetum</i>	0.02	1.81	0.17	0.15	8	27.54
		<i>Jacobaea vulgaris</i>	0.03	0.03	0.05	0.28	1	0.31

Family	Species	Flower units % (n= 227,081)	Visits % (n= 3097)	Contribution to pollen production (%)	Contribution to nectar production (%)	Number of insect types hosted	Number of visits for 100 flower units
	<i>Lapsana communis</i>	0.03	0.00	0.02	0.03	0	0.00
	<i>Matricaria discoidea</i>	0.68	0.42	0.55	0.00	3	0.17
	<i>Sonchus asper</i>	<0.01	0.00	0.00	0.00	0	0.00
	<i>Taraxacum</i> agg.	0.10	0.26	0.60	2.19	3	0.69
	<i>Tripleurospermum inodorum</i>	0.14	0.06	0.78	1.78	2	0.13
Brassicaceae	<i>Sinapis alba</i>	<0.01	0.00	0.00	na	0	0.00
Caryophyllaceae	<i>Cerastium fontanum</i>	0.05	0.00	0.00	0.01	0	0.00
	<i>Stellaria media</i>	<0.01	0.00	0.00	0.00	0	0.00
Convolvulaceae	<i>Convolvulus sepium</i>	0.01	0.06	na	1.10	2	1.60
	<i>Ervilia hirsuta</i>	0.38	0.03	na	0.09	1	0.02
	<i>Trifolium dubium</i>	0.08	0.00	na	0.00	0	0.00
Faba-ceae	<i>Trifolium hybridum</i>	0.35	0.03	0.04	0.00	1	0.02
	<i>Trifolium repens</i>	24.46	1.97	1.31	2.68	5	0.02
	<i>Trigonella officinalis</i>	<0.01	0.00	na	0.00	0	0.00
	<i>Vicia sativa</i>	0.02	0.00	na	0.05	0	0.00
Geraniaceae	<i>Geranium molle</i>	0.01	0.00	0.00	0.00	0	0.00
	<i>Geranium robertianum</i>	<0.01	0.00	na	0.00	0	0.00
Lamiaceae	<i>Galeopsis tetrahit</i>	<0.01	0.00	0.00	na	0	0.00
	<i>Lamium purpureum</i>	0.75	0.13	0.03	0.72	3	0.05
Papaveraceae	<i>Fumaria officinalis</i>	0.43	0.03	0.01	0.09	1	0.02
	<i>Papaver rhoeas</i>	0.02	0.00	0.48	0.00	0	0.00
Plantaginaceae	<i>Plantago lanceolata</i>	0.22	0.00	0.00	na	0	0.00
	<i>Veronica persica</i>	0.07	0.00	0.00	0.00	0	0.00
	<i>Fallopia convolvulus</i>	0.07	0.00	na	0.04	0	0.00
Polygonaceae	<i>Persicaria lapathifolia</i>	1.17	0.45	na	na	6	0.11
	<i>Persicaria maculosa</i>	0.06	0.00	0.00	0.02	0	0.00

Family	Species	Flower units % (n= 227,081)	Visits % (n= 3097)	Contribution to pollen production (%)	Contribution to nectar production (%)	Number of insect types hosted	Number of visits for 100 flower units
	<i>Polygonum aviculare</i>	1.09	0.00	0.01	0.03	0	0.00
Primu- laceae	<i>Lysimachia arvensis</i>	0.02	0.00	na	na	0	0.00
Ranun- cula- ceae	<i>Ranunculus acris</i>	0.15	0.23	0.65	0.05	3	0.40
	<i>Asperula cynanchica</i>	<0.01	0.00	na	na	0	0.00
Rubia- ceae	<i>Galium mollugo</i>	0.02	0.00	na	0.00	0	0.00
Solana- ceae	<i>Solanum nigrum</i>	0.12	0.00	na	na	0	0.00
Viola- ceae	<i>Viola tricolor</i>	0.05	0.00	na	na	0	0.00

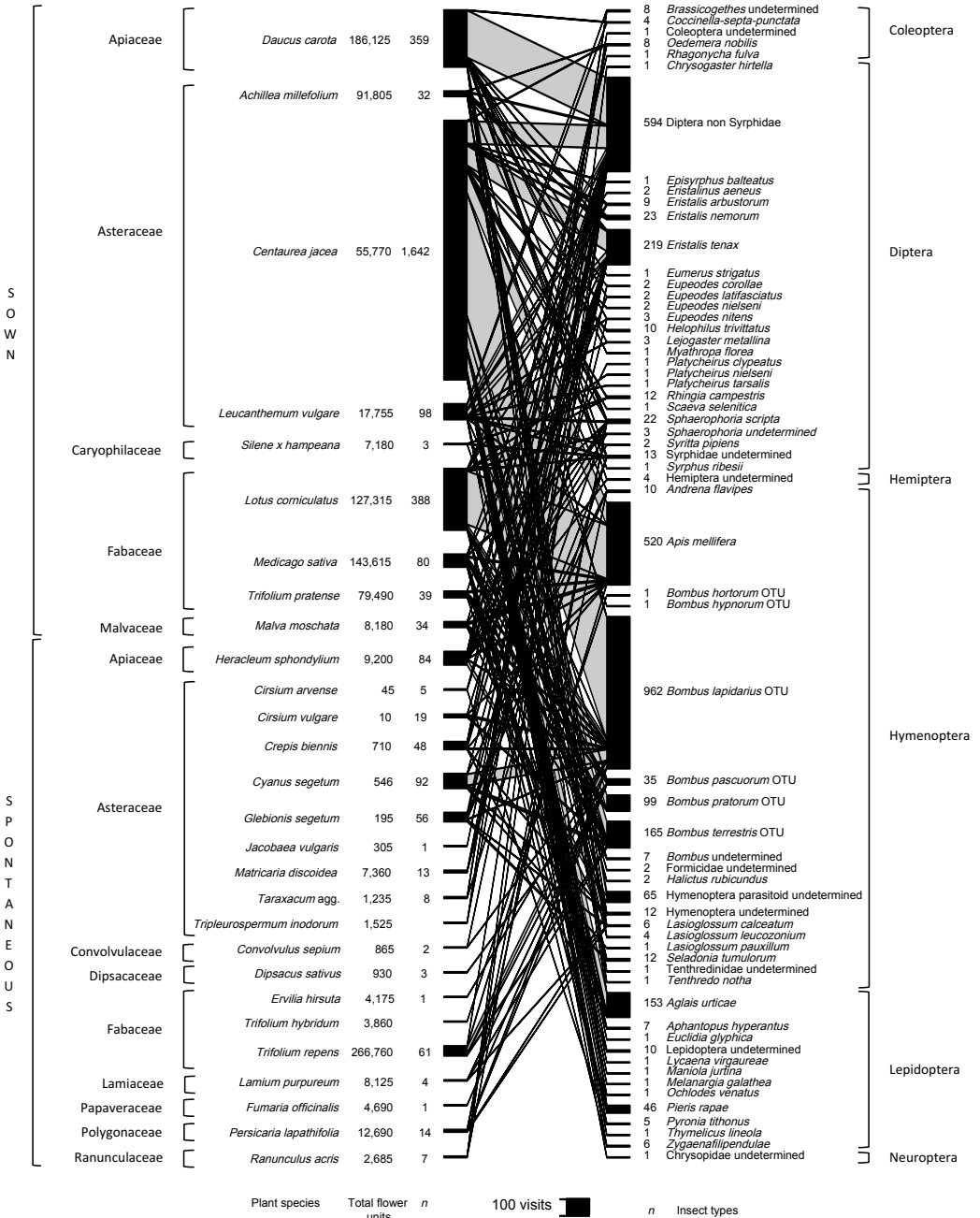


Figure 10. Global interaction network among flowers and insects over the four pollinator flowered strips studied in 2014 and 2015 ($n=3097$). Size of boxes are proportional to 'n' the number of insect visits. 'Total flower units' refers to the total number of flower units observed.

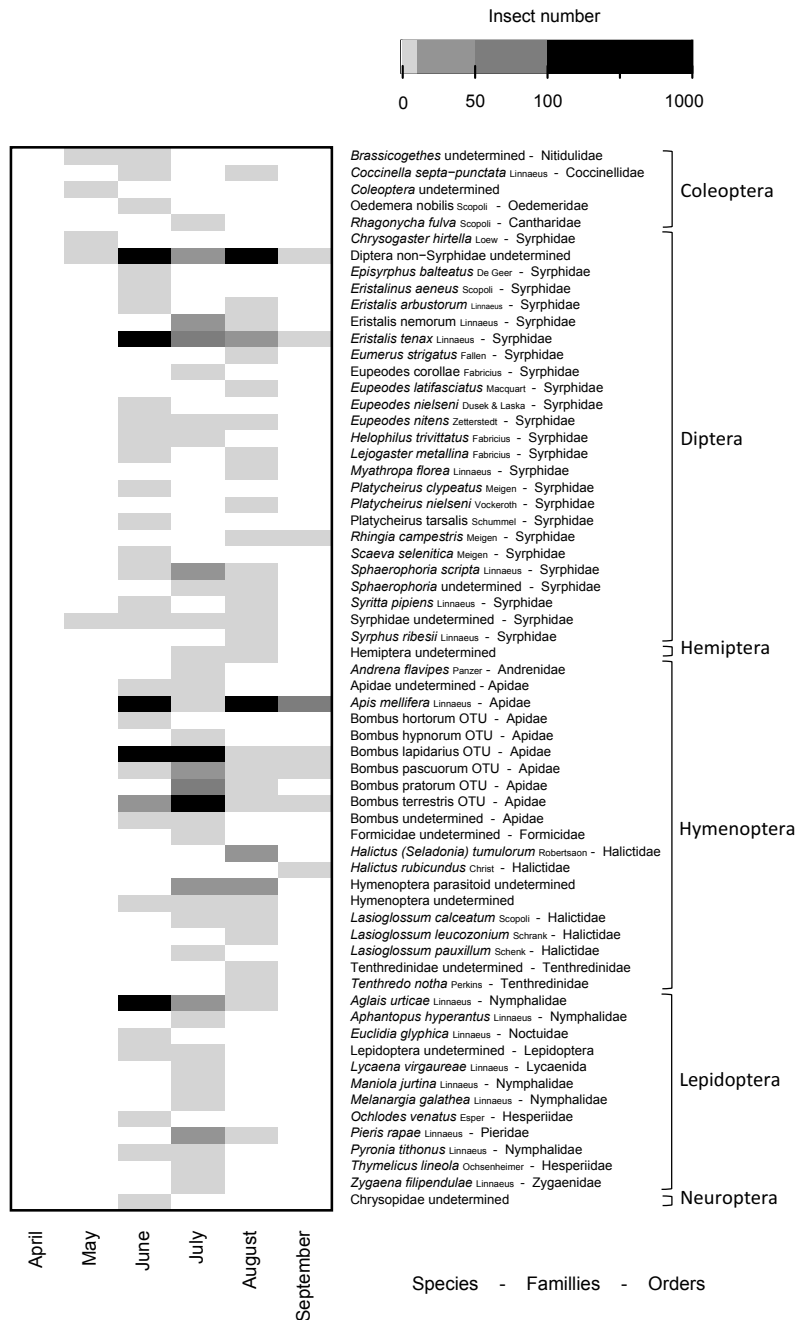


Figure 11. Insect visitor diversity and abundance across seasons over the four pollinator flower-strips in 2014 and 2015 (total number of observed insect–flower interactions (N=3097)). Within each order, species are in alphabetical order.

D. Insect fidelity

We identified a total of 18 plant species from the 49 collected pollen loads. The plant species present in the strips accounted for 97.5% of the collected pollen with 53.8% for the sown species. Most of the collected pollen loads contained a spontaneous species, *Trifolium repens* (80%), and two sown species, *Centaurea jacea* (59%) and *L. corniculatus* (55%, Table 11). The Fabaceae family was the predominant pollen supplier representing 77.1% of collected pollen grains, especially *Trifolium repens* (39.3%). The Asteraceae family was the second-most-common pollen supplier with 18.2% of collected pollen grains.

Table 11. Pollen species presented in the pollen loads of the three-main bumblebee OTU observed on four pollinator flower-strips in 2014 and 2015. Percentage in pollen load and in OTU diet refers to the pollen grain numbers. Only species representing more than 2% of pollen load composition were considered. Values are means $\pm$ SD. Sown species are in **bold**. * indicates species from outside of the flower-strips. N= 49

Bombus OTU	Plant species	Plant family	Number of pollen loads containing the species	% in pollen load	% in OTU diet
<i>Bombus lapidarius</i> n=34	Brassicaceae sp.	Brassicaceae	1	39.0	1.2
	<i>Centaurea jacea</i>	Asteraceae	21	35.2 $\pm$ 41.8	19.9
	<i>Lotus corniculatus</i>	Fabaceae	20	54.4 $\pm$ 45.7	32.8
	<i>Morus alba</i> L.*	Moraceae	1	8.7	0.2
<i>Bombus pascuorum</i> n=7	<i>Trifolium repens</i>	Fabaceae	25	59.3 $\pm$ 43.4	44.6
	<i>Lotus corniculatus</i>	Fabaceae	6	45.2 $\pm$ 36.3	38.6
	<i>Medicago lupulina</i>	Fabaceae	1	57.9	8.7
	<i>Medicago sativa</i>	Fabaceae	1	78.9	10.9
	<i>Trifolium hybridum</i>	Fabaceae	2	26.8 $\pm$ 17.9	7.5
	<i>Trifolium pratense</i>	Fabaceae	5	12.9 $\pm$ 11.6	9.2
	<i>Trifolium repens</i>	Fabaceae	5	29.9 $\pm$ 27.6	21.7
<i>Bombus terrestris</i> n=8	<i>Centaurea jacea</i>	Asteraceae	3	73.0 $\pm$ 10.3	26.8
	<i>Chenopodium quinoa</i> Willd*	Chenopodiaceae	1	91.8	15.7
	<i>Daucus carota</i>	Apiaceae	2	5.4 $\pm$ 6.9	1.2
	<i>Medicago lupulina</i>	Fabaceae	3	15.7 $\pm$ 19.1	6.2
	<i>Medicago sativa</i>	Fabaceae	1	6.2	1.1
	<i>Raphanus sativus</i> L.*	Brassicaceae	1	19.7	1.7
	<i>Tilia</i> sp.*	Tiliaceae	1	10.4	1.6
	<i>Trifolium dubium</i>	Fabaceae	1	6.5	0.8
	<i>Trifolium pratense</i>	Fabaceae	2	44.3 $\pm$ 61.9	10.4
	<i>Trifolium repens</i>	Fabaceae	4	74.2 $\pm$ 49.1	33.4

Experimental trial of *Chenopodium quinoa* (5 ha) and *Morus alba* (few individuals) were in the vicinity of our strips C and D respectively in 2015.

On average, each pollen load included pollen from 3.5 different plant species (e.g. 3.1 ± 1.1 for *Bombus lapidarius* OTU, 4.6 ± 1.0 for *Bombus pascuorum* OTU and 4.1 ± 1.9 for *Bombus terrestris* OTU). Two pollen loads coming from *Bombus lapidarius* OTU individuals caught on *Lotus corniculatus* contained only pollen of this plant species.

VI. DISCUSSION

During our two years of observations, we mostly recorded *Bombus* (41% of the visits) and Diptera (30%) visitors in (Belgian) flower-strips, dominated by *Centaurea jacea* (53% of the visited flower units). The spontaneous plant species, despite their contribution to the nectar sugar production (60%) were poorly visited (14% of the visits) and by only 50% of the observed insect types. We observed six non-Apidae wild bee species in very few number (1.1% of the visits). In the collected *Bombus* pollen loads, *Trifolium repens* (spontaneous) was the most collected species, even if sown species provided more than 50% of the *Bombus* pollen diet.

A. Asteraceae provided most of the flower resources

Asteraceae were the most visited family with 65% of visits. Notably, *Centaurea jacea* was the most-visited species for nectar collection, as also recorded in a previous study on flower-strips in Sweden and Switzerland (Haaland and Gyllin 2010; Hennig and Ghazoul 2011; Sutter et al. 2017). This species attracted the largest insect diversity in the present study. Asteraceae pollen represented 82% of the available pollen in the pollinator flower-strips whereas it constituted 18% of the *Bombus* pollen diet (loads). Species of the Asteraceae family are known to be good nectar suppliers, both in quality and quantity (Pywell et al. 2004, 2011; Hicks et al. 2016). However, this family provides poor-quality and low-attractive pollen, that does not provide all the requisite essential amino acids, leading to poor larvae development for numerous insect species (Goulson et al. 2005; Hanley et al. 2008; Forcone et al. 2011; Nicolson and Human 2013; Somme et al. 2015; Spear et al. 2016; Vanderplanck et al. 2016).

The majority of the pollen collected by the *Bombus* visitors came from species present on the strips (97.5%), mainly sown species (53.8%). The other collected plant species could be used to improve the seed mixes. Fabaceae was the most abundant family, i.e. the best pollen supplier, in the *Bombus* pollen loads (77% of pollen grains collected). This family provides good quality pollen for insects (Forcone et al. 2011; Moerman et al. 2017). Bumblebee individuals largely collected pollen of *Trifolium repens*. This pollen has a high content of proteins and essential amino acids (Hanley et al. 2008), with a high degree of digestibility by bees (Liolios et al., 2015). The sown plant mix does not include this species, and its presence depends on the seed bank of each site or spontaneous seed dispersal. Despite the predominance of the Asteraceae flower resources, we found only one Asteraceae species in *Bombus* pollen loads. *Bombus* essentially used strips species (14 of the 18

species found in pollen loads). The spontaneous species were of particular interest, representing 46% of the *Bombus* pollen diet. Thus, the various flower species of the pollinator flower-strips provided opportunities for mixing different pollen sources, which is particularly interesting for nutrition and health of some bee species (Di Pasquale et al. 2013). In summer, the pollinator flower-strips can provide sufficient pollen resources for insects. Nevertheless, to increase the quality of pollinator flower-strips, independently of the spontaneous species, we recommend including *Trifolium repens* in the seed mix, even if this species has to be used at a low density to preserve the plant mix balance (Warren 2000).

We found positive correlation among the number of insect interactions with a plant species and the density of flower resources it provided. The quantity and the nutritional value of the flower resources are first clues to assess the value of a plant species for insects. Nevertheless, several other parameters like resources accessibility, colour, odours, and flower shape (Giurfa et al. 1994; Dafni et al. 1997; Campbell et al. 2010) are known to influence insect flower choices strongly. The plant spatial organisation of the strips could explain the prevalence of visits to some species. The two most-visited species for nectar collection, *Centaurea jacea* and *Daucus carota*, have long flowering periods and provided densely grouped flowers (capitulum and umbels respectively) on the top of long rigid stems (>80 cm) that constituted the highest stratum of the strips. These species were locally the most visible and accessible, present in the upper layer of the strip. To optimise the flower-strip, plant mixes have to provide flowers visited by insects. Thus, the number of visits per flower unit could help to select the sown species. Nevertheless, the most interesting species regarding these criteria are weed pest (*Cirsium* species, Pauly and Coppée 2017) or cornfield annuals (*Cyanus segetum*, *Glebionis segetum*) not adapted to perennial strip management. The next species, *Crepis biennis*, *Convolvulus sepium* or *Heracleum sphondylium* could nevertheless be interesting to be added or favoured in the plant mixes. In the same way, the density of sown species with a low flower unit visit rate (e.g. *Achillea millefolium* or *Medicago lupulina*) could be reduced in the seed mix. Nevertheless, more research should be conducted to determine an optimal flower unit visit rate per plant species.

B. Floral visitor abundance and diversity

The observed visitor diversity, more than 110 insect morphotypes, was within the top range of other observations in European farmland flowered areas as 34 to 131 pollinator species in Germany (Fründ et al. 2010; Ebeling et al. 2011) and 25 in England (Campbell et al. 2017). The wild bee diversity included six non-*Bombus* wild bee species and eight *Bombus* species. These species, all polylectic, represent a small fraction of the Walloon Hymenoptera diversity of 300 non-*Bombus* wild bee species (Rasmont et al. 2005) and 30 *Bombus* species (Pauly and Rasmont 2010). The number of Hymenoptera species observed was similar to other studies on European sown strips (Carreck and Williams 2002; Carvell et al. 2004; Aviron et al. 2009; Ebeling et al. 2011; Wood et al. 2017) and in European fields near semi-natural habitat

(Le Féon et al. 2010). Nevertheless, this low wild bee diversity observed on pollinator strip AES could reflect the lack of habitat elements on strips (lack of floral resource diversity or nesting areas) or insufficient area at the landscape scale. A more complex landscape, with a higher density of connected semi-natural elements, could help filling this gap (Mitchell et al. 2013; Defra 2014; Carvell et al. 2016).

The scarcity of non-*Bombus* wild bees (1.1% of the visits) was characteristic of intensively cropped farmland area with a low proportion of semi-natural habitats (Holzschuh et al. 2008; Le Féon et al. 2010; Wood et al. 2017). But Apidae species, especially *Bombus* species (41% of visits), chiefly *Bombus lapidarius* OTU, and *Apis mellifera* (17%), were the foremost visitors on the pollinator flower-strips. Such predominance of polylectic bee visitors may support pollination services at the landscape scale as demonstrated in other studies (Kleijn et al. 2015; M'Gonigle et al. 2015; Wood et al. 2017). The presence of *Apis mellifera*, despite the distance from apiaries (3 km), emphasises the attractiveness of the pollinator flower-strips for insects on a landscape scale.

The visitor abundance we observed (29.2 visits/100m²/25 min from June to September), was comparable to those in other pollinator favourable areas in summer. Blaauw and Isaacs (2014) recorded 32.6 visits/100m²/25 min in sown wild-flowers plots (even if they recorded only bees and Syrphidae) and Forup and Memmott (2005) observed 28.6 visits/100m²/25 min in meadows. Oppositely, the insect abundance we observed was higher than in areas without specific pollinator-friendly management studied by Baldock et al. (2015) in the UK (6.7, 11.1 and 13.0 visits/100m²/25 min respectively in urban areas, farmlands, and nature reserves).

Syrphidae (Diptera) represented 11% of observed visitors on our studied strips with 25 different species. These species represent 8% of the 310 Walloon Syrphidae species (Speight et al. 2015). Syrphidae use several habitats during their life cycle and are highly mobile among habitats (Sommaggio 1999), which could explain their higher diversity than bees even in intensively managed landscape. *Eristalis tenax* was the most frequently observed Syrphidae, as in previous studies in farmland areas (Rader et al. 2012). These Syrphidae are complementary pollinators to Hymenoptera and improve overall pollination services (Ssymank et al. 2008; Jauker et al. 2009; Rader et al. 2015). In addition to their importance in pollination services, 50% of observed Syrphidae species have an aphidophagous larval stage (Speight 2016). Therefore, they might offer additional pest management ecosystem service for surrounding crops (Ssymank et al. 2008).

C. Differences of phenology between insects and flowers

All the observed bee species are early emerging, from March to April (Benton 2006; Falk 2015). But, nearly no flowers were available in April (0.03 flower units/m²) and only a few in May (16.10 flower units/m²). This flower scarcity probably explained the absence of Hymenoptera individuals during spring and

the small number of *Bombus* species observed (Scheper 2015). Also, as flower density and diversity decreased from August to September (from 115.48 to 29.65 flower units/m² and from 15.8 ± 9.5 to 9.5 ± 5.5 species per strip), available resources for late insect individuals were also reduced. In the current study, most of the observed *Bombus* species were early-nesting ones (March to April, (Benton 2006; Falk 2015). Nevertheless, we observed the first bumblebees in June. Spring flower resources are critical for insect population growth (Osborne et al. 2008; O'Rourke et al. 2014; Moquet et al. 2015, 2017) and autumn resources are essential for their overwintering success. Providing flower resources during summer months helps to support insects, while continuity in resource provision is necessary to sustain populations (Roulston and Goodell 2011; Schellhorn et al. 2015; Scheper et al. 2015). Adding early- and late-blooming flower resources could, therefore, bolster insect abundance and diversity (Haaland and Gyllin 2010; Garbuzov and Ratnieks 2014; Schellhorn et al. 2015; Wood et al. 2017). We recommend introducing local spring or autumn blooming species, both attractive for insects and producing abundant flower resources, like *Lamium purpureum*, *Ranunculus acris* or *Taraxacum* agg. (Baude et al. 2016; Hicks et al. 2016), in strip seed mixes. As threes can also provide valuable spring resources (Aupinel et al. 2001), we also recommend coupling flower strips with semi-natural landscape elements as hedges or with other AES favouring threes to increase the duration of flower resource availability.

D. Durability and diversity of flower-strips

On the observed pollinator flower-strips, all ten entomophilous sown species were still dominant eight years after sowing (70% of flower units). Most of the previously studied flower-strips of Europe contained more sown species (about 20 species) and up to 52 spontaneous species (Engels et al. 1994; Bokenstrand et al. 2004; Carvell et al. 2006; Haaland et al. 2011; Hicks et al. 2016; Campbell et al. 2017). However, the diversity of sown species was better conserved in our strips (100%) than in other studies after the same period (Bokenstrand, Lagerlöf and Torstensson, 2004; 69% of the sown species present after ten years). Consequently, the current Walloon species mix and management are suitable for a mid to long-term period of at least eight years.

VII. CONCLUSION

Our study revealed that the Walloon pollinator flower-strips provided flower resources mainly during summer to insect species. Observed insects were mostly common polylectic Hymenoptera (honeybees and bumblebees) and hoverflies which are good candidates for supplying pollination services. But the support for insect diversity was moderate. The sown plant species provided most of the nectar sugar, but only half of the pollen collected by insects. The spring flower resources were scattered, and some sown species were not or poorly used by insects. Thus flower-strips could be improved. The most visited and highly accessible sown species *Centaurea jacea*, *Daucus*

carota and *Lotus corniculatus* have to be kept in the seed mix. Species like *Trifolium repens*, whose pollen is largely collected by *Bombus*, *Lamium purpureum*, *Ranunculus acris* or *Taraxacum* agg. with early and extended flowering period, could be sown instead of poorly visited sown species as *Achillea millefolium* or *Medicago lupulina*. Moreover, we recommend combining this AES, along with other measures as AES supporting hedgerows that provide complementary resources in the early and late seasons and increase landscape connections. Studying the insect dispersal from the strips to the countryside could elucidate the potential for ecosystem services in the surrounding area and exchanges among habitats that could help to set up strips in the best location according to landscape elements.

VIII. AUTHORS' CONTRIBUTIONS

PO and ALJ conceived the ideas and designed the methodology; PO and JT collected and analysed the data; PO and ALJ led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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X. CONFLICT OF INTEREST

The authors declare having no conflicts of interest.

XI. SUPPLEMENTAL DATA

Supplemental table S2. Pollen and nectar production measured to complete Baude et al. (2016) and Hicks et al. (2016) databases. Values are mean $\pm$ SD. 'na' indicates the absence of flower resource data.

Families	Species	Nectar μg sugar/day per flower unit	Pollen μL per flower unit
Asteraceae	<i>Centaurea jacea</i>	1177.75 $\pm$ 1761.20	(Hicks et al. 2016)
	<i>Cirsium vulgare</i>	22548.09 $\pm$ 10542.81	na
	<i>Cyanus segetum</i>	1479.49 $\pm$ 487.56	(Hicks et al. 2016)
	<i>Glebionis segetum</i>	931.21 $\pm$ 735.17	5.079 $\pm$ 2.001
	<i>Jacobaea vulgaris</i>	1098.90 $\pm$ 937.50	(Hicks et al. 2016)
Brassicaceae	<i>Sinapis alba</i>	na	0.822 $\pm$ 0.437
Caryophyllaceae	<i>Silene x hampeana</i>	(Hicks et al. 2016)	0.801 $\pm$ 0.015
Convolvulaceae	<i>Convolvulus sepium</i>	1534.23 $\pm$ 630.02	na
Fabaceae	<i>Lotus corniculatus</i>	67.98 $\pm$ 42.59	(Hicks et al. 2016)
	<i>Medicago sativa.</i>	40.28 $\pm$ 58.85	0.058 $\pm$ 0.002
Malvaceae	<i>Malva moschata</i>	823.75 $\pm$ 755.13	(Hicks et al. 2016)
Polygonaceae	<i>Polygonum aviculare</i>	(Baude et al. 2016)	0.006 $\pm$ 0.001

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Chapter 2: *Agri-environment schemes targeted at farmland bird populations benefit pollinating insects*

Bird-strip AES benefit insect pollinators.

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I. ABSTRACT

1. Farmland biodiversity has declined over the past few decades. European Common Agricultural Policy encourages farmers to set up agri-environment schemes (AES) that support biodiversity and associated ecosystem services, like pollination. Although few AES are specifically devoted to pollinating insects, many could provide suitable resources for pollinators. In Belgium, bird-strip AES contain mixed-plant communities that might prove attractive to insects, although their ability to support pollinators has not yet been evaluated.

2. The main objective of this research was to assess the opportunity of bird strips to feed pollinators. We identified and quantified flower resources, flower-visiting insects and insect-flower interaction networks for two years on four bird strips in intensive agro-ecosystems in Belgium.

3. The bird-strips contained both sown and spontaneous plant species. The spontaneous *Cirsium* species, with its high nectar production, offered complementary resources to the sown species, *Raphanus sativus* and *Phacelia tanacetifolia*. Most of the flower visitors considered as pollinators belonged to Hymenoptera and Diptera orders.

4. In conclusion, bird strips provided flower resources to pollinators, even in the absence of annual sowing.

II. KEYWORDS

Field margins, floral resources, seed strips, bees, syrphids, *Cirsium*

III. INTRODUCTION

Pollinator abundance and diversity are decreasing worldwide, reducing the pollination ecosystem service offer (Kells *et al.*, 2001; Marshall & Moonen, 2002; Kluser & Peduzzi, 2007; Burkle *et al.*, 2013; Garibaldi *et al.*, 2013; Morandin & Kremen, 2013). The decline is largely linked to changes in agricultural management from the last 60 years (Kluser & Peduzzi, 2007; Potts *et al.*, 2009; Kluser *et al.*, 2010). The agricultural intensification, in particular pesticide use (Godfray *et al.*, 2015; Goulson *et al.*, 2015), field consolidation, field margins and hedgerows removal contributed to landscape simplification (MacDonald & Johnson, 2000). Simplified landscapes have less plant species (Kleijn & Raemakers, 2008; Scheper *et al.*, 2014) and less semi-natural areas to provide flower food resources and nesting habitats for insects (Kennedy *et al.*, 2013; Deguines *et al.*, 2014; Bretagnolle & Gaba, 2015; Goulson *et al.*, 2015).

In the European Union, the second pillar of the Common Agricultural Policy subsidises agri-environment schemes (AES) to compensate for landscape simplification and support farmland biodiversity (European Commission, 2017; Wood *et al.*, 2017). But AES still need to be optimized to increase the gain in biodiversity they provide (Merckx *et al.*, 2009). Most of the assessments have focused on a single purpose (e.g. annual cornfield conservation, erosion limitation, beetle banks, or skylark nesting plots in cereal fields), although a single scheme could serve several purposes. Improving habitat for one target species often improves the habitat for a larger group of non-target species (Mckenzie *et al.*, 2013).

Flower-strips are common AES facilities in farmland (Marshall *et al.*, 2006; Haenke *et al.*, 2009; Haaland & Gyllin, 2010, 2011; Haaland *et al.*, 2011; Feltham *et al.*, 2015; Wood *et al.*, 2015; Campbell *et al.*, 2017; Sutter *et al.*, 2017). Such schemes aim to provide food and refuge area to wild fauna, often birds or insects. In farmland, flower-strips are often implemented with the aim to support pollination or pest management services to surrounding fields (Balzan *et al.*, 2014, 2016; Feltham *et al.*, 2015; Tschumi *et al.*, 2016; Campbell *et al.*, 2017). But when established in intensively managed farmland, flower-strips were mainly dominated by few types of interactions, few common insect species visiting only some of the flowering plant species present on the strips (Pywell *et al.*, 2005; Carvell *et al.*, 2007). Therefore, the interest and attractiveness of such flower-strips can be questioned (Kovács-Hostyánszki *et al.*, 2016).

In Wallonia (Belgium), pollinator flower-strip AES are specifically designed for conservation of flower-visiting insects and especially pollinators (Terzo & Rasmont, 2007; Service public de Wallonie, 2012). The most effective and admit pollinating insects are Hymenoptera, mainly bees (Kevan & Baker, 1983; Free, 1993; Willmer, 2011), Syrphidae Diptera (Ssymank *et al.*, 2008; Inouye *et al.*, 2015) and Lepidoptera (Crepet, 1984; Weiss, 1995; Willmer, 2011). Other categories of AES, such as Walloon bird-strip AES, could also be important for insects (Carvell *et al.*, 2007), even if their role to provide

appropriate resources for pollinating insects has not been evaluated. Bird-strip AES are designed for grain-eating farmland birds, providing food (seeds) and shelter during the winter (Natagriwal, 2016). Bird-strip AES can include entomophilous flowering plant species sown in a 'feeding section' (e.g. *Helianthus annuus*, *Medicago sativa*, *Phacelia tanacetifolia*, *Raphanus sativus* etc., Carreck *et al.*, 1999). Such species can provide flower resources to insects. The bird-strips present two other sections, a tall grass 'refuge section' remaining uncut for several consecutive years, and an optional harrowed section. The presence of stubble and hollow-stem plants (Poaceae and Brassicaceae) and the bare soil access provide potential nesting structures, especially for solitary bees (Westrich, 1996; Cane, 1997; Falk, 2015) and other small animals (Kells & Goulson, 2003; Cole *et al.*, 2015).

To effectively support insect abundance and diversity, in addition to nesting opportunities, a candidate scheme must provide dietary resources year after year, without interruption (Schellhorn, Gagic & Bommarco 2015; Scheper 2015; Dainese, Potts & Smith 2016; Moquet *et al.* 2017). Even if the sown species would probably be dominant, as for other annually sown schemes, plant community will mix both spontaneous native plant species and sown species (Fiedler *et al.*, 2008). Therefore, it would be relevant to evaluate the relative contribution of spontaneous and sown species in the provision of flower resources for insects in bird-strip AES.

In 2015 and 2016, we studied four bird-strip AES in southern Belgium to evaluate their ability to feed pollinating insects. We addressed the following questions: (i) What are the flower resources provided by bird-strips? (ii) What is the importance of sown vs spontaneous plant species? (iii) What are the insects feeding on bird-strip flowers? (iv) Which species are visited by insects? And (v) Are flower resources resilient to annual changes?

To assess the diversity and quantity of available resources, we recorded flower diversity and density throughout the year and estimated pollen and nectar quantity. To evaluate attractiveness to pollinators, we recorded the diversity of insects visiting the strips and the plant species that they used.

IV. MATERIALS AND METHODS

A. Studied sites

From April to September 2015 and 2016, we studied four bird strips in Hesbaye, southern Belgium (50°36'N, 4°34'E, 100 to 150 m a.s.l., Table 5). This region is characterised by the high fertility of the soils (Witte *et al.*, 2009). The agricultural landscapes are classical open fields dominated by cereal (44%), sugar beet (*Beta vulgaris*, 20%), potato (*Solanum tuberosum*, 5%) and maize (*Zea mays*, 5%) crops, intermingled with meadows (13%) (Witte *et al.*, 2009). We chose strips in the open field land, at least two km apart from other landscape elements such as apiaries, orchards, forests, hedges, or villages to minimise interactions with honeybee hives or semi-natural habitats. All strips were at least 3 km apart to avoid pseudo-replication. The studied strips were 320 to 600 m long.

Strips are organized into two or three longitudinal sections: (i) a 2 to 3 m wide unmown grass refuge section, sown in 2008 with 20 kg/ha of a mix of *Dactylis glomerata* (50% seed weight), *Schedonorus arundinaceus* (49%) and *Trigonella* sp. (1%). (ii) a 3 to 9 m wide feeding section, sown in 2015 with 40 kg/ha of a mix of one cereal, *Triticum aestivum* (65% of seed weight), and three entomophilous species, *Fagopyrum esculentum* (25%), *Phacelia tanacetifolia* (3%) and *Raphanus sativus* (7%). In 2016, self-seeding was effective for the three species on strip B, for *Phacelia tanacetifolia* and *Raphanus sativus* in strip A and only for *Raphanus sativus* in strip C. None of these species were observed in strip D in 2016. (iii) The optional 2 m wide unsown and harrowed section was present on two strips (Table 12).

Table 12. The four studied bird-strip AES located in Condroz, Belgium.

Strip	Locality	Location (Lat. Long.)	Length (m)	Width (m)
A	Opprebais	50°41'05"N 4°47'09"E	570	12
B	Opprebais	50°40'25"N 4°48'41"E	500	12
C	Gembloux	50°32'53"N 4°38'08"E	600	9
D	Goesnes	50°27'10"N 5°11'53"E	320	12

B. Climatic data

Climatic data were provided by the Belgian Royal Meteorological Institute (IRM). The data were collected hourly at the Ernage station (Gembloux) from April to September 2015 and 2016. Precipitation was summed monthly, and sunshine was recorded daily. Temperatures were recorded daily, and monthly means were calculated (mean, minimum mean, and maximum mean, Table 13).

C. Experimental design

On each strip, a permanent 100 m long, 1 m wide transect was established in the centre of each section (2 to 3 per strip). Each transect was observed once a month from April to September in 2015 and 2016. All observations were conducted on sunny and warm days. Due to bad weather, no field observation was conducted in June 2016. A total of 11 observations sessions were performed per site: 6 in 2015 and 5 in 2016.

D. Floral resource quantities

For all entomophilous species, we recorded flower unit densities using 1 m² quadrats every 5 m along each transect according to Hicks *et al.* (2016). Plant species were identified according to APG III (Tison & de Foucault, 2014). For Asteraceae, flower unit corresponds to a capitulum while for all the other species a flower unit corresponds to a single flower. The three species sowed in 2015 (*Fagopyrum esculentum*, *Phacelia tanacetifolia*, and *Raphanus sativus*) constituted the "sown" species group. We grouped all the other

species into the “spontaneous” species group, including not only unsown species, but also plants that had regrown from previously sown mixes (i.e. *Helianthus annuus*, *Cyanus segetum*, and *Trifolium incarnatum*).

Table 13. Climatic data from the Ernage station, Gembloux, Belgium. Values are mean $\pm$ SD or median with inter-quartile ranges (25th–75th percentiles). * indicates significant differences at 95% confidence. Data provided by the IRM.

Period	Parameter	Value	2015	2016	Stat values
From April to September	Sunshine (min/d)	mean	435.8 260.8	$\pm 378.2 \pm 242.2$	$t_{364} = 2.1889^*$
	Mean temp (°C)	mean	12.30 ± 3.79	11.85 ± 4.26	$t_{350} = -1.2574$
	Mini mean temp (°C)	mean	6.45 ± 3.46	7.29 ± 4.56	$t_{350} = -0.64079$
	Max mean temp (°C)	mean	17.2 ± 4.57	16.16 ± 4.72	$X^2_1 = 10.099$ $P < 0.01$
		median	17.0 (14.4–19.6)	16.5 (12.8–20.1)	
	Raining days		37	60	
	Precipitations (mm/9h)	mean	0.51 ± 2.21	1.45 ± 2.52	$X^2_1 = 15.944^*$ $P < 0.01$
During insect activity hours (9:00 am to 6:00 pm) from April to June		median	0.0 (0.0–1.6)	0.0 (0.0–0.0)	
	Mean temp (°C)	mean	15.52 ± 4.42	14.36 ± 4.66	$t_{169} = 1.666^*$
	Raining days		21	45	
	Heavy rains (>5 mm/9h)		2	10	

For species with previously recorded nectar sugar and pollen production, we used data from Baude *et al.* (2016) and Hicks *et al.* (2016) (Table 3). For species without data available, but representing more than 2% of all the flower units, we evaluated flower resources following the same methods as Baude *et al.* (2016) and Hicks *et al.* (2016).

To estimate nectar production, while excluding visitors, we placed pollination bags (Delnet Pollination, Middletown, USA) on a minimum of 15 flowers per species 24 hours before sampling. Nectar was collected with 1- μ L glass capillary tubes (Hirschmann Laborgerate, Eberstadt, Germany). Nectar volumes were then estimated by measuring the length of the nectar column in the capillary tube. Sugar concentrations were measured with a low-volume hand refractometer (Eclipse Hand held Refractometer, Bellingham & Stanley Ltd, Tunbridge Wells, UK) and expressed as the percentage of sucrose (w/w) (Prys-Jones & Corbet, 2011).

To estimate pollen production, we sampled flowering plants. Open flowers were removed, and branches were individually stored in water in the lab. The

following morning, all the freshly dehiscent anthers were collected. Pollen was extracted in a 70% ethanol solution by three successive rinsing and ultrasonication cycles. Pollen solutions were centrifuged for 10 minutes at 3600 rpm. The pollen residue was dried overnight at room temperature and dissolved in a known volume of 70% ethanol. Pollen grains were counted in a known volume of solution using a 0.10 mm deep haemocytometer (assistant, Glaswarenfabrik Karl Hecht GmbH & Co KG, Sondheim/Rhön, Germany) under a light microscope.

E. Insect observations

We monthly recorded, per strip section, insect-flower interactions during two consecutive walks (at least 10 min apart) along the transects. All insects visiting an open flower were collected and recorded.

When possible, insects were identified in the field (e.g. *Apis mellifera*) and released. Due to close morphological characteristics, bumblebee individuals were identified up to the Operational Taxonomic Unit (OTU) (Terzo & Rasmont, 2007). As *Bombus* individuals were numerous in the field, we sampled at least one individual per OTU per strip and per run for precise lab identification. All the other individuals were individually collected for further lab identification.

Bombus (Terzo & Rasmont, 2010), Lepidoptera (Skinner & Wilson, 2009; Tolman & Lewington, 2009), and Syrphidae flies (Verlinden, 1994; Stubbs & Falk, 2002; Speight & Sarthou, 2016) were identified up to the species by the authors; the other Hymenoptera were identified by a taxonomist of the Royal Institute of Natural Sciences of Brussels.

F. Pollen loads

Bumblebees are common insects able to fly several hundreds of meters to find flower resources in different landscape elements (Le Féon, 2010). Thus, we focused on this genus to determine which flower species were visited for pollen collection, and to assess the proportion of pollen collected from outside the strips. We sampled 26 pollen loads from *Bombus* individuals. One corbicular pollen load was collected per individual, kept at -20°C then acetolysed (Erdtman, 1954, 1960; Hesse & Waha, 1989; Moquet *et al.*, 2015). Using a light microscope, around 500 pollen grains were counted and identified per pollen load. We identified the pollen using our lab's reference collection and Reille (1992, 1995). Marginal species, < 2.0% pollen-load content, were not included in the quantitative analysis (Free, 1970; Westrich & Schmidt, 1986).

G. Statistical analyses

All the analyses were run in R version 3.2.4 GUI 1.67 (available at www.r-project.org). Bumblebees were grouped by OTU morphotypes. The non-Syrphidae Diptera were classified into a single group. For the other insects, a

group corresponded to a single species.

Insect–flower interaction networks were visualised using the {bipartite} R-package. We used insect species as higher trophic level and plant species as lower trophic level. Grey-coloured heat maps were generated using the “heatmap.2” function of the {gplot} R-package.

We conducted mean values comparison (95% confidence) following several steps. Normality was tested with the Shapiro-Wilk normality test (“shapiro.test” function of the {stats} R-package). In the case of non-normal distribution, we used the Kruskal-Wallis test (“kruskal.test” function of the {stats} R-package). When normality was accepted, we tested variance equality (“var.test” function of the {vegan} R-package) using the paired Student’s *t*-test or Welsh test according to the equality, or inequality, of the variances (“t.test” function of the {stats} R-package). The *t*-values were compared to the *t* table values of the corresponding degree of freedom, with 95% confidence level.

For normally distributed data, we provided means values $\pm$ SD. For non-normally distributed data, we provided median values with inter-quartile ranges (25th–75th percentiles).

V. RESULTS

A. Climatic variation between years

Precipitations differed between 2015 and 2016, sunshine was significantly lower in 2016 and the raining days were more numerous (60 vs 37 d) (Table 14). The climatic differences were strong in April, May, and June with monthly precipitation respectively 6.1, 1.4, and 3.0 times higher in 2016 than 2015 ($\chi^2_1 = 15.964$, $P < 0.01$).

During insect activity (9:00 am to 6:00 pm) from April to June, rainy days were more numerous in 2016 than 2015 (45 vs 21 d). Precipitation was three times higher in 2016 than 2015, and the heavy rains (> 5 mm/9 h) were five times more numerous (10 vs 2 d).

B. Flower diversity

We recorded a total of 56 flowering species (53 spontaneous and three sown) belonging to 17 families in the strips (Table. 15). Mean diversity was 17.5 ± 7.8 species per strip. Over the two years, the nine Fabaceae species provided the highest number of flower units (35.3%) followed by the five species of Brassicaceae (21.8%) and by four species of Polygonaceae (17.7%) (Fig. 4). Nevertheless, most of the observed flower species (47 to 70% of species per strip) each provided less than 1% of the total flower units per strip.

Table 14. Climatic data from the Ernage station, Gembloux, Belgium. Values are mean $\pm$ SD or median with inter-quartile ranges (25th–75th percentiles). * indicates significant differences at 95% confidence. Data provided by the IRM.

Period	Parameter	Value	2015	2016	Stat values
From April to September	Sunshine (min/d)	mean	435.8 $\pm$ 260.8	378.2 $\pm$ 242.2	$t_{364} = 2.1889^*$
	Mean temp (°C)	mean	12.30 $\pm$ 3.79	11.85 $\pm$ 4.26	$t_{350} = -1.2574$
	Mini mean temp (°C)	mean	6.45 $\pm$ 3.46	7.29 $\pm$ 4.56	$t_{350} = -0.64079$
	Max mean temp (°C)	mean	17.28 $\pm$ 4.57	16.16 $\pm$ 4.72	$X^2_1 = 10.099$
		median	17.0 (14.4–19.6)	16.5 (12.8–20.1)	$P < 0.01$
	Raining days		37	60	
During insect activity hours (9:00 am to 6:00 pm) from April to June	Precipitations (mm/9h)	mean	0.51 $\pm$ 2.21	1.45 $\pm$ 2.52	$X^2_1 = 15.944^*$
		median	0.0 (0.0–1.6)	0.0 (0.0–0.0)	$P < 0.01$
	Mean temp (°C)	mean	15.52 $\pm$ 4.42	14.36 $\pm$ 4.66	$t_{169} = 1.666^*$
	Raining days		21	45	
	Heavy rains (>5 mm/9h)		2	10	

Across all four strips, flower unit density was 34.9 ± 58.1 flower units/m². Most flowering species (84%) had very low flower unit density over the seasons regardless of the year (< 1 flower unit/m²) (Fig. 12). The highest flower unit densities were attained with *Trifolium incarnatum*, with up to 215 and 109 flower units/m² in strip C in July and September, respectively, followed by *Polygonum aviculare* with up to 45 flower units/m² in August and *Raphanus sativus* with up to 40 flower units/m² in July in strip B.

In total, the three sown entomophilous species, *Fagopyrum esculentum*, *Phacelia tanacetifolia*, and *Raphanus sativus*, represented 25.4% of the 81,539 flower units observed over the two years.

Spontaneous and sown species provided more floral units in 2015 than in 2016, respectively nine and two times more. Among the 56 total observed flowering species, 48 were observed in 2015 and 32 in 2016. No significant differences in flower diversity per strip were observed between the two years ($t_6 = 1.7273$). The three sown species provided 20.9% of the flower units in 2015 and 50.4% in 2016. These three species were all present in 2015, except in strip C where *Phacelia tanacetifolia* did not germinate.

Global flower density significantly differed between the two years and was five times lower in 2016 (4.8 (1.5–22.9) flower units/m²) than 2015 (42.1 (0.0–89.0), $X^2_1 = 0.84877$, $P = 0.3569$).

Mean flowering period of recorded species (at least one flower unit in one strip) was 1.6 ± 1.0 months in 2015 and 1.5 ± 0.7 in 2016. The flowering

periods were 4.0 ± 0.0 in 2015 and 3.0 ± 1.7 in 2016 for the sown species only. Four species were flowering during most of the 11 months of observation: *aphanus. sativus* and *Veronica persica* in 8 observation sessions, *Polygonum aviculare* and *Tripleurospermum inodorum* in 7 sessions. The blooming season started in June 2015 and May 2016 with 28.9 ± 29.0 and 2.9 ± 5.9 flower units/m², respectively. Peak flowering occurred from July to September: 47.9% of the total flower units were observed in July, 18.3% in August and 23.1% in September (Table 15).

Table 15. Monthly flower unit densities (/m²) and flower resources (24h pollen and nectar production / m²) of sown and spontaneous plant species in the four studied bird-strip AES in 2015 and 2016. Nectar production corresponds to the mg of sugar produced per day per m². Values are means $\pm$ SD. No data were recorded in June 2016 due to bad weather.

Year	Month	Flower unit density (/m ²)		Pollen μ L/day/m ²		Nectar mg/day/m ²	
		Sown species	Spontaneous species	Sown species	Spontaneous species	Sown species	Spontaneous species
2015	April	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	May	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	June	0.92 $\pm$ 0.95	14.45 $\pm$ 19.25	0.09 $\pm$ 0.06	11.81 $\pm$ 19.10	0.09 $\pm$ 0.09	0.06 $\pm$ 0.09
	July	43.98 $\pm$ 38.72	28 $\pm$ 17.98	13.3 $\pm$ 11.58	11.27 $\pm$ 17.26	9.24 $\pm$ 9.78	6.65 $\pm$ 9.07
	August	9.17 $\pm$ 6.41	73.46 $\pm$ 83.95	1.73 $\pm$ 1.76	17.29 $\pm$ 36.19	1.26 $\pm$ 0.90	9.45 $\pm$ 14.17
	Sept.	5.00 $\pm$ 9.19	63.84 $\pm$ 126.81	2.17 $\pm$ 4.11	11.31 $\pm$ 25.13	0.66 $\pm$ 1.21	4.77 $\pm$ 9.15
2016	April	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	May	0.23 $\pm$ 0.46	2.71 $\pm$ 5.43	0.10 $\pm$ 0.21	0.00 $\pm$ 0.00	0.03 $\pm$ 0.06	0.97 $\pm$ 1.93
	June	Na	Na	Na	Na	Na	Na
	July	18.85 $\pm$ 34.52	12.03 $\pm$ 15.66	8.27 $\pm$ 15.15	2.35 $\pm$ 3.01	2.58 $\pm$ 4.71	2.83 $\pm$ 4.14
	August	5.30 $\pm$ 7.58	9.59 $\pm$ 4.06	2.28 $\pm$ 3.37	1.09 $\pm$ 0.77	0.69 $\pm$ 1.00	2.73 $\pm$ 2.30
	Sept.	1.31 $\pm$ 1.52	3.53 $\pm$ 2.61	0.58 $\pm$ 0.67	1.15 $\pm$ 1.51	0.18 $\pm$ 0.21	1.15 $\pm$ 0.83

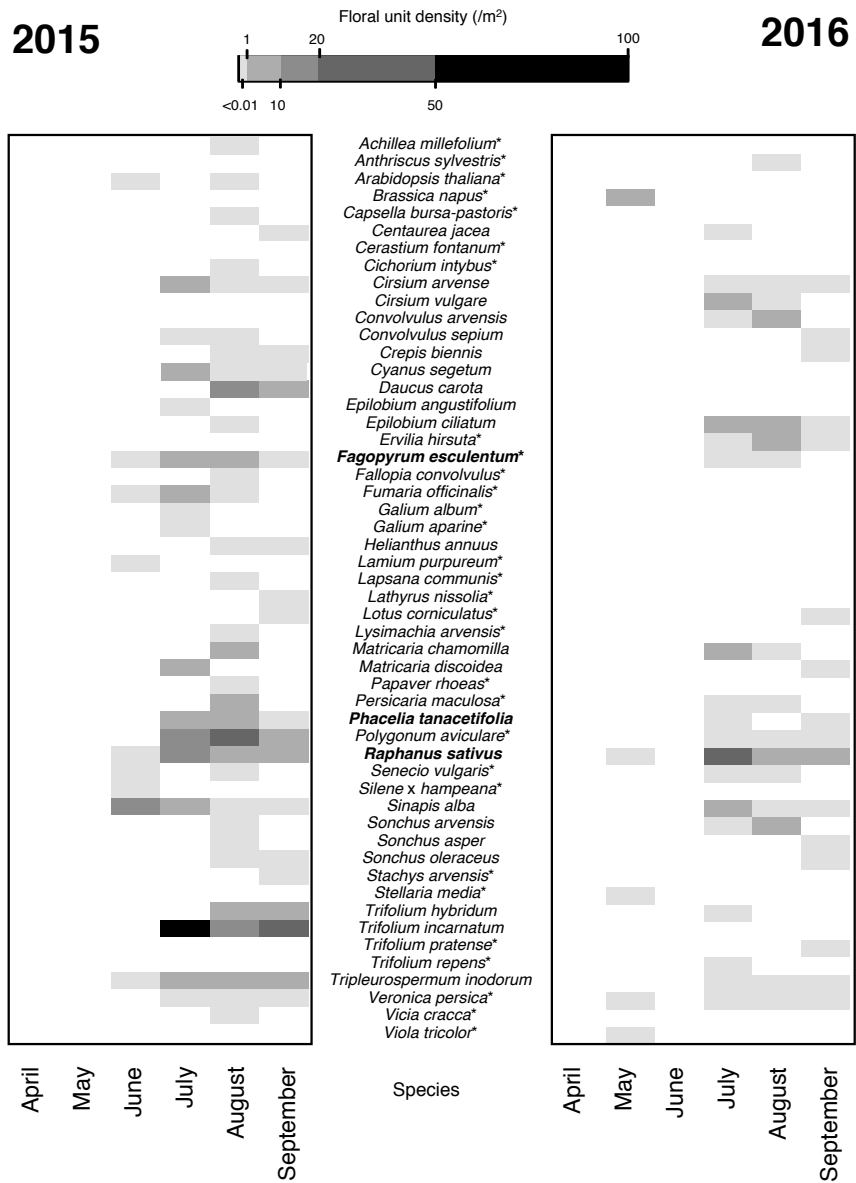


Figure 12. Mean flower unit densities per m² per plant species across the seasons for the four studied bird-strip AES in 2015 (left) and 2016 (right). Species are in alphabetical order; sown species are in **bold**; *, species without any insect interaction recorded. Grey level of rectangles corresponds to the flower unit density. No observation session was conducted in June 2016 due to unfavourable weather conditions.

C. Flower resources

Overall, most of the flower resources were provided by four families dominated by spontaneous species. Nectar sugar was mainly provided by flowers from the Asteraceae (75.8%), Brassicaceae (8.5%), and Boraginaceae (6.2%) families. Pollen was mainly provided by flowers from the Brassicaceae (44.7%), Asteraceae (37.0%), Fabaceae (10.7%), and Boraginaceae (6.3%). Regardless of year, July was the month with the highest production of flower resources (Table 16).

Table 16. Flower resources of the three sown plant species and 53 spontaneous species flowering in the four studied bird-strip AES in 2015 and 2016. Species nomenclature followed APG III nomenclature (Tison & de Foucault, 2014). We set apart plants species on the ones we observed insects visiting flowers (Species with insect interaction record) and plant species without any observation of insects visiting the flowers (Species without insect interaction record). Data from our results, from (Hicks *et al.*, 2016)*, from (Baude *et al.*, 2016)**, from (Cawoy *et al.*, 2009)' and from (Szabo & Najda, 1985)". Values are mean $\pm$ SD.

	Origin	Families	Species	Mean unit (/m ²)	floral density	Nectar sugar (μ g/flower unit/24h)	Pollen (μ L/flower unit/24h)
Species with insect interaction record	Sown	Boraginaceae	<i>Phacelia tanacetifolia</i> Benth.	1.86 $\pm$ 8.86		328.46 $\pm$ 187.84	0.335 $\pm$ 0.160
		Brassicaceae	<i>Raphanus sativus</i> L.	4.79 $\pm$ 12.23		131.54 $\pm$ 79.44	0.444 $\pm$ 0.162
		Apiaceae	<i>Daucus carota</i> L.	1.78 $\pm$ 10.58		27.18 $\pm$ 32.44*	0.019 $\pm$ 0.003*
		Asteraceae	<i>Centaurea jacea</i> L.	0.21 $\pm$ 0.91		1177.75 $\pm$ 1761.20	6.404 $\pm$ 2.030*
			<i>Cirsium arvense</i> Scop.	0.00 $\pm$ 0.02		2608.94 $\pm$ 239.08*	0.598 $\pm$ 0.210*
			<i>Cirsium vulgare</i> Ten.	0.73 $\pm$ 2.93		1534.23 $\pm$ 630.02	na
			<i>Crepis biennis</i> L.	0.07 $\pm$ 0.33		na	na
			<i>Cyanus segetum</i> Hill.	0.00 $\pm$ 0.01		1479.49 $\pm$ 487.56	3.534 $\pm$ 0.500*
			<i>Helianthus annuus</i> L.	0.01 $\pm$ 0.02		na	na
			<i>Matricaria chamomilla</i> L.	0.56 $\pm$ 2.18		0.60 $\pm$ 1.29**	na
			<i>Matricaria discoidea</i> DC.	1.46 $\pm$ 6.65		0.28 $\pm$ 0.89**	0.430 $\pm$ 0.072*
			<i>Sonchus arvensis</i> L.	0.16 $\pm$ 0.75		3.34 $\pm$ 4.11**	na
			<i>Sonchus asper</i> Hill	0.02 $\pm$ 0.08		593.83 $\pm$ 16.8*	0.939 $\pm$ 0.230*
			<i>Sonchus oleraceus</i> L.	0.04 $\pm$ 0.25		568.84 $\pm$ 16.02*	1.326 $\pm$ 0.256*
			<i>Taraxacum</i> agg.	0.00 $\pm$ 0.00		2137.20 $\pm$ 286.43*	2.823 $\pm$ 0.661*
			<i>Tripleurospermum inodorum</i> L.	0.70 $\pm$ 2.78		1406.81 $\pm$ 81.86*	2.955 $\pm$ 0.226*
		Brassicaceae	<i>Sinapis alba</i> L.	1.84 $\pm$ 6.37		na	0.822 $\pm$ 0.437
Spontaneous							

Origin	Families	Species	Mean unit (/m ²)	floral density (µg/flower unit/24h)	Nectar sugar (µL/flower unit/24h)	Pollen (µL/flower unit/24h)
Species without insert interaction record	Convolvulaceae	<i>Convolvulus arvensis</i> L.	0.18 ± 0.78	351.82 ± 188.29**	na	
		<i>Convolvulus sepium</i> L.	0.21 ± 1.06	1543.23 ± 630.02	na	
	Fabaceae	<i>Trifolium dubium</i> Sibth.	0.00 ± 0.00	0.00 ± 0.00**	na	
		<i>Trifolium hybridum</i> L.	0.49 ± 2.44	1.00"	0.053"	
		<i>Trifolium incarnatum</i> L.	14.64 ± 69.29	31.15 ± 38.8	0.080 ± 0.010	
		<i>Trifolium pratense</i> L.	0.02 ± 0.16	48.37 ± 34.28*	0.020 ± 0.002*	
	Onagraceae	<i>Epilobium angustifolium</i> L.	0.01 ± 0.04	na	na	
		<i>Epilobium ciliatum</i> Rafin	0.42 ± 1.57	144.71 ± 26.62*	0.010 ± 0.003*	
	Sown	Polygonaceae	<i>Fagopyrum esculentum</i> Moench	1.53 ± 3.76	90'	0.017 ± 0.006
	Spontaneous	Apiaceae	<i>Anthriscus sylvestris</i> Hoffman	0.01 ± 0.06	11.33 ± 14.26**	na
		Asteraceae	<i>Achillea millefolium</i> L.	0.01 ± 0.09	28.48 ± 34.38*	1.128 ± 0.215*
			<i>Cichorium intybus</i> L.	0.00 ± .000	na	na
			<i>Lapsana communis</i> L.	0.00 ± 0.00	99.35 ± 7.88*	0.331 ± 0.033*
			<i>Leucanthemum vulgare</i> Lam.	0.03 ± 0.22	292.65 ± 459.43*	15.918 ± 3.900*
			<i>Senecio vulgaris</i> L.	0.05 ± 0.22	0.19 ± 0.83**	0.175 ± 0.019*
		Brassicaceae	<i>Arabidopsis thaliana</i> Heynh.	0.00 ± 0.02	na	na
			<i>Brassica napus</i> L.	0.15 ± 1.03	541.29 ± 580.43	na
			<i>Capsella bursa-pastoris</i> Med.	0.03 ± 0.15	9.05 ± 1.6*	0.001 ± 0.000*
		Caryophyllaceae	<i>Cerastium fontanum</i> Baumg.	0.01 ± 0.03	11.56 ± 1.66**	0.035 ± 0.002*
			<i>Silene x hampeana</i> Meusel et K. Werner	0.01 ± 0.05	62.97 ± 63.01*	0.801 ± 0.015
			<i>Stellaria media</i> Vill.	0.02 ± 0.15	11.92 ± 2.90*	0.001 ± 0.000*
		Fabaceae	<i>Ervillea hirsuta</i> Opiz	0.24 ± 0.76	26.76 ± 7.26*	0.006 ± 0.004*
			<i>Lathyrus nissolia</i> L.	0.00 ± 0.01	na	na
			<i>Lotus corniculatus</i> L.	0.01 ± 0.06	67.98 ± 42.59	0.146 ± 0.012*
			<i>Trifolium repens</i> L.	0.02 ± 0.12	12.13 ± 8.12*	0.028 ± 0.002*
			<i>Vicia cracca</i> L.	0.00 ± 0.02	484.40 ± 411.27**	na
		Lamiaceae	<i>Lamium purpureum</i> L.	0.01 ± 0.04	107.73 ± 23.60*	0.020 ± 0.004*
			<i>Stachys arvensis</i> L.	0.00 ± 0.03	na	na

Origin	Families	Species	Mean unit (/m ²)	floral density	Nectar sugar (µg/flower unit/24h)	Pollen (µL/flower unit/24h)
	Onagraceae	<i>Epilobium montanum</i> L.	0.01 ± 0.08		36.57 ± 3.98*	0.053 ± 0.014*
	Papavera- ceae	<i>Fumaria officinalis</i> L.	0.23 ± 1.24		21.90 ± 6.62*	0.010 ± 0.001*
		<i>Papaver rhoeas</i> L.	0.00 ± 0.01		0.57 ± 0.57*	13.343 ± 2.813*
	Plantagina- ceae	<i>Veronica persica</i> Poiret	0.21 ± 0.51		4.69 ± 0.41*	0.031 ± 0.006*
	Polygonaceae	<i>Fallopia convolvulus</i> Á Löve	0.02 ± 0.12		65.08 ± 32.29**	na
		<i>Persicaria maculosa</i> S.F.	0.18 ± 0.80		29.59 ± 5.82**	0.003 ± 0.000*
		<i>Polygonum aviculare</i> L.	3.34 ± 13.75		3.05 ± 4.97**	0.006 ± 0.001
	Primulaceae	<i>Lysimachia arvensis</i> U. Manns et Andreb.	0.02 ± 0.13		na	na
	Rubiaceae	<i>Galium album</i> Mill.	0.01 ± 0.06		7.06 ± 1.17*	0.005 ± 0.002*
		<i>Galium aparine</i> L.	0.01 ± 0.05		9.48 ± 3.52**	na
	Violaceae	<i>Viola tricolor</i> L.	0.05 ± 0.38		na	na

D. Nectar

Over the two years, two spontaneous Asteraceae species, *Cirsium arvense* and *vulgare*, produced most (>63%) of the available nectar (Table 10). The three sown species, *Fagopyrum esculentum*, *Phacelia tanacetifolia* and *Raphanus sativus*, provided 15.6% of the nectar sugar. The sown species provided 19.0% of the nectar in 2015 but only 10.0% in 2016. Daily nectar production per m² was significantly higher in 2016 (56.3 (5.7–293.4) mg/m²) than 2015 (38.1 (0–127.4) mg/m²; $\chi^2_1 = 0.56068$, $P = 0.454$).

E. Pollen

The three sown species provided 32.3% of the available pollen (Table 17). The sown species provided 24.4% of the pollen in 2015 but 71.9% in 2016. Daily pollen production per m² was significantly higher in 2015 (109.4 (0.0–178.5) µL/m²) than 2016 (66.1 (1.3–78.6) µL/m², $\chi^2_1 = 0.20668$, $P = 0.6494$).

Table 17. Monthly flower unit densities (/m²) and flower resources (24h pollen and nectar production / m²) of sown and spontaneous plant species in the four studied bird-strip AES in 2015 and 2016. Nectar production corresponds to the mg of sugar produced per day per m². Values are means ± SD. No data were recorded in June 2016 due to bad weather.

Year	Month	Flower unit density (/m ²)		Pollen µL/day/m ²		Nectar mg/day/m ²	
		Sown species	Spontaneous species	Sown species	Spontaneous species	Sown species	Spontaneous species
2015	April	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	May	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	June	0.92 ± 0.95	14.45 ± 19.25	0.09 ± 0.06	11.81 ± 19.10	0.09 ± 0.09	0.06 ± 0.09
	July	43.98 ± 38.72	28 ± 17.98	13.3 ± 11.58	11.27 ± 17.26	9.24 ± 9.78	6.65 ± 9.07
	August	9.17 ± 6.41	73.46 ± 83.95	1.73 ± 1.76	17.29 ± 36.19	1.26 ± 0.90	9.45 ± 14.17
	Sept.	5.00 ± 9.19	63.84 ± 126.81	2.17 ± 4.11	11.31 ± 25.13	0.66 ± 1.21	4.77 ± 9.15
2016	April	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	May	0.23 ± 0.46	2.71 ± 5.43	0.10 ± 0.21	0.00 ± 0.00	0.03 ± 0.06	0.97 ± 1.93
	June	Na	Na	Na	Na	Na	Na
	July	18.85 ± 34.52	12.03 ± 15.66	8.27 ± 15.15	2.35 ± 3.01	2.58 ± 4.71	2.83 ± 4.14
	August	5.30 ± 7.58	9.59 ± 4.06	2.28 ± 3.37	1.09 ± 0.77	0.69 ± 1.00	2.73 ± 2.30
	Sept.	1.31 ± 1.52	3.53 ± 2.61	0.58 ± 0.67	1.15 ± 1.51	0.18 ± 0.21	1.15 ± 0.83

F. Flowers visited

Insect visits were observed on 25 of the 56 recorded species (Table 16). Twenty out of 46 plant species were visited in 2015 and 15 out of 31 in 2016. In both years, *Cirsium arvense* flower units received most of the insect visits (54.9% of all interactions) despite its low density (Table 18). The second and third most-visited species were *Phacelia tanacetifolia* and *Raphanus sativus* in 2015, and *Sonchus arvensis* and *Raphanus sativus* in 2016. The three sown species received 17.2% of the insect visits. No insect visits were observed on one of the three sown species, *Fagopyrum esculentum*.

Five species, all belonging to the Asteraceae, presented a global flower unit visit rate (total visits/total flower units) higher than 0.10: *Centaurea jacea* (0.13 in 2015, 0.50 in 2016), *Cirsium arvense* (0.28 in 2015, 0.19 in 2016), *Crepis biennis* (0.40 in 2016), *Helianthus annuus* (0.20 in 2016) and *Sonchus arvensis* (1.07 in 2015).

Table 18. Annual contributions of the ten most dense, most visited or main flower resource producer plant species. These ten species represented all together 80% of the flower units and more than 90% of the flower-resources and flower-insect interactions. Values are % of the total annual observations over the four sites. Sown species are in **bold**.

Plant species	% of flower units		% pollen production		% nectar sugar production		% of interactions (N=1523)	
	2015 (n=346,715)	2016 (n=60,930)	2015	2016	2015	2016	2015 (n=1150)	2016 (n=373)
SOWN (n= 3 species)	20.9	50.4	24.2	71.9	19.0	10.0	18.7	12.3
SPONTANEOUS (n= 53 species)	79.1	49.6	75.8	81.1	81.0	90.0	81.3	87.7
<i>Cirsium arvense</i>	0.8	1.5	4.4	2.9	26.1	5.6	58.1	45.0
<i>Cirsium vulgare</i>	0.2	2.3	na	na	26.0	76.1	1.0	3.5
<i>Daucus carota</i>	5.0	0.0	0.4	0.0	0.7	0.0	0.6	0.0
<i>Phacelia tanacetifolia</i>	5.7	1.1	7.3	1.2	9.8	0.6	10.3	0.0
<i>Polygonum aviculare</i>	12.7	3.7	0.3	0.1	0.2	0.0	0.0	0.0
<i>Raphanus sativus</i>	9.8	48.8	16.6	70.6	6.7	9.4	8.4	12.3
<i>Sinapis alba</i>	6.3	5.5	19.7	14.7	na	na	1.9	3.2
<i>Sonchus arvensis</i>	0.0	3.0	na	na	0.0	0.0	2.8	18.0
<i>Trifolium incarnatum</i>	40.5	0.0	12.6	0.0	6.6	0.0	8.1	0.0
<i>Tripleurospermum inodorum</i>	1.9	0.4	21.3	4.0	13.9	0.9	1.0	2.4

G. Insect visitors

During our study, we recorded a total of 1523 insect-flower interactions. We identified 1389 insects in the field and caught 134 insect individuals for lab identification. We observed 60 different groups of insect flower visitors: 43 in 2015 and 37 in 2016 (Fig. 13 & 14). The non-syrphid flies comprised 17 morphotypes belonging to six families. More than a quarter (26.8%) of the observed insect species morphotypes were represented by a single individual (Fig. 13).

Removing the 2015 June observations (as no fieldwork was performed in June 2016), we observed 67.3% of the visits in 2015. Bumblebee individuals represented 48.7% of the recorded individuals in 2015 and only 11.0% in 2016. We identified three species among the captured *Bombus* morphotypes: *B. lapidarius*, *B. pascuorum*, and *B. terrestris*.

Five solitary bee species (0.8% of total insects recorded) were observed in 2015 (*Andrena flavipes*, *Andrena* sp., *Halictus rubicundus*, *Hylaeus annularis* and *Lasioglossum calceatum*), but none were observed in 2016. We observed nearly as many *Apis mellifera* in 2015 (26, 2.2% of the visitors) as in 2016 (27, 7.2% of the visitors).

Estimation of pollinating insect populations in agro-ecosystems: comparing oilseed-rape fields and AES flower-strips.

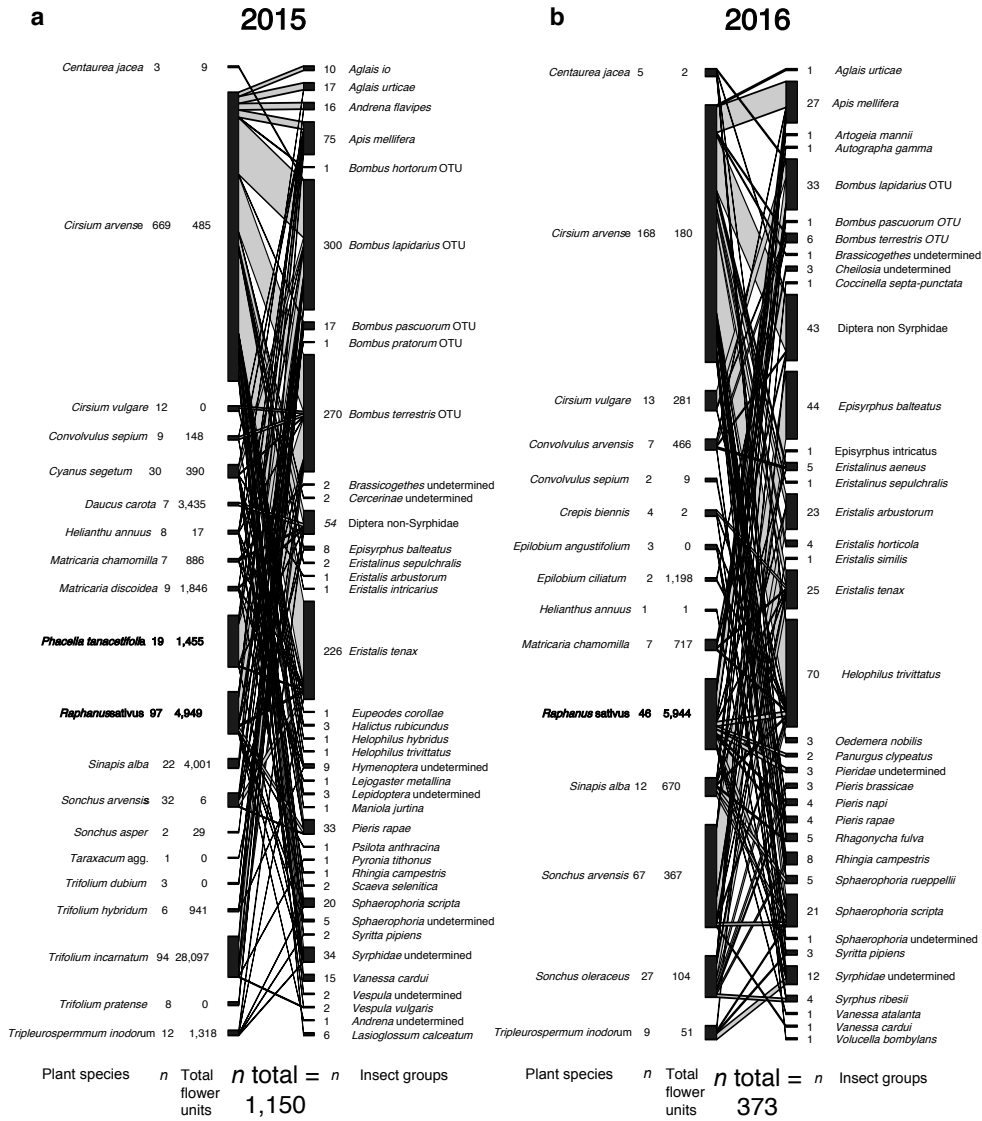


Figure 13. Global interaction networks among flowers and insects across the four studied bird-strip AES in 2015 (a) and 2016 (b). A total of 1523 interactions were recorded. Sown species are in bold. The height of boxes is proportional to the number of insect visits (*n*). Total flower units refer to the total number of flower units observed. Zero indicates that the species was not dense enough (<5 flower units /100 m² per observation run) to be observed on sampled quadrats, even if present on the transect. Species are ordered by alphabetical order; sown species are in bold.

Diptera (both Syrphidae and non-Syrphidae) visitor densities were similar in 2015 (307 Syrphidae and 54 non-syrphid flies) and 2016 (233 Syrphidae and 45 non-syrphid flies). Consequently, the relative proportion of Diptera was twice as high in 2016 (74.5%) than 2015 (35.5%). Syrphidae diversity was also higher in 2016 (19 species) than 2015 (13 species), and only ten species were present both years. *Eristalis tenax* represented the main Syrphidae in 2015 (54.6%) whereas *Helophilus trivittatus* was the most observed in 2016 (24.9%).

We recorded only four Lepidoptera species visiting the bird-strip flowers.

H. Insect–flower interactions

Flower visits were mainly observed in summer, with 54.1% occurring in July and 28.4% in August. The first visits were observed in June, representing 11.2% of all the recorded visits (Fig. 14). The mean flower-visit abundances were 0.0 in April and May, 0.4 visits/100m²/25 min in June, 5.2 visits/100m²/25 min in July, 3.9 visits/100m²/25 min in August and 0.9 visits/100m²/25 min in September.

Annual insect-flower interactions can be summarised by global bipartite networks (Fig. 13). Of the 61 insect groups observed, 23 visited sown species, and 53 visited spontaneous species. A third of the observed insect groups (19) visited both sown and spontaneous species. Only six groups of insects exclusively visited the sown species, and 36 visited only the spontaneous species. In 2015 the sown species hosted only 33% of the insect species, 43% in 2016 and 38% over the two years. Most of the insect diversity visited spontaneous plant species (Fig. 13).

The number of visitors was three times lower in 2016 than in 2015, and insect–flower interactions were dispatched differently among species, but networks maintained similar complexity between the two years. In 2015 and 2016 networks had 1.8 links per species. Nevertheless, the connectance varied from 0.14 in 2015 to 0.17 in 2016 and a web asymmetry from 0.3 to 0.4. *Cirsium arvense* attracted the largest diversity of insects, with 79.5% of the observed diversity in 2015 and 59.5% in 2016. In 2015 two other plant species attracted more than 20% of the insect diversity, *Raphanus sativus* (29.5%) and *Cyanus segetum* (20.5%). In 2016 four other plant species attracted more than 20.0% of the insect diversity, *Raphanus sativus* (43.2%), *Sonchus oleraceus* (27.0%), *Sonchus arvensis* (24.0%) and *Sinapis alba* (21.6%) (Fig. 13).

In the other hand, some insect groups visited diverse flower species. *Bombus lapidarius* OTU was observed on 60.0% of the visited species; *Bombus terrestris* OTU and *Eristalis tenax* were each observed on 48.0%, and *Apis mellifera* and *Helophilus trivittatus* were each observed on 44.0% (Fig. 13). As many as 93.1% of the non-*Bombus* wild bee visits were recorded on spontaneous species, with *Cirsium arvense* accounting for 89.7% of the visits. The sown species *Raphanus sativus* accounted for 6.9% (Fig. 13).

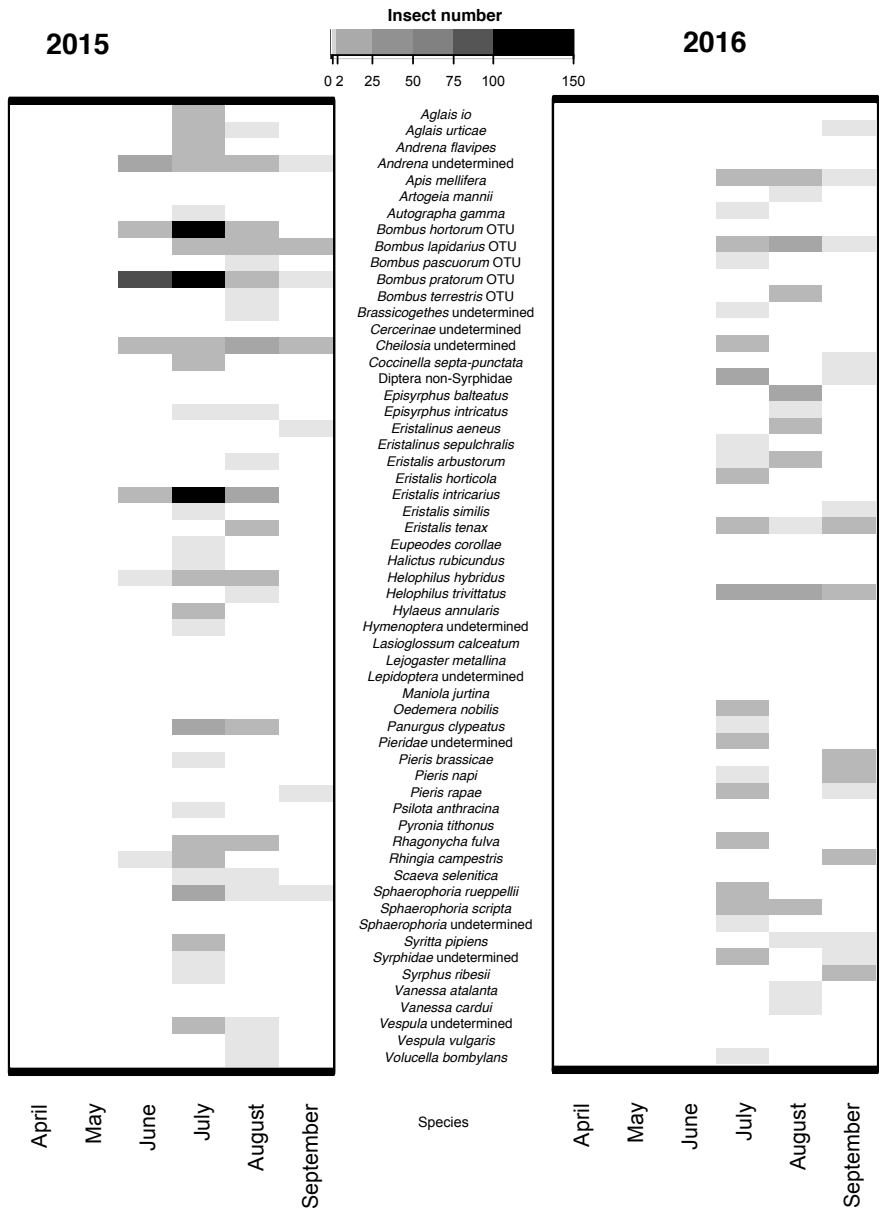


Figure 14. Insect flower visitor diversity and number across seasons across the four bird-strip AES in 2015 (left) and 2016 (right): total number of insects observed on flowers (n=1523). Species are in alphabetical order. Grey level of rectangles corresponds to the number of insects observed. No observation session was conducted in June 2016 due to unfavourable weather conditions.

I. Insect fidelity

We identified a total of ten plant species in *Bombus* pollen loads. The most collected pollens were *Phacelia tanacetifolia*, 43.1% of the collected pollen grains, and *Raphanus sativus*, 45.0% (two sown species, Table 19). Fidelity of *Bombus* individuals was high. In each pollen load, the dominant species represented $94.0 \pm 8.2\%$ of the content. Pollen from species outside of the strips was marginal (2.3% of the collected pollen, Table 19).

Table 19. Pollen load composition (% of collected pollen grains) and importance of each plant species (all pollen loads grouped together) in diet of the three main *Bombus* OTU caught in the four studied bird-strip AES in 2015 and 2016. Values are means $\pm$ SD for the pollen load composition and %. Only species representing more than 2% of pollen load composition were considered. *, species from outside of the strips, sown species are in bold. N=26

Plant family		Boragi- naceae	Brassicaceae		Fabaceae			Poa- ceae	Rhinan- theae	Solana- ceae	
Plant species		<i>Phacelia tanacetifolia</i>	<i>Brassica nigra</i>	<i>Raphanus sativus</i>	<i>Lotus corniculatus</i>	<i>Medicago sativa</i>	<i>Trifolium incarnatum</i>	<i>Trifolium repens</i>	<i>Poaceae</i> sp.	<i>Pedicularis</i> sp.	<i>Solanum tuberosum</i>
OTU											
Mean % in pollen load (Number of pollen loads)	<i>Bombus lapidarius</i> n=12	9.9 ± 7.6 (4)	99.5 (1)	96.2 ± 6.9 (10)			71.1 (1)				
	<i>Bombus pascuorum</i> n=1	8.4 (1)		89.2 (1)		2.2 (1)					
	<i>Bombus terrestris</i> n=13	95.7 ± 2.2 (11)		99.4 (1)	4.1 ± 2.7 (6)			3.4 (1)	14.5 (1)	13.6 (1)	71.1 (1)
	<i>Bombus lapidarius</i> n=12	3.7	3.7	81.4			6.0				
% in <i>Bombus</i> OTU diet	<i>Bombus pascuorum</i> n=1	8.4		89.2		2.2					
	<i>Bombus terrestris</i> n=13	81.1		7.88	2.4			0.4	1.1	1.1	5.5

VI. DISCUSSION

A. Effects of annual climatic variations

Periods of sunshine were 1.5 times shorter, and precipitation was 2.8 times more abundant in 2016 than 2015. In 2016, we observed three times fewer interactions than in 2015. These wet weather conditions, especially in spring, negatively affected plant and insect communities and their interactions (Williams, 1940, 1961; Willmer & Stone, 2004; Baldock *et al.*, 2015).

a) Plant community: spontaneous species more affected than sown species

In 2016, plant diversity was reduced by approximately one-third (from 46 to 31 species) compared with 2015. The absence of soil disturbance was deleterious to annual species (e.g. *Cyanus segetum*, *Fagopyrum esculentum* and *Phacelia tanacetifolia*,) that need annual soil turnover to germinate (Dyer, 1995). On the contrary, insect-attractive perennial species such as *Cirsium* and *Convolvulus* species (Free *et al.*, 1975; Carvell *et al.*, 2006) were favoured. Moreover, *R. sativus* flowered similarly between the two years; this species is known for its self-seeding capacity (Warwick & Francis, 2005).

Surprisingly in 2015, when the annual seed mix was sown, sown species provided a low proportion of available pollen (24%), whereas in 2016 when only spontaneous sowing occurred, these species (from the sown species group) provided 72% of the available pollen. Pollen production resilience was excellent, as pollen production was less reduced than the flower unit number.

b) Insect communities were more affected than plant communities

In 2016, we recorded nearly 15 times fewer bumblebee individuals than in 2015. Hymenoptera populations decrease when flower resource availability decreases (Kim, 1999; Scheper *et al.*, 2015; Thomson, 2016). Spring leak of resources affects populations for the entire year (Williams, 1940; Williams *et al.*, 2012). The ability of bird-strip AES to favour the foundation of bumblebee colonies is therefore reduced. Solitary bees were even more affected by weather as we did not observe any in 2016.

The number of Syrphidae individuals remained stable between the two years. Although the Syrphidae population is usually stable from year to year, with the dominant species remaining dominant (Owen & Gilbert, 1989), here we observed a switch in dominant species. The reduction in flower unit abundance was compensated by a diversification of the plant species visited by Syrphidae individuals (10 in 2015 vs 16 in 2016). The sown species were marginally used by Syrphidae (4.4% of the visits). The spontaneous species made the strips suitable for Syrphidae (Branquart & Hemptinne, 2000), regardless of climatic variations.

B. Strip quality

a) Diverse flower resources for insects

Across the four studied strips, spontaneous plant species were dominant, providing most of the flower units (75%). These spontaneous plant species attracted most of the observed insect diversity (88%) and abundance (82%). Nevertheless, the sown species were the most visited by *Bombus* for pollen collection. The pollen quality is predominant in the choices of bumblebees and explains their flower fidelity (Leonhardt & Blüthgen, 2012; Ruedenauer *et al.*, 2016). The high fidelity to sown species we observed in *Bombus* pollen diet probably reveal the poor pollen quality of the other plant species. Thus, the sown species were critical to providing pollen to *Bombus* and the spontaneous plant species to provide nectar.

The failure of the sown species to establish was mitigated by the growth of spontaneous species. In our strips, the diversity of sown entomophilous plants was low, with only three species, whereas the diversity of spontaneous entomophilous plants (52 species) was among the highest of the studied strips in Europe. Usually, numbers of spontaneous species ranged from ten to 60 (Engels *et al.*, 1994; Bokenstrand *et al.*, 2004; Carvell *et al.*, 2006; Haaland *et al.*, 2011; Hicks *et al.*, 2016; Campbell *et al.*, 2017). Insect visitors were observed on 25 plant species, but mostly visited Asteraceae (69%).

Despite Asteraceae species being attractive to insects due to their nectar resources (Hicks *et al.*, 2016), for many bee species the Asteraceae pollen quality is poor and lacks attractiveness because some essential amino acids are missing (Goulson *et al.*, 2005; Hanley *et al.*, 2008; Forcone *et al.*, 2011; Nicolson & Human, 2013; Somme *et al.*, 2015; Spear *et al.*, 2016; Vanderplanck *et al.*, 2016).

All of the observed *Bombus* spp. were spring emergent (March to April) (Benton, 2006; Falk, 2015) and need flower resources early in the season. The poor flower resources in spring in the bird-strips AES (first flower units observed in May) reduced their attractiveness during this season (Dicks *et al.*, 2015).

b) Importance of *Cirsium arvense*

Insect-flower observations revealed that only a few plant species attracted the majority of insects (Carvell *et al.*, 2006; Haaland & Gyllin, 2010). Despite its low density (0.6 flower unit/m²), *C. arvense* hosted 55% of the interactions. The attractiveness of this species, regardless of year, can be explained by a combination of high nectar production (up to 2.6 mg of sugar/d/flower unit) and plant morphology (i.e. tall stems with highly visible purple capitula) (Kirk & Howes, 2012). This species has been previously recorded as very attractive for numerous insects (Knuth, 1908; Kirk & Howes, 2012; Vray *et al.*, 2017), especially in farmlands, where other flower resources are rare (Haaland & Gyllin, 2010). Nevertheless, *C. arvense* is an aggressive and hard to control weed with a strong potential for field colonisation. This species is reputed as agronomically problematic species and regulated by state law. In several countries (Belgium, France, USA), eradication is compulsory during flowering

(Welton *et al.*, 1929; Hill, 1983; Vray *et al.*, 2017). Despite its capacity to provide flower resources to numerous insects, integrating such species in AES is not realistic.

One other Asteraceae species, *Centaurea jacea*, even scattered (5×10^{-3} flower units /m²) in the strips, had a higher rate of visits than *Cirsium arvense*. These two perennial Asteraceae have similar morphology and flower resources. In the pollinator field-strip scheme, *Centaurea jacea* key role is to attract and feed insects (Haaland & Gyllin, 2010), thus making it a good alternative to *C. arvense*. Moreover, *C. jacea* has a tuff morphology with long stems, well adapted to the refuge section, allowing birds to intersperse through the plants.

c) Brassicaceae and Fabaceae resources

For insect nutrition, the quality of a pollen consists of high polypeptides, sterols and protein content with all the essential amino acids. Boraginaceae, Brassicaceae and Fabaceae are families recognised to produce high-quality pollen for insects, highly attractive and digestible (Williams & Christian, 1991; Cook *et al.*, 2003; Hanley *et al.*, 2008; Huang, 2012; Liolios *et al.*, 2016). Nevertheless, for a balanced diet, numerous insect species must mix different pollen sources (Di Pasquale *et al.*, 2013; Eckhardt *et al.*, 2014). Provide various high-quality pollen sources is therefore important in insect targeted schemes, but the exact requirement could strongly vary, even among closely related bee species (Sedivy *et al.*, 2011; Leonhardt & Blüthgen, 2012; Vaudo *et al.*, 2016).

The spontaneous Brassicaceae and Fabaceae plant species offered few flowers on strips (< 9% of all flower units). Conversely, *Raphanus sativus*, the main sown Brassicaceae species in the studied strips, produced abundant flowers. This species, with pollen largely collected by *Bombus* spp., and *Phacelia tanacetifolia*, are known to have attractive, highly nutritional pollen that supports good Hymenoptera larva development (Williams & Christian, 1991; Cook *et al.*, 2003; Hanley *et al.*, 2008). Their presence in bird-strips is thus a clue to promote these strips as interesting for insects.

d) Bird-strip AES attracted mainly polylectic insect visitors

The visitor abundance we observed (2.6 visits/100m²/25 min from June to September), was lower to those in pollinator favourable areas in summer. Blaauw and Isaacs (2014) recorded 32.6 visits/100m²/25 min in sown wild-flowers plots (even if they recorded only bees and Syrphidae) and Forup and Memmott (2005) observed 28.6 visits/100m²/25 min in meadows. The observed insect abundances in bird-strips were even lower than in the UK areas without specific pollinator-friendly management studied by Baldock *et al.* (2015) (6.7, 11.1 and 13.0 visits/100m²/25 min respectively in urban areas, farmlands, and nature reserves).

We observed only a small subsample of the Walloon diversity, three of the 30 *Bombus* species present in Wallonia (Pauly & Rasmont, 2010), five of the 300 non-*Bombus* wild bee species (Rasmont *et al.*, 2005), 24 of the 310 Syrphidae

species (Speight *et al.*, 2015) and four of the 115 Lepidoptera species (Dopagne, 2015). But, the observed flower-visitor insect diversity (74 morphotypes) was the same as on a pollinator field-strip AES (Ouvrard, personal observations, 2015) and within the range of other observations on European farmland flowered areas dedicated to insects (131 to 25) (Fründ *et al.* 2010; Ebeling *et al.* 2011; Campbell *et al.* 2017). The observed diversity was higher than the flower-visitor diversity across urban, farmland or nature reserve landscape without specific pollinator-friendly management in a recent UK study (Baldock *et al.*, 2015, 32, 48 and 46 respectively). The solitary bee diversity (five species) was similar to another flowered-strip scheme nearby (Ouvrard, personal observations, 2015) and higher than diversity find in several countries (Baldock *et al.*, 2015; Scheper *et al.*, 2015, mean bee species diversity from 0.8 to 3.1).

The pool of insects observed was mainly driven by four common polylectic species (*Apis mellifera*, *Eristalis tenax*, *Bombus lapidarius* and *Bombus terrestris* OTUs) that represented 63% of the visitors. The dominance of a these common Hymenoptera and Syrphidae species is typical in flowered field-strips (Rader *et al.*, 2012; Kleijn *et al.*, 2015; Wood *et al.*, 2017). These polylectic insects are known as pollinating insects (Free, 1993; McGregor, 2009; Willmer, 2011), and their presence in high numbers may support effective landscape-level pollination services (Kleijn *et al.*, 2015; Wood *et al.*, 2017). Moreover, one-third of the 24 observed Syrphidae species have a carnivorous larval stage (Speight, 2016) that can provide additional pest management services (Ssymank *et al.*, 2008).

e) Recommendations

Fagopyrum esculentum provided only 0.2% of the pollen, 1.6% of the nectar, and was not visited by insects. Except if the seeds produced are important for birds, this species could be removed from the sown mix. Oppositely, adding early flowering species, such as *Erysimum* spp. or *Vicia faba*, to the feeding section, or *Lamium* species, particularly attractive for bumblebees (Sikora & Kelm, 2012), to the refuge section, could help fill the spring gaps of resources. Moreover, we highly recommend adding *Centaurea jacea* to the seed mix for the refuge section to increase nectar resources and replace *C. arvensis*, which must legally be destroyed. Implementing such simple improvement could increase the provision of flower resources and made the bird-strip AES even more interesting to insects.

VII. CONCLUSION

Management of bird-strip AES allows spontaneous self-seeded plant species to grow and flower. These species provided flower resources to most of the insect visitors during the summer, but the sown species were particularly important to provide pollen to insects. One sown plant species, *Raphanus sativus*, is particularly valuable due to the quantity and quality of its pollen, and its resilience to climatic conditions. Nevertheless, spontaneous plant species increased nectar quantity in the bird-strips.

Although designed for birds, bird-strip AES attract flower-visitor insect species as diverse as in several other flower-strips dedicated to insects. These insects were attracted in the summer, providing pollination ecosystem service. Nevertheless, the addition of an early annual flowering Fabaceae species in the feeding section and an Asteraceae species in the perennial refuge section could be meaningful improvements to make this scheme even more interesting for insects.

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Chapter 3: Landscape structure is more determinant for flower-visiting insects than the type of managed strip agri-environment schemes

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I. ABSTRACT

1. From several years, numerous agri-environmental schemes (AES) were deployed to mitigate declines in farmland biodiversity. They linked eco-system services in intensively cultivated farmlands. In Belgium, some AES strips were implemented for pollinators and birds, but their interest for flower-foraging insects has not been compared according to landscape composition yet.

2. The primary objective of this research is to compare floral resources and insect communities of two types of AES strips, the bird-strips and the pollinator-strips in Condroz and Hesbaye, two biogeographical regions of Belgium. We identified and quantified flowering resources and flower-visiting insects while analysing landscape composition in a two-km buffer area around each studied strip. We monthly recorded densities and diversities of flowers, and flower-visiting insects during three years on six pollinator-strips and four bird-strips in intensively cultivated farmland.

3. The sown and spontaneous plant communities of both types of strips provided nectar and pollen to numerous insect species, especially common polylectic species, known as efficient pollinators. Floral resources of both types of strips were mainly produced in summer and by sown and spontaneous Asteraceae species. Extra-strip available floral-resources are so particularly required by insects. These resources should provide spring resources and allow insects to find pollen complementary to the essential-amino-acid-deficient Asteraceae pollen.

The local flower-foraging insect diversities were richer than the flower-visiting insect diversities observed on flowers. The insect diversities were more affected by the biogeographical region than by the type of implemented AES. In their current configuration, the AES strips were not sufficient to compensate the anthropogenic landscape perturbations.

4. *Synthesis*: the Belgian bird- and pollinator-strip AES are both effective AES that provide floral resources to pollinating insects in summer and that support ecosystem services in intensively cultivated farmland. Nevertheless, the spring flower resources remain weak, and insects have to find floral resources

outside the strips until June. The surrounding landscape was more important than the type of AES strips to explain insect communities' diversity. Therefore, the location of AES strips within the landscape is of particular importance to improve the strips effectiveness to support insects.

II. KEYWORDS

Bird-strips, seed strips, insect conservation, Agri-environmental scheme, landscape structure

III. HIGHLIGHTS

- Bird- and pollinator-strips AES provided valuable floral resources for insect
- Most of the supported insect species are known to be effective pollinators
- Insect communities were more affected by landscape composition than type of AES strips
- Surrounding landscape is of particular interest to provide spring floral resources
- AES strips were not sufficient to compensate anthropogenic landscape perturbations

ABBREVIATIONS ^{1,2}

IV. INTRODUCTION

Since the 50's, farming intensification has led to a worrying decline in farmland biodiversity and population sizes of both plant and animal species (Benton et al., 2003). The reduction of crop diversity, the excessive use of chemicals (Godfray et al., 2015; Goulson et al., 2015), the destruction of hedges and field margins (MacDonald and Johnson, 2000) rarefied nesting sites and feeding resources for wild fauna as birds, small mammals and insects (Deguines et al., 2014; Kennedy et al., 2013). Anthropogenic habitat disturbance (habitat simplification, increasing urbanisation and agricultural intensification) increases the extinction risk for species and conducts to simplification of insect communities (Carman and Jenkins, 2016). The pollination and pest management ecosystem services these insect populations previously widely provided to crops and wild plants are now imperilled (Kells et al., 2001; Marshall and Moonen, 2002; Morandin and Kremen, 2013). The supplying of such ecosystem services now often requests specific management from landowner and farmers.

¹ AES: Agri-Environmental Schemes

² OTU: Operational Taxonomic Unit

In Europe, the Common Agricultural Policy subsidises several Agri-Environmental Schemes (AES) to support farmland biodiversity by the conservation, the restoration or the creation of semi-natural areas in intensively cropped farmland areas (European Commission, 2017; Wood et al., 2017). Among them, the managed field strips and their numerous variations, as the bird- and the pollinator-strips, provide refuges to insects (Ouvrard et al., 2017b; Ouvrard and Jacquemart, 2017).

Insects feed and nest on diverse semi-natural elements. In different landscape contexts, various insect communities are expected. We worked in two Belgian regions with distinct land use characteristics, but the same AES were implemented. We hypothesised that i) both abundance and diversity of flower foraging insects differ among regions due to key landscape features such as crop typology, ii) the observed visitor communities reflected the interest of a strip for the local insect communities. The closer the visitor community on a strip to the flower foraging insects of the region, the more efficient the strip to compensate lost landscape foraging resources. iii) the effectiveness of an AES is not linked to the strip only but to the cross effect of the strip and the surrounding landscape (both local flora and fauna).

We studied two AES in two biogeographic regions. The pollinator-strips AES was specifically implemented to support flower-visitor insects. The bird-strips AES was implemented to help farmland birds thanks to seed provision. To record insect diversity, we applied pan-trapping and netting as these two methods are complementary to assess the pollinator diversity (Cane et al., 2000; Roulston et al., 2007).

V. MATERIALS AND METHODS

A. *Studied strips*

Monthly, from April to September 2014 to 2016, we studied ten strips that were managed according to South Belgium AES regulation. Four pollinator-strips and one bird-strip located in Condroz (50°22'N, 5°13'E, 250 to 300 m a.s.l., Table 1), two pollinator-strips and three bird-strips located in Hesbaye (50°36'N, 4°34'E, 100 to 150 m a.s.l., Table 20). Strips were observed in Condroz between 2014 and 2016 and between 2015 to 2016 in Hesbaye. We omitted strips with direct neighbouring apiaries, orchards, forests, hedges, or villages to avoid the influence of honeybee *Apis mellifera* hives or non-agricultural habitats.

Condroz and Hesbaye are both intensively cultivated farmland areas but with a different crop typology. Crops in Hesbaye are dominated by a mix of cereals (44%), sugar beetroot (*Beta vulgaris*, 20%), potato (*Solanum tuberosum*, 5%) and maize (*Zea mays*, 5%), intermingled with meadows (13%) (Witte et al., 2009). Whereas meadows (47%) dominate the Condroz agricultural landscape, followed by the cereals (37%), oilseed rape (*Brassica napus*, 5%), sugar beetroot (*Beta vulgaris*, 4%) and potato (*Solanum tuberosum*, 4%) crops (Castiau et al., 2011).

The studied schemes were set up by farmers following the local Wallon AES management plans. Studied managed strips were nine to 21 m width and several hundred meters long (Table 1). The pollinator-strip AES intended to provide flower resources to flower-feeding insects, including an uncut area that provides nesting sites. The bird-strip AES aimed to provide winter seeds to birds, including a tall-grass refuge area. The pollinator-strips were sown with a flower-rich hay-meadow type perennial plant mix. This mix consisted of four native species of grasses (85% of the mix, *Agrostis capillaris*, *Festuca rubra*, *Poa* spp.) and 10 entomophilous wildflowers; mainly belonging to Asteraceae (e.g. *Achillea millefolium*, *Centaurea jacea*, *Leucanthemum vulgare*) and Fabaceae (e.g. *Medicago lupulina*, *Medicago sativa*, *Lotus corniculatus*, and *Trifolium pratense*). The additional sown flower species were *Daucus carota*, *Malva moschata* and *Silene x hampeana*. The pollinator-strips were divided into three longitudinal sections managed with different mowing regimes (Le Roi et al., 2010). The bird-strips were also divided into three longitudinal sections but with distinct sown mixes. An un-mown 'refuge' section is sown for several years with a combination of *Dactylis glomerata* (50%), *Schedonorus arundinaceus* (49%) and *Melilotus* sp. (1%). A feeding section is sown with a mix containing one cereal, *Triticum aestivum* (65%) and three entomophilous species *Fagopyrum esculentum* (25%), *Phacelia tanacetifolia* (3%) and *Raphanus sativus* (7%). An optional un-sown annually harrowed section can be added (Le Roi et al., 2010).

Each studied pollinator-strip was sown in 2008. Refuge sections of the studied bird-strips were sown in 2008, and their feeding sections were sown in 2015. Harrowed sections were implemented on two of the four studied bird-strips (n° 1 and 2, Table 20).

B. Landscape composition

We analysed the primary landscape structure on a two-km radius buffer area around the studied strips. Maps were drawn on Quantum GIS 2.18.9 (QGIS), based on the 'Google hybrid map' and checked on the field. We grouped forests, groves and large hedges in 'wooded' area. We designated meadows, both grazed and mown, clearing and grass strips as 'grassland'. We named cultivated areas 'crops'. We grouped all the villages, houses, gardens, public areas (e.g. cemetery, car park), industrial buildings, farms and their surrounding as 'urban' areas. We omitted the motorways areas and to simplified the maps; we fairly shared the road areas out adjacent areas.

Surfaces of each land use class were calculated using the QGIS '\$area' function and expressed as a percentage of total buffer area (Elton and Jason, 2015).

Table 20. Location of the 10 studied strips in the two Belgian biogeographical regions (Condroz and Hesbaye) from 2014 to 2016.

Site	Area	Locality	Years of observation	Location Latitude, Longitude	Length (m)	Width (m)
Pollinator strip 1	Condroz	Ciney	2014-2015	50°18'25"N, 5°08'43"E	1000	12
Pollinator strip 2	Condroz	Ciney	2014-2015	50°16'54"N, 5°07'54"E	560	12
Pollinator strip 3	Condroz	Havelange	2014-2015	50°21'42"N, 5°17'20"E	773	21
Pollinator strip 4	Condroz	Ciney	2014-2015	50°17'38"N, 5°00'13"E	1000	20
Pollinator strip 5	Hesbaye	Thines	2015-2016	50°36'10"N 4°22'18"E	290	18
Pollinator strip 6	Hesbaye	Opprebais	2015-2016	50°40'17"N 4°48'41"E	330	14
Bird strip 1	Hesbaye	Opprebais	2015-2016	50°41'05"N 4°47'09"E	570	12
Bird strip 2	Hesbaye	Opprebais	2015-2016	50°40'25"N 4°48'41"E	500	12
Bird strip 3	Hesbaye	Gembloux	2015-2016	50°32'53"N 4°38'08"E	600	9
Bird strip 4	Condroz	Goesnes	2015-2016	50°27'10"N 5°11'53"E	320	12

C. Observations

Observations were carried out between 9:00 am to 06:00 pm under favourable weather conditions for optimal insect activity (temperatures above 10°C with at least 50% of clear sky, no rain and the wind speed under 5 on Beaufort's scale, Baldock et al., 2015; Ouvrard et al., 2017a). Order and time of observation varied among sampling runs to avoid confounding of daytime and strip.

In the centre of each strip section, a permanent 100-m long one-m width standardised transect was established to ensure consistency in survey area among sampling sessions.

a) Flower resources and abundance

Every 5 m along each transects, on one m², we identified every entomophilous plant species (Lambinon and Verloove, 2012; Rameau and Dumé, 2008; Rose, 2004; Tison and de Foucault, 2014). We then recorded densities of open floral units. For species of the Asteraceae family, a floral unit corresponded to a capitulum. We counted as an open floral unit every

capitulum with at least one open floret. For all the other species, a floral unit corresponds to one single flower.

We compiled flower resources (pollen and nectar) daily productions from published data (Cawoy et al., 2009; Hicks et al., 2016; Kuberappa et al., 2007; Ouvrard et al., 2017a, 2017b; Ouvrard and Jacquemart, 2017; Szabo and Najda, 1985).

b) Insect visiting flowers

Plant-insect interactions were recorded using standardised transect walks (Hicks et al., 2016; Ouvrard et al., 2017b; Ouvrard and Jacquemart, 2017). On each field session, we recorded interactions on every single transect along two consecutive walks of at least 10 min apart, in the same way. Only the insects actively visiting open flowers were recorded.

Bombus individuals, particularly numerous in our surveys, were identified on the field up to Operational Taxonomic Unit (OTU, Terzo and Rasmont 2007), using their colour patterns. We subsampled *Bombus* individuals for precise lab identification up to the species. We identified directly on the field some species readily recognisable such as *Aglais urticae* (Lepidoptera), *Apis mellifera* (Hymenoptera), and *Eristalis tenax* (Syrphidae, Diptera). All the other insects were individually caught and pinned for lab identification up to the species level for Hymenoptera (Archer, 2014; Falk, 2015; Goulet and Huber, 1993; Patiny and Terzo, 2010; Terzo and Rasmont, 2010a, 2010b; Wright, 1990), Lepidoptera (Skinner and Wilson, 2009; Tolman and Lewington, 2009), Coleoptera (Auber, 1960; Unwin, 1984) and Syrphidae Diptera (Speight and Sarthou, 2016; Stubbs and Falk, 2002; Verlinden, 1994). Non-Syrphidae Diptera were identified up to the family level (Oosterbroek, 2006; Stubbs and Falk, 2002) but the number of different morphotypes was nevertheless recorded.

Bumblebees were grouped by OTU for further statistical analyses. Non-Syrphidae Diptera were round up in a single group. For the other insects, a group corresponded to a single species.

c) Insect diversity

Pan-traps were used on our ten selected strips to assess the insect diversity (Vrdoljak & Samways, 2012; Westphal & al., 2008). For each observation session, we exposed two sets of pan-traps for non-raining 24h in the centre of each strip, 50 m apart. We conducted pan trapping in 2014 and 2015. Each set of pan-traps was an assemblage of a group of three polypropylene boxes (14 x 10 x 3 cm). We painted each box with a specific UV reflecting painting (SPARVAR, de SPRAY-COLOR GmbH, Merzenich, Germany). Blue (ref. 3107LF), white (ref. 3108) and yellow (ref. 3104) colours were used to offer the largest attracting spectrum for insects as possible (Westphal et al., 2008). We filled each pan-trap with 420 mL of 0.5% of sent less detergent (Froggy ecological, Wermer & Merts GmbH, Mainz, Germany) water solution. We fixed each set of pan-traps at the flower level with an adjustable height metallic frame.

After 24h on the field, we closed the pan-traps and refrigerated them at 2°C. Within 48h, we sorted the insects. We counted, but we did not identify the non-Syrphidae Diptera, Coleoptera and parasitoid Hymenoptera as they are recognised as only occasional or no pollinating insects (Willmer, 2011). We pinned all the other specimens, and we identified them up to the species level.

D. Statistical analyses

We performed all statistical analyses using the R software (version 3.2.4 GUI 1.67, R Core Team, 2016). Insect–flower interaction networks were visualised with the ‘bipartite’ R-package. We considered insect species as higher trophic level and plant species as lower trophic level.

Non-metric MultiDimensional Scaling (NMDS) were conducted to represent the pairwise dissimilarities among records grouped by strip and year as tightly as possible. We used the ‘metaMDS’ function of the {vegan} R-package. We gave means with their standard errors.

Recorded floral units per species, number of visitors per group of insects, number of visited floral units per species and land use data were compared by Kruskal-Wallis rank sum test (using the ‘kruskal.test’ function of the {asbio} R-package). These comparisons were followed by a *post-hoc* test using the Tukey method for p-value calculation (using ‘posthoc.kruskal.nemenyi.test’ function of the {asbio} R-package).

Insect diversities among biogeographic regions, type of AES and catching methods were compared by a permutational multivariate analysis of variance using distances matrices (permanova), with the ‘adonis’ function of the {vegan} R-package. We used a Bray-Curtis distance matrix and 999 permutations.

VI. RESULTS

A. Landscape composition

In the two-km buffer areas around the studied strips, landscape compositions were significantly different between the two biogeographic regions (Condroz and Hesbaye, Fig. 15). Hesbaye has a higher urbanization rate ($14.7 \pm 5.9\%$), more crops ($65.9 \pm 9.6\%$) compared to grasslands ($15.5 \pm 4.2\%$) and wooded areas ($3.9 \pm 2.0\%$) than Condroz ($2.6 \pm 1.4\%$; $46.5 \pm 9.9\%$, $37.8 \pm 8.1\%$ and $13.0 \pm 6.9\%$ respectively, $p < 0.03$).

At the strip level, the landscape was constituted of $8.6 \pm 7.5\%$ urban areas, $56.2 \pm 13.8\%$ crops, $26.6 \pm 13.2\%$ grasslands and $8.5 \pm 6.8\%$ wooded areas in all cases.

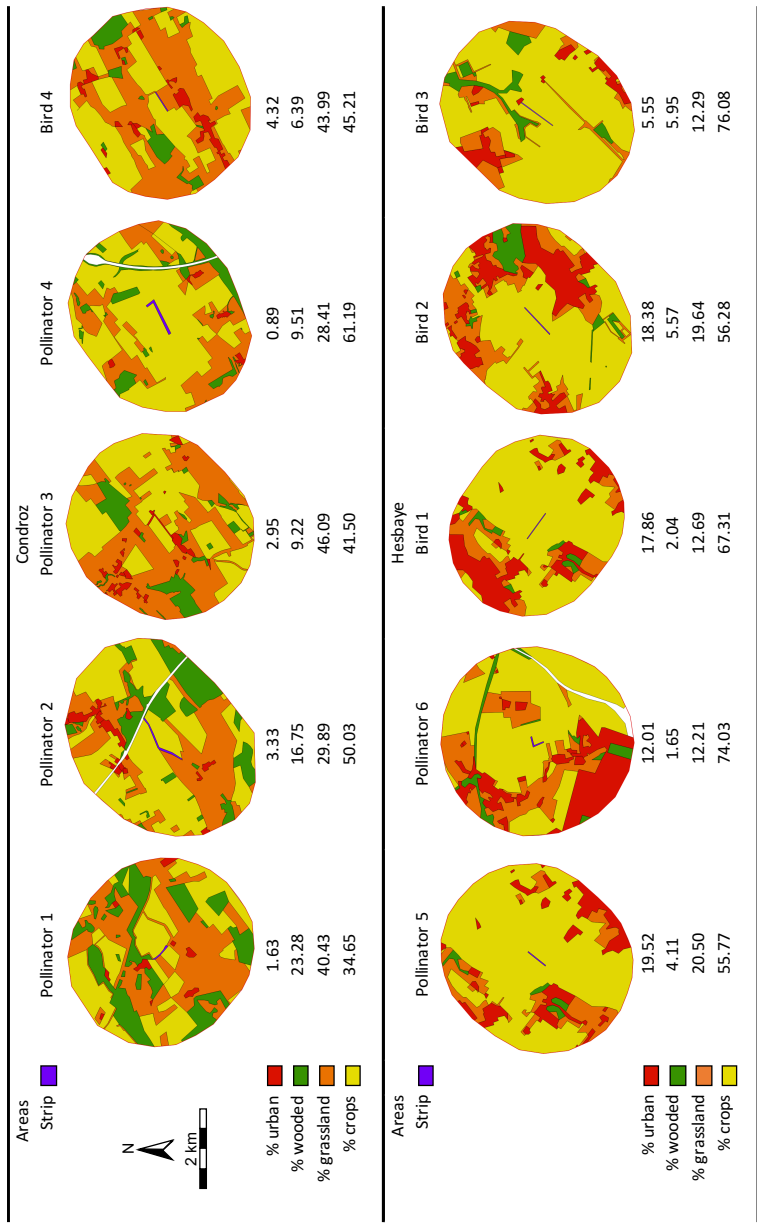


Figure 15. Soil occupation on two-km radius buffer area around the 10 studied strips in the two biogeographical regions. Values are percentages of each type of soil occupation per buffer area. Strips are in the centre of each map. Motorways areas (white) were omitted.

B. Flower diversity and abundance

We observed 76 flowering plant species with 54 species in the Condroz and 58 species in Hesbaye. Among these plant species, we found 52 species in bird-strips and 58 in pollinator-strips. The global biogeographical regions and strip types combined, monthly flower diversity increased along the season with 7, 21, 35, 50, 56 and 42 flowering plant species from April to September respectively.

The available floral resources varied between the two biogeographical regions as we observed significant differences in floral composition and floral unit densities ($p < 0.01$) (Fig. 16). In Condroz, three Fabaceae species dominated flower communities, *Trifolium repens* (20.8% of the floral units observed), *Trifolium incarnatum* (11.5%), and *Medicago sativa* (10.3%) and one Apiaceae, *Daucus carota* (16.6%). In Hesbaye the plant communities were dominated by one Brassicaceae, *Raphanus sativus* (19.6% of the floral units), one Polygonaceae, *Polygonum aviculare* (14.9%) and one Fabaceae *Lotus corniculatus* (10.9%). The global floral densities were higher in Condroz than in Hesbaye (80.0 ± 135.6 floral units per m^2 vs 29.7 ± 67.6 respectively, $p = 0.009$).

We observed no significant variation in floral composition and floral unit densities between bird- and pollinator-strips ($p = 0.80$). But the global floral densities were significantly higher on the pollinator-strip AES (71.2 ± 126.6 floral units per m^2) than on the bird-strip AES (17.9 ± 34.4 , $p = 0.024$).

We recorded significant variations in floral composition and floral unit densities among the 'pollinator 1' strip and 'pollinator 5', 'pollinator 6' and 'fauna 3' strips only ($p < 0.01$). The strip 'pollinator 1' had significantly higher floral richness (42 species vs 16, 12 and 14 respectively).

C. Visited flowers

Flowers species visited by insects varied between Condroz and Hesbaye as we observed significant differences of flower visited communities (the species diversity and the number of floral units visited) between biogeographical regions ($p < 0.01$). In Condroz, insects visited three main plant species, *Centaurea jacea* (43.8% of the visits), *Lotus corniculatus* (12.1%), and *Daucus carota* (11.6%). In Hesbaye, the most visited plant species were more diverse with five main species, *Centaurea jacea* (22.9% of the visits), *Cirsium arvense* (18.1%), *Lotus corniculatus* (10.8%), *Raphanus sativus* (10.3%), and *Leucanthemum vulgare* (10.1%, Fig. 16).

No significant difference was observed between type of strips ($p = 0.48$) nor among strips ($p > 0.1$).

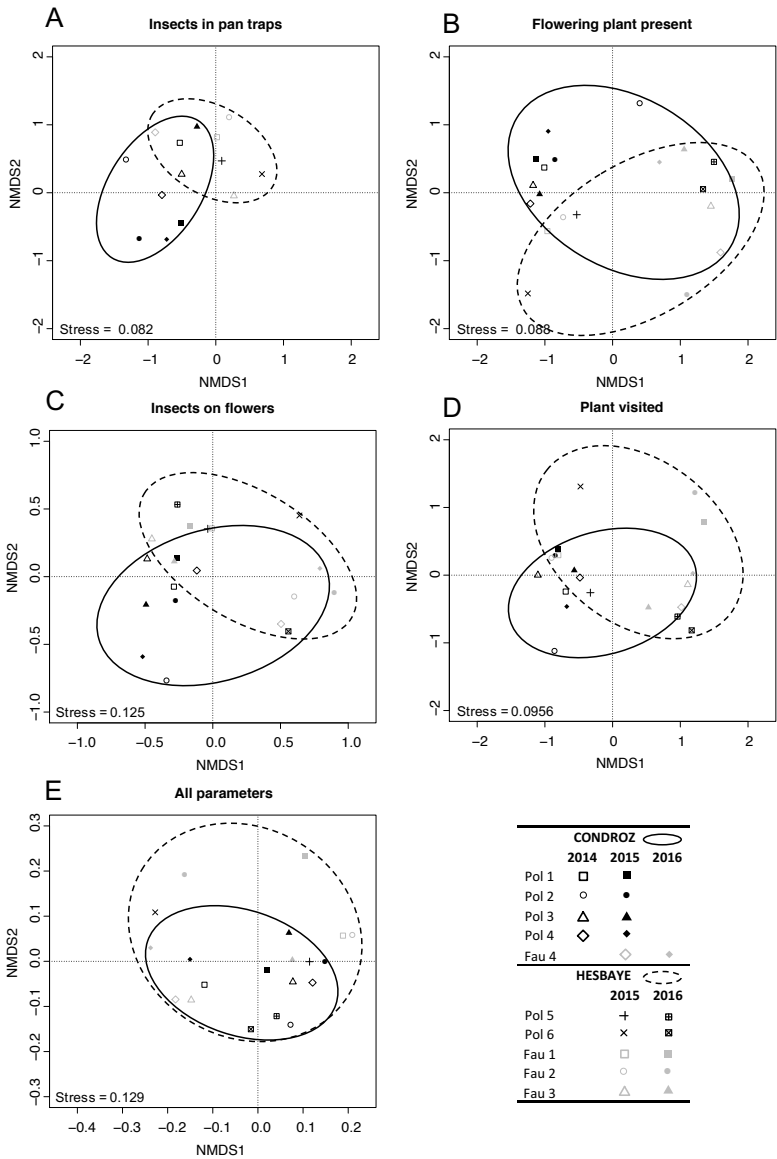


Figure 16. Non-metric MultiDimensional Scaling (NMDS). Based on the diversity and the number of pan-trapped insect species from 2014 to 2016 over the ten studied strips (**A**), flowering plant species diversity and floral unit density (**B**), visitor insect species diversity and number (**C**), visited plant species diversity and number of visits per species (**D**). The mean number of floral units per 100m², the number of plant species, the number of visited plant species, the number of insect visitor species, the mean number of visits per 100m², the mean daily production of pollen and nectar per m² were used for the last (**E**) graph. Strips and years are identified by symbols as

presented on the graph. Strips in Condroz are circled with a solid line, strips in Hesbaye are circled with a dotted line.

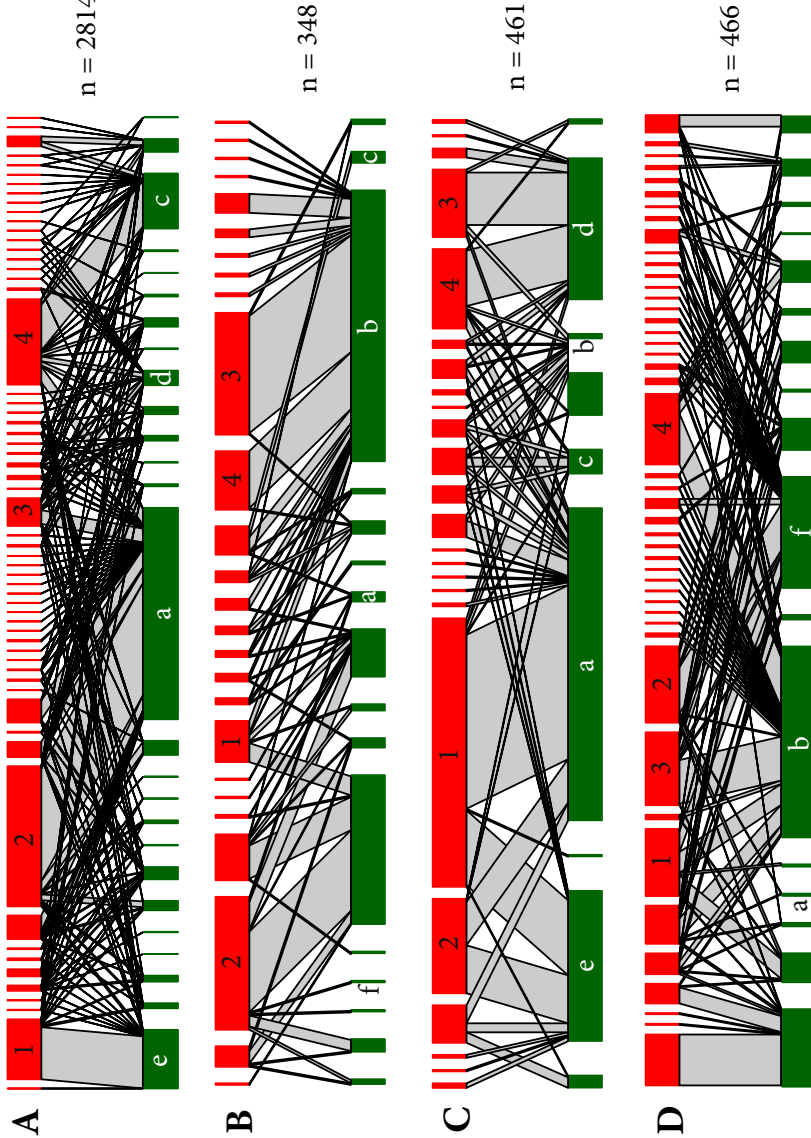


Figure 17. Insect-flower interaction networks among flowers and insects over the 10 studied strips based on: A. the four pollinator-strips in Condroz, B. the bird-strip in Condroz, C. the two pollinator-strips in Hesbaye and, D. the three bird-strips in Hesbaye. Each rectangle corresponds to a plant species (green) or an insect group (red). Rectangle sizes are proportional to the number of interactions observed. Main flowering plant species are indicated: a) *Centaurea jacea*, b) *Cirsium arvense*, b) *Daucus carota*, d) *Leucanthemum vulgare*, e) *Lotus corniculatus* and f) *Raphanus*

stivus. Main insect types are indicated 1) *Apis mellifera*, 2) *Bombus lapidarius* OTU, 3) *Eristalis tenax* and 4) non-Syrphidae Diptera.

D. Flower resources

Whatever the strip, the type of strip or the biogeographical regions, spring (April – May) resources amounts were meagre < 0.1% and 2.2% of the nectar available over the year, respectively in April and May, and < 0.1 and 1.1% of the pollen available along the year. July was the most resource-abundant month of the year (59.4% of nectar and 47.0% of pollen produced, Table 21).

Table 21. Floral resources (pollen and nectar) repartition among months in the three studied years. Values are percentages per year. n= 4 in 2014, 10 in 2015 and 6 in 2016.

	2014		2015		2016	
	Pollen (%)	Nectar (%)	Pollen (%)	Nectar (%)	Pollen (%)	Nectar (%)
April	na	na	0.02	0.13	0.00	0.00
May	1.70	0.78	0.02	2.35	5.71	2.55
June	48.87	31.93	28.08	7.97	na	na
July	39.31	50.19	51.22	56.77	70.56	73.76
August	6.50	11.07	14.32	23.58	20.80	21.07
September	3.62	6.04	6.33	9.21	2.92	2.63

No data were available for April 2014 and June 2016

Over the year, nectar-sugar quantities produced were significantly lower in Condroz than in Hesbaye (7.3 ± 11.2 mg/m²/day vs 6.8 ± 17.1 , $p = 0.027$). Nevertheless, species providing floral resources and their relative importance varied. In Condroz, the primary nectar producers were mostly sown Asteraceae and Fabaceae species. *Centaurea jacea* provided 37.4% of the total nectar production. *Lotus corniculatus* (8.2%), *Tripleurospermum inodorum* (7.6%) and *Trifolium incarnatum* (7.4%) were also high nectar producers. In Hesbaye, spontaneous Asteraceae species produced most of the nectar resources. The first nectar producer was *Cirsium vulgare* (43.6% of the total nectar produced on Hesbaye's strips), followed by *Centaurea jacea* (15.4%), *Raphanus sativus* (10.4%), *Cirsium arvense* (8.1%) and *Tripleurospermum inodorum* (6.3%).

Over the year, the nectar-sugar productions were similar between the type of strips (7.2 ± 11.8 for pollinator-strips vs 6.9 ± 17.8 for bird-strip, $p = 0.057$). Nevertheless, the plant communities providing most of the nectar differed more between the type of strips than between biogeographical regions.

In bird-strips, most of the nectar was produced by spontaneous Asteraceae (*Cirsium vulgare* 40.2%, *Tripleurospermum inodorum* 12.4%, and *Cirsium arvense* 11.1%), one sown Fabaceae (*Trifolium incarnatum*, 10.2%), and one sown Brassicaceae (*Raphanus sativus* 9.7%). In pollinator-strips two sown

species only dominated the nectar production, one Asteraceae (*Centaurea jacea* 49.6%) and one Fabaceae (*Lotus corniculatus* 10.5%).

The pollen production was significantly higher in Condroz ($17.8 \pm 31.0 \mu\text{L}/\text{m}^2/\text{day}$) than in Hesbaye (14.3 ± 30.7 , $p = 0.043$) and higher in pollinator-strips ($20.8 \pm 36.3 \mu\text{L}/\text{m}^2/\text{day}$) than in bird-strips (8.8 ± 17.8 , $p = 0.022$).

In Condroz the pollen production was dominated by two sown Asteraceae species (*Centaurea jacea*, 33.2% and *Leucanthemum vulgare*, 23.5%). In Hesbaye two Brassicaceae species adding further to the two previous Asteraceae (*Centaurea jacea* 22.0%, *Raphanus sativus* 20.9%, *Leucanthemum vulgare* 20.2%, and *Sinapis alba* 15.7%).

In bird-strips, the primary pollen producers were two Brassicaceae species (*Raphanus sativus* 23.0%, and *Sinapis alba*, 18.3%), one Asteraceae (*Tripleurospermum inodorum* 19.6%) and one Fabaceae species (*Trifolium incarnatum*, 11.0%). On pollinator-strips, two Asteraceae species only produced most of the pollen (*Centaurea jacea* 40.2% and *Leucanthemum vulgare* 28.1%).

E. Insect visiting flowers

We observed significant differences of insect visitor communities (the species diversity and the number of observed interactions) between biogeographical regions ($p = 0.018$).

In Condroz, all observations pooled together, we observed a mean instant-number of insects of 9.6 ± 18.4 per 100 m^2 and only 3.6 ± 8.1 in Hesbaye. In Condroz we recorded 26 groups of insects belonging to Syrphidae Diptera species, vs 23 in Hesbaye. A total of 24 groups of insects were identified as Hymenoptera in Condroz while 13 only in Hesbaye. We observed 14 groups of insects belonging to Lepidoptera in Condroz vs 9 in Hesbaye.

In Condroz, we recorded 70 different groups of insects. *Bombus lapidarius* OTU (31.1% of the recorded interactions), the group of non-Syrphidae Diptera (18.1%) and *Apis mellifera* (13.2%) dominated the visitor communities.

In Hesbaye, we observed 52 different groups of insects. *Apis mellifera* was dominant with 25.6% of the interactions, followed by *Bombus lapidarius* OTU (13.9%), the groups of non-Syrphidae Diptera (12.2%) and *Eristalis tenax* (11.7%, Fig. 17).

Among the biogeographical regions, the insect diversities were not the same. Condroz had a richer insect diversity, 39 groups of insects were observed in Condroz only. Nevertheless, in Hesbaye we recorded 21 groups of insects not shared with Condroz. Within the four *Bombus* OTUs recorded, we identified nine species in Condroz but only four in Hesbaye (Table 22).

We observed no difference in insect visitor communities (the species diversity and the number of observed interactions) between the type of strips ($p = 0.094$).

Table 22. *Bombus* Operational Taxonomic Units (OTU) composition observed in the ten studied strips in the two biogeographical studied regions (Condroz and Hesbaye). N=61

OUT	Species	Condroz	Hesbaye
<i>Bombus hortorum</i> n=1	<i>Bombus ruderatus</i>	1	0
	<i>Bombus humilis</i>	2	1
<i>Bombus lapidarius</i> n=31	<i>Bombus lapidarius</i>	17	6
	<i>Bombus ruderarius</i>	4	0
	<i>Bombus rupestris</i>	1	0
<i>Bombus pascuorum</i> n=23	<i>Bombus hypnorum</i>	1	0
	<i>Bombus pascuorum</i>	16	3
	<i>Bombus pratorum</i>	3	0
<i>Bombus terrestris</i> n=6	<i>Bombus terrestris</i>	1	5

F. Insect diversity

In 2015, a total of 22,948 insects were caught by pan-traps. Most of them (22,328) were non-Syrphidae Diptera, Coleoptera or parasitoid Hymenoptera. The 620 remaining specimens were identified and belonged to 108 different species (Supplemental table S3). For 40 species, we caught only one individual of each.

Hymenoptera dominated the sampling diversity with 60 species and 58.9% of the identified specimens, followed by Syrphidae Diptera (31 species, 34.4%). Lepidoptera accounted for 6.6% of the insects with, 16 species (Supplemental table S3).

Apidae (46.6% of caught Hymenoptera), Halictidae (34.3%) and Andrenidae (15.3%) were the most represented Hymenoptera families. Most of the caught Lepidoptera were Noctuidae (78.1%).

Species recorded with the higher strength (≥ 20 specimens) belonged to the Hymenoptera and the Diptera families. The most represented Hymenoptera species were *Bombus terrestris* (8.6%), *Apis mellifera* (6.6%), *Lasioglossum laevigatum* (5.5%), *Bombus lapidarius lapidarius* (4.5%), and *Andrena haemorrhoa* (3.2%). The most represented Diptera species were three Syrphidae species, *Eristalis tenax* (9.4%), *Episyrphus balteatus* (3.6%) and *Sphaerophoria scripta* (3.2%).

We observed significant differences of pan-trapped insect communities (diversity and number) between biogeographical regions ($p = 0.010$). Condroz region presented a higher diversity of species, with 25 Syrphidae species, 38 Hymenoptera species and 12 Lepidoptera species than in Hesbaye (17, 37 and seven respectively, Supplemental table S1).

an-trapped insect communities (diversity and number) were not significantly different between types of strips ($p = 0.165$), nor among strips (all $p > 0.38$). Conversely, we noticed significant differences between the diversity of pan-trapped insects and the diversity of visitors by biogeographical regions and by type of strips (all p -values < 0.03). Indeed, the similarities are scattered as, over the insect families observed both in pan-traps and on flowers, only 29 groups out of 153 were both pan-trapped and recorded as visitors (Supplemental tables S2 & S3). Nevertheless, pan-trapped and visitor insect diversities were not significantly different at the strip level ($p = 1$).

VII. DISCUSSION

A. *Landscape composition*

The landscape composition and differences of land use among biogeographical regions can explain some of the variations of insect communities (Krauss et al., 2003; Steffan-Dewenter et al., 2002).

The observed Syrphidae Diptera community diversity variation among biogeographic regions (48 species in Condroz, 32 in Hesbaye) could be explained by the variation of woodlands (13% in Condroz, 4% in Hesbaye). Numerous Syrphidae species need nesting resources and feeding resources for larvae provided by woody species (Moquet et al., 2017a). In the same way, the different proportions of woodlands, meadows and urban areas (13, 38 and 3 respectively in Condroz, versus 4, 15 and 15 in Hesbaye) in the two-km buffer areas around the strips could explain the differences of Lepidoptera (31 species in Condroz, 10 in Hesbaye) and Hymenoptera communities (71 species in Condroz, 46 in Hesbaye). Woodlands and meadows can provide valuable food resources and attractive nesting areas for Lepidoptera and Hymenoptera. Whereas urban areas are highly perturbed areas that are less favourable for farmland insects (Fox, 2013; Fuentes-Montemayor et al., 2011; Hines and Hendrix, 2005; Kämper et al., 2016; Rundlöf and Smith, 2006; Watson et al., 2011). Moreover, *Brassica napus*, was more abundant in Condroz than in Hesbaye (5% of the cropped areas in Condroz versus less than 1% in Hesbaye, Castiau et al., 2011; Witte et al., 2009). *Brassica napus* is a spring mass-flowering crop that could be a valuable spring resources for insects (Westphal et al., 2003).

As expected, the biogeographical region with the height anthropogenic habitat disturbance (habitat simplification, increasing urbanisation and agricultural intensification) showed the simplest insect communities (Carman and Jenkins, 2016).

Some conservation or restoration schemes are known to mitigate the adverse impacts of anthropogenic landscape disturbances on insect populations (Forup et al., 2008; Forup and Memmott, 2005; Kearns et al., 1998; Marshall et al., 2006), but their effectiveness was linked to the surrounding landscape. The proximity of the diverse and complementary landscape elements is essential to ensure connectivity among them and emphasising the AES effectiveness (Albrecht et al., 2007; Becher et al., 2016). As the observed

insect communities on AES strips were poorer in the most anthropogenic disturbed region than in the areas with less urban matrix and more woodlands, the implemented AES were not sufficient to fully compensate the surrounding landscape simplification.

AES strips' plant species composition and the AES landscape density should be improved to increase the beneficial AES effect to mitigating the decline of insect diversity and abundance. Moreover, the AES should be promoted within a geographically larger planning to be more effective and coherent. The AES should be built not on a field- or a farm-scale, but at a landscape scale to create landscape heterogeneity and connectivity among landscape elements.

B. Flower resources quantities and qualities

In both types of studied AES, both sown (mainly *Centaurea jacea* and *Leucanthemum vulgare*) and spontaneous Asteraceae species (especially *Cirsium arvense*, *C. vulgare* and *Tripleurospermum inodorum*), represented a large part of the available resources (43% of all the available pollen and 78% of nectar). Nevertheless, these species did not provide the highest number of floral units (only 16% of all floral units). The large quantities of sugar-rich nectar produced by species of this family are valuable resources for numerous flower-feeding insects (Hicks et al., 2016). Unfortunately, the pollen provided by these species is known to be of low quality, missing or having lower concentration in several essential amino acids (especially Methionine and Tryptophan) than needed for a successful Hymenoptera larvae development (Forcone et al., 2011; Goulson et al., 2005; Hanley et al., 2008; Nicolson and Human, 2013; Somme et al., 2015; Spear et al., 2016; Vanderplanck et al., 2016). Larvae require high-quality pollen or a mix of complementary pollens from diverse plant species and families (Di Pasquale et al., 2013; Eckhardt et al., 2014). As in summer, observed insects on the strips collected nearly no pollen from surrounding landscape resources (pers. obs.), the AES strips should provide plant communities that allow insects to mix pollens to a non-deficiency essential amino acids diet. Nor the landscape resources nor the spontaneous species of strips (mainly Asteraceae) help reducing the global essential amino acids deficit of pollen diet available on AES strips, (Marshall et al., 2006).

Insect communities differed between biogeographical regions. Therefore, the current sown mixes should be redesigned to provide more high-quality pollen resources. Several Brassicaceae or Fabaceae species are known to produce high-quality pollen (Hanley et al., 2008; Huang, 2012; Liolios et al., 2015). Additional species from these two families could be added to the seed mixes sown on strips. These supplemental resources may increase plant species and plant families diversity (Balzan et al., 2016, 2014) to mitigate or compensate the high prevalence of resources from sown and spontaneous Asteraceae species. Moreover, as insect communities were different in distinct biogeographical regions, AES should be adapted regionally at a landscape scale (Fuentes-Montemayor et al., 2011). These strip

improvements should increase the functional complementarity among plant species and include specific adaptations to local insect communities.

Whatever the differences between type of strips or biogeographical regions, the mean available sugar-nectar resource densities (7 ± 14 mg of sugar/m²/day over the year) or even the available sugar-nectar resource densities at peak strip production (up to 104 mg of sugar/m²/day) were small compared to resources densities of mass-flowering crops. Indeed, the mean daily nectar-sugar production of *Brassica napus* crops during its blooming period, in the same biogeographical regions, was 14 times higher (up to 1496 mg of sugar/m²/day, Ouvrard et al., 2017a) than peak AES strips production.

In the same way, the mean daily pollen production (16 ± 31 μ L of pollen/m²/day over the year, fluctuating up to 167 μ L of pollen/m²/day during the peak flowering) was rather low (around 2400 μ L of pollen/m²/day in *Brassica napus* fields, Ouvrard et al., 2017a). At peak flowering 1 m² of AES strips provided up to 24 honeybee pollen loads (around 7 μ L each, pers. obs.) when 1 m² of *Brassica napus* provided 343 honeybee pollen loads.

A large-scale implementation of AES should help provide sufficient flower resources for insects at a landscape scale.

Bird-strip AES provided a lower density of resources than pollinator-strip AES. It appeared that the feeding sections of the bird-strip AES (a third to half of a strip area), provided very few flowers (8% of the total bird-strip AES flowers). Particular efforts should be conducted on this section to increase the interest of bird-strip AES in supporting insect communities while maintaining the required specifications for birds. Adding some perennial species such as *Centaurea jacea*, *Trifolium pratense* and *Medicago sativa* could help both increasing the quantity of provided flower resources in bird-strip AES and providing high-quality Fabaceae pollen that could increase the insect breeding stock (Rundlöf et al., 2014).

C. Flower resources timing

Although most of the observed *Bombus* species were early-emerging species (April – May, Benton, 2006; Falk, 2015), we observed the first individuals in June on the studied strips. Indeed, floral resources were so scattered in April (0.1 ± 0.3 floral unit/m²) and May (7.5 ± 20.5 floral unit/m²) that the strips presented a weak interest for *Bombus* in spring. The emerging time is a crucial period for *Bombus* as the colony settlement is conditioned by the gynes' capacity to find a lot of early flower resources and nesting site (Hines and Hendrix, 2005; Kämper et al., 2016; Moquet et al., 2015). Mass-flowering crops, as *Brassica napus* fields (for nectar, Kämper et al., 2016; Requier, 2013) and woody species as *Acer* or *Salix* species (for pollen, Kämper et al., 2016; Moquet et al., 2015), could provide a major amount of valuable flower resources in spring for *Bombus* (Herrmann et al., 2007; Kämper et al., 2016; Moquet et al., 2015; Stanley and Stout, 2014; Westphal et al., 2003). It would be necessary to have such flower resources available at the landscape scale, around the strips, to supplement AES strips resources. Such additional flower

resources should ensure the continuity of flower resources availability from early spring to autumn (Havens and Vitt, 2016). The foraging range of wild flower-foraging insects is reduced (from less to few hundred meters, Le Féon, 2010). Therefore, to improve their efficiency, strips should be set up near additional sources of flower resources, especially spring ones. Before implementing AES strips, it should be interesting to localise such flower sources in the landscape to set up AES strips near them. Such reflection on AES strips location should increase the continuity in floral resources availability and accessibility for flower-foraging insects all along the flying season.

D. Insect visiting flowers

Bird- and pollinator-strip AES hosted various flowers-foraging insects (153 morphotypes). We caught numerous flower-foraging insects morphotypes by pan-traps (104) that we did not record as flower-visitors (75). We observed many flower-visitor species (40) only once. The studied AES were attractive to some insects, mainly the most common generalist Hymenoptera and Syrphidae, but poorly attractive for numerous other species. We observed no species of European Red Data Book of bee species (Nieto et al., 2014). This observation confirms that current intensively managed farmlands exclusively host common species, even where specific biodiversity improvement schemes are set up (Kleijn et al., 2006).

As suggested in another study (Carman and Jenkins, 2016), additional resources provided by AES strips may not have been abundant enough to attract more individuals, or not diverse enough to match the specific needs of the insects caught by pan-traps but not recorded as insect-visitors.

VIII. CONCLUSION

Both studied AES provided pollen and nectar to many insect species, mainly common polylectic species, known as very efficient pollinators. Nevertheless, observed insect communities on AES strips were more impacted by their surrounding landscape than by the type of implemented AES strips. The more grassland and woodland in the surrounding (two-km buffer), the more diverse the insect communities on AES strips. Bird- and pollinator AES strips are not sufficient to compensate landscape anthropogenic perturbations, in their current configuration.

We observed numerous flower-feeding insect species on the strips that we never recorded as flower-visitors. Floral resources were mainly provided during summer and by sown and spontaneous Asteraceae species. It would be of particular interest to set up AES strips in the vicinity of early flowering areas such as woodland, hedges or spring mass-flowering crops, to create a functional resource continuity from early spring to autumn. We recommend adding high-quality pollen species in the sown mixes, such as some Brassicaceae or Fabaceae species. These additional species should increase the floral resources diversity and compensate the poor quality of Asteraceae

pollen. Moreover, as bird-strips provided fewer resources than pollinator-strips, bird-strip sowed mixes should be quantitatively improved, especially their refuge section. The addition of some perennial species such as *Centaurea jacea*, *Trifolium pratense* and *Medicago sativa* should be considered.

Whatever the differences among the type of AES strips, quantities of floral resources provided per square meter remain low. Therefore, the studied AES strips should be implemented on vast areas to provide a significant amount of resources at the landscape scale. AES location is also of particular importance to create functional floral resources continuity in time and space and to help to mitigate the decline of flower-feeding insects, related to landscape simplification.

IX. AUTHORS' CONTRIBUTIONS

PO and ALJ conceived the ideas and designed the methodology; PO collected and analysed the data; PO and ALJ led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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XI. CONFLICT OF INTEREST

The authors declare having no conflicts of interest.

XII. REFERENCES

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XIII. SUPPLEMENTAL MATERIAL

Table S2. Insect species caught by pan-traps in 2015 in the ten studied strips in Condroz and Hesbaye. N= 620. * indicate species also recorded as a flower visitor.

Order	Family	Genus	Species	Condroz	Hesbaye
Hymenoptera		Cheilosia	<i>Cheilosia longula</i> Zetterstedt	1	0
		Episyrphus	<i>Episyrphus balteatus</i> De Geer	20	2*
		Eristalinus	<i>Eristalinus sepulchralis</i> Linnaeus	3*	6*
		Eristalis	<i>Eristalis arbustorum</i> Linnaeus	8*	0*
		Eristalis	<i>Eristalis horticola</i> De Geer	3*	0*
		Eristalis	<i>Eristalis nemorum</i> Linnaeus	0*	1
		Eristalis	<i>Eristalis similis</i> Fallén	2	1*
		Eristalis	<i>Eristalis tenax</i> Linnaeus	21*	37*
		Eumerus	<i>Eumerus strigatus</i> Fallén	0*	2
		Eupeodes	<i>Eupeodes corollae</i> Fabricius	8*	4*
		Eupeodes	<i>Eupeodes luniger</i> Meigen	3	0
		Eupeodes	<i>Eupeodes nitens</i> Zetterstedt	2*	2
		Ferdinandea	<i>Ferdinandea cuprea</i> Scopoli	2	0
		Helophilus	<i>Helophilus pendulus</i> Linnaeus	1	0
		Helophilus	<i>Helophilus trivittatus</i> Fabricius	0*	1*
		Melanostoma	<i>Melanostoma mellinum</i> Linnaeus	12	5
		Orthonevra	<i>Orthonevra geniculata</i> Meigen	0	2
		Pipizella	<i>Pipizella viduata</i> Linnaeus	1	0
		Platycheirus	<i>Platycheirus ambiguus</i> Fallén	1	0
		Platycheirus	<i>Platycheirus manicatus</i> Meigen	2	0
		Platycheirus	<i>Platycheirus melanopsis</i> Loew	1	0
		Platycheirus	<i>Platycheirus scutatus</i> Meigen	0	2
		Rhingia	<i>Rhingia campestris</i> Meigen	15*	4*
		Riponnensia	<i>Riponnensia splendens</i> Meigen	0	1
		Sphaerophoria	<i>Sphaerophoria scripta</i> Linnaeus	15*	5*
		Sphaerophoria	<i>Sphaerophoria</i> spp.	5	5
		Syrirta	<i>Syrirta pipiens</i> Linnaeus	2*	0*
		Syrphus	<i>Syrphus vitripennis</i> Meigen	1	0
		Volucella	<i>Volucella bombylans</i> Linnaeus	1	0*
		Xylota	<i>Xylota segnis</i> Linnaeus	1	1
			Syrphidae	132	81
			Diptera Total	132	81
		Waiting for identification by taxonomists			
			Hymenoptera Total	191	174
Le	Lycaenidae	Heodes	<i>Lycaena phlaeas</i> Linnaeus	1	0

Order	Family	Genus	Species	Condroz	Hesbaye
Meco	Lycaenidae			1	0
	Noctuidae	Autographa	<i>Autographa gamma</i> Linnaeus	12	6*
		Callistege	<i>Callistege mi</i> Clerck	1	0
		Noctua	<i>Noctua pronuba</i> Linnaeus	1	0
		Xestia	<i>Xestia c-nigrum</i> Linnaeus	2	0
		-	Noctuidae undetermined	10	2
	Noctuidae			24	8
	Nymphalidae	Aglais	<i>Aglais urticae</i> Linnaeus	0*	3*
		Aglais	<i>Inachis io</i> Linnaeus	1	0
	Nymphalidae			1	3
	Pieridae	Colias	<i>Colias crocea</i> Geoffroy	0	1
		Pieris	<i>Pieris brassicae</i> Linnaeus	1	0*
		Pieris	<i>Pieris rapae</i> Linnaeus	0*	2*
	Pieridae			1	3
	Lepidoptera Total			27	14
Panorpidae	Panorpa	<i>Panorpa communis</i> Linnaeus	0	1	
Panorpidae			0	1	
Mecoptera Total			0	1	
n=				350	270

Table S3. Insect species recorded as visitors in 2014, 2015 and 2016 in the ten studied strips in Condroz and Hesbaye. N= 4089. * indicate species also pan-trapped in 2015.

Order	Family	Genus	Species	Condroz	Hesbaye
Coleoptera	Cantharidae	Rhagonycha	<i>Rhagonycha fulva</i> Scopoli	2	5
			Cantharidae	2	5
	Coccinellidae	Coccinella	<i>Coccinella septa-punctata</i> Linnaeus	4	2
			Coccinellidae	4	2
	Nitidulidae	Brassicogethes	<i>Brassicogethes</i> undetermined	2	3
			Nitidulidae	2	3
	Oedemeridae	Oedemera	<i>Oedemera nobilis</i> Scopoli	8	9
			Oedemeridae	8	9
			Coleoptera Total	16	19
	-	-	Diptera non Syrphidae indetermined	199	15
Diptera			Undetermined	199	15
	Anthomyiidae	-	Anthomyiidae undetermined	15	3
			Anthomyiidae	15	3
	Axymyiidae	-	Axymyiidae undetermined	9	0
			Axymyiidae	9	0
	Bibionidae	-	Bibionidae undetermined	2	0
			Bibionidae	2	0
	Bombyliidae	-	Bombyliidae undetermined	1	0
			Bombyliidae	1	0
	Calliphoridae	-	Calliphoridae undetermined	5	21
		Lucilia	<i>Lucilia caesar</i> Linnaeus	0	1
			Calliphoridae	5	22
	Ceratopogonidae	-	Ceratopogonidae undetermined	1	0
			Ceratopogonidae	1	0
	Clusiidae	-	Clusiidae undetermined	1	0
			Clusiidae	1	0
	Empididae	-	Empididae undetermined	96	55
			Empididae	96	55
	Faniidae	-	Faniidae undetermined	2	0
			Faniidae	2	0
	Megachilidae	-	Megachilidae undetermined.	0	1
			Megachilidae	0	1
	Muscidae	-	Muscidae undetermined	128	8
			Muscidae	128	8

Order	Family	Genus	Species	Condroz	Hesbaye
	Sarcophagidae	-	Sarcophagidae undetermined	12	2
			Sarcophagidae	12	2
	Scathophagi- dae	-	Scathophagidae undetermined	14	0
			Scathophagidae	14	0
Syrphidae		Cheilosia	<i>Cheilosia</i> undetermined	0	3
		Chrysogaster	<i>Chrysogaster hirtella</i> Loew	1	0
		Episyrphus	<i>Episyrphus balteatus</i> De Geer	0*	45*
		Eristalinus	<i>Eristalinus aeneus</i> Scopoli	2	22
		Eristalinus	<i>Eristalinus sepulchralis</i> Linnaeus	2*	16*
		Eristalis	<i>Eristalis arbustorum</i> Linnaeus	12*	28
		Eristalis	<i>Eristalis horticola</i> De Geer	2*	2
		Eristalis	<i>Eristalis intricarius</i> Linnaeus	0	1
		Eristalis	<i>Eristalis nemorum</i> Linnaeus	23	0*
		Eristalis	<i>Eristalis similis</i> Fallén	0*	1*
		Eristalis	<i>Eristalis tenax</i> Linnaeus	258*	108*
		Eumerus	<i>Eumerus strigatus</i> Fallén	1	0*
		Eupeodes	<i>Eupeodes corollae</i> Fabricius	2*	3*
		Eupeodes	<i>Eupeodes latifasciatus</i> Macquart	2	0
		Eupeodes	<i>Eupeodes nielsenii</i> Dušek & Láska	2	0
		Eupeodes	<i>Eupeodes nitens</i> Zetterstedt	3*	0*
		Helophilus	<i>Helophilus hybridus</i> Loew	1	0
		Helophilus	<i>Helophilus trivittatus</i> Fabricius	47	44*
		Lejogaster	<i>Lejogaster metallina</i> Fabricius	3	1
		Myathropa	<i>Myathropa florea</i> Linnaeus	1	0
		Platycheirus	<i>Platycheirus clypeatus</i> Miegen	1	0
		Platycheirus	<i>Platycheirus nielsenii</i> Vockeroth	1	0
		Platycheirus	<i>Platycheirus podagratus</i> Zetterstedt	0	1
		Platycheirus	<i>Platycheirus tarsalis</i> Schummel	1	0
		Psilota	<i>Psilota anthracina</i> Meigen	0	1
		Rhingia	<i>Rhingia campestris</i> Meigen	16*	5*
		Sphaerophoria	<i>Sphaerophoria rueppellii</i> Weidemann	5	1
		Sphaerophoria	<i>Sphaerophoria scripta</i> Linnaeus	29*	30*
		Sphaerophoria	<i>Sphaerophoria</i> spp.	3	3
		Syritta	<i>Syritta pipiens</i> Linnaeus	4*	3
		-	Syrphidae undetermined	16	16
		Syrphus	<i>Syrphus intricatus</i> De Vockeroth	0	1
		Syrphus	<i>Syrphus ribesii</i> Linnaeus	1	4

Order	Family	Genus	Species	Condroz	Hesbaye
		Volucella	<i>Volucella bombylans</i> Linnaeus	0*	1
			Syrphidae	441	340
	Tachinidae	-	Tachinidae undetermined	79	9
			Tachinidae	79	9
	Trichoceridae	-	Trichoceridae undetermined	4	0
			Trichoceridae	4	0
	Xenasteiidae	-	Xenasteiidae undetermined	1	0
			Xenasteiidae	1	0
	Xiphydriidae	-	Xiphydriidae undetermined.	5	0
			Xiphydriidae	5	0
			Diptera Total	1013	455
Hemiptera-	-		Hemiptera undetermined	4	0
			Hemiptera Total	4	0
	-	-	Hymenoptera undetermined	18	1
			Undetermined	18	1
	Andrenidae	Andrena	<i>Andrena flavipes</i> Panzer	22*	3*
		Andrena	<i>Andrena</i> undetermined	0	1
			Andrenidae	22	2
		-	<i>Apidae undetermined</i>	4	0
		Apis	<i>Apis mellifera</i> Linnaeus	417*	237*
		Bombus	<i>Bombus hypnorum</i> OTU	1	0
		Bombus	<i>Bombus lapidarius</i> OTU	998	129
		Bombus	<i>Bombus pascuorum</i> OTU	48	5
		Bombus	<i>Bombus pratorum</i> OTU	99	1
		Bombus	<i>Bombus</i> undetermined	2	0
		Bombus	<i>Bombus terrestris</i> OTU	183	40
			Apidae	1752	412
	Colletidae	Hylaeus	<i>Hylaeus anularis</i> Kirby	0	1*
			Colletidae	0	1
	Formicidae	-	Formicidae undetermined	2	0
			Formicidae	2	0
		Halictus	<i>Halictus rubicundus</i> Christ	3*	2
		Lasioglossum	<i>Lasioglossum calceatum</i> Scopoli	11*	0
		Lasioglossum	<i>Lasioglossum leucozonium</i> Schrank	4*	0*
		Lasioglossum	<i>Lasioglossum pauxillum</i> Schenck	1*	0
		Lasioglossum	<i>Seladonia tumulorum</i> Linnaeus	12*	0
			Halictidae	31	4
	-	-	Hymenoptera parasitoid undetermined	65	0
			Hymenoptera parasitoïde	65	0

Order	Family	Genus	Species	Condroz	Hesbaye
Lepidoptera	Megachilidae	Megachile	<i>Megachile centuncularis</i> Linnaeus	0	1
			Megachilidae	0	1
	Philanthidae	Cercerinae	Cercerinae undetermined	0	2
			Philanthidae	0	2
	Tenthredinidae	Tenthredinidae	Tenthredinidae undetermined	1	0
		Tenthredinidae	<i>Tenthredo notha</i> Klug	1	0
			Tenthredinidae	2	0
	Vespidae	Vespula	<i>Vespula vulgaris</i> Linnaeus	2	0
			Vespidae	2	0
	Hymenoptera Total			1893	423
	-	-	Lepidoptera undetermined	10	0
			Lepidoptera undetermined	10	0
	Hesperiidae	Ochlodes	<i>Ochlodes venatus</i> Bremer & Grey	1	0
		Thymelicus	<i>Thymelicus lineola</i> Ochsenheimer	1	0
			Hesperiidae	2	0
	Lycaenidae	Lycaena	<i>Lycaena virgaureae</i> Linnaeus	1	0
			Lycaenidae	1	0
	Noctuidae	Autographa	<i>Autographa gamma</i> Linnaeus	0*	1*
		Euclidia	<i>Euclidia glyphica</i> Linnaeus	1	0
			Noctuidae	1	1
	Nymphalidae	Aglais	<i>Aglais urticae</i> Linnaeus	152	4*
		Aphantopus	<i>Aphantopus hyperantus</i> Linnaeus	7	0
		-	Nymphalidae undetermined	1	0
		Melanargia	<i>Melanargia galathea</i> Linnaeus	1	0
		Pyronia	<i>Pyronia tithonus</i> Linnaeus	5	0
		Vanessa	<i>Vanessa atalanta</i> Linnaeus	0	1
		Vanessa	<i>Vanessa cardui</i> Linnaeus	0	1
			Nymphalidae	166	6
	Pieridae	Artogeia	<i>Artogeia mannii</i> Mayer	0	1
		-	Pieridae undetermined	0	4
		Pieris	<i>Pieris brassicae</i> Linnaeus	0*	3
		Pieris	<i>Pieris napi</i> Linnaeus	0	4
		Pieris	<i>Pieris rapae</i> Linnaeus	47	10*
			Pieridae	47	22
	Satyridae	Maniola	<i>Maniola jurtina</i> Linnaeus	1	0
			Satyridae	1	0
	Zygaenidae	Zygaena	<i>Zygaena filipendulae</i> Linnaeus	6	0
			Zygaenidae	6	0
	Lepidoptera Total			234	29

Order	Family	Genus	Species	Condraz	Hesbaye
Neuroptera	Chrysopidae	-	Chrysopidae undetermined	1	0
			Chrysopidae	1	0
			Neuroptera Total	1	0
n=				3162	927

Discussion

I. OILSEED RAPE POLLINATION AND RELATIONSHIPS WITH POLLINATING INSECTS

Although oilseed rape is a crop often recorded as entomophilous and very attractive for flower-feeding insects, we recorded few flowers (1.1%) visited by insects in fields, but a low pollination deficit. During the last 20 years, oilseed rape breeding selection was mainly based-on seed yield improvements that could potentially reduce the insect dependency of the crop. Nevertheless, this honey crop remains more attractive for honey bees than for wild insects.

During our observations, we recorded only *Apis mellifera* visiting oilseed rape flowers (1.1% of the flowers). We observed no wild insects on *Brassica napus* flowers. The small number of visited flower (around 275/100m²/h) was within the range of visitation rate in previous oilseed rape fields observations in intensively farmed areas without honeybee hives nearby (from 117 to 1368 visits/100m²/h, Bartomeus et al., 2015, 2014; Blažytė-Čereškienė et al., 2010; Morandin and Winston, 2005; Stanley et al., 2013). In different contexts, as in Ireland where grassland is dominant (61% of land cover, Central Statistics Office, 2016; Stanley et al., 2013), or in small experimental plots (<100m²) as in Pakistan or France (Ali et al., 2011; Brunel et al., 1994), oilseed-rape-flower insect visitors were more diverse (up to 17 pollinator species). In such context, flower visitation rates on oilseed rape flowers were higher (up to 1000 times). In these cases and when many honeybee hives are installed close to the fields (Durán et al., 2010; Lindström et al., 2016b), insect pollination could impact the yield.

The 12,200 ha of Belgian oilseed rape fields (in 2014, FAOSTAT, 2016), produced around 280 billions flowers a day. The 32,200 honeybee hives registered in the country (FAOSTAT, 2016), have the potential to visit ca 10 billion flowers a day (van der Steen, 2015). Thus, we were not surprised to observe a low flower visit rate by *A. mellifera*, explained by the dilution effect over *B. napus* fields (Holzschuh et al., 2011). This dilution effect increased in the last fifteen years as in Belgium the oilseed rape cropped area increased from 4,800 ha to 12,200 from 2000 to 2014 and the honeybee hives increased only from 29,000 to 32,200 during the same period (FAOSTAT, 2016). Nevertheless, we were surprised to observe no other insect species visiting oilseed rape flowers. The insect pollination service was thus only provided by *A. mellifera* but with a low intensity in oilseed rape fields.

We recorded an excellent self-fertility indices (SFI) of modern *Brassica napus* cultivars (SFI_{fruit} = 0.7 and SFI_{seed} = 0.8), higher than self-fertility of cultivars cropped 40 years ago (Williams, 1978, SFI_{fruit} = 0.7 and SFI_{seed} = 0.5). Moreover, despite the low number of visited flowers recorded, we observed little differences between open-pollinated and hand-supplied pollinated plants (= pollen limitation, PL) on fruits (PL_{fruit} = 0.1) and seeds (PL_{seed} = 0.2). The comparison with the literature is difficult as most of the pollen limitation measures were conducted under greenhouse conditions (PL_{fruit} = 0.3 and PL_{seed} = 0.5, Williams, 1978; Williams et al., 1986) or between plants under

mesh cages without insects and under cages with numerous insects. Pollen limitation on fruits and seeds could thus be high (up to $PL_{\text{fruit}} = 0.8$ and $PL_{\text{seed}} = 0.9$, Jauker et al., 2012). While these observations assess the need for pollen vector (including the wind), our observation assesses the pollen limitation in open field condition. The low pollination deficit we observed is the expression of a low need of additional pollination vector under field condition in Belgium, despite the low proportion of flowers visited by insects. Thus, the studied hybrid cultivars seem adapted to a reduced provision of pollination services by insects. Such result is in opposition to the classical recommendations of adding two to four beehives per ha to reach high oilseed rape yields but no data established the maximum floral visitation required (McGregor, 2009). As found by Gallai et al. (2009) these recommendations are largely based on personal communication and observations pre-70's. But during the last 50 years, oilseed rape cultivars evolved considerably (Friedt and Snowdon, 2009).

The first notable breeding evolution of oilseed rape happened in the late 80's with the creation of high oil quality cultivars, the "0" (simple zero) cultivar group (erucic-acid-free) and the "00" (double zero) cultivar group (erucic-acid-free and low glucosinolate content). The second main evolution was in the 90's when breeders had been interested in the hybrids to increase yields (Diepenbrock, 2000; Westcott and Nelson, 2001). Firstly, hybrid cultivars were male sterile and therefore cultivated in association with one or two lines of non-hybrid cultivars. These were the CHL (Composite Hybrid-Line(s)) or CHH (Composite Hybrid-Hybrid) groups. Then the first restored (full fertile) hybrid cultivars were created in the early 2000's (Pierre and Renard, 2010; Pinochet and Bertrand, 2000). Based on these new high-quality restored hybrid cultivars, breeders could focus their selection on yields criteria. Selection of new cultivars was from then on based on their seed yield productivity, including their resistance to environmental conditions such as water stress or nitrogen availability. In addition to the seed yield, the classical selection criteria are based among other on high morphological criteria as primary branch numbers, fruit number per plant or length of main raceme (Lu et al., 2011). These criteria correspond to those implied in compensation mechanisms occurring in a context of low pollination service. A low provision of pollination service can induce more numerous and longer branches per plants, more abundant flowers and longer flowering period conducting to a similar seed yield as plants with fewer flowers but more pollinated (Mesquida et al., 1988). Such compensation mechanisms can prevent detection of statistically significant yield variations linked to pollination services (Mesquida et al., 1988). Thus, the current European breeding programs select plants potentially more adapted to a low insect pollination services provision. That could be of particular interest in areas as in Belgium, where a deficit of insect pollination services was identified (Schulp et al., 2014). The presence of predisposition to effective compensation mechanisms in the studied cultivars could explain our low pollen limitation observations. Moreover, we worked on a plant section only, and yet compensatory mechanisms could be more effective at the whole plant level (Williams and Free, 1979). Thus, we probably

under-estimated the compensation capacities, and the pollination deficit at the full plant level is perhaps even lower than calculated.

In Belgium, the combination of very high yields (4.0 tons/ha, that is to say, two times the mean yield worldwide, FAOSTAT, 2016; FEDIOL, 2016) and very low insect pollination, confirms the hypothesis of an adaptation of the new cultivars to a low pollinator environment. Therefore, the oilseed rape pollinator-dependence ratio of 0.25 provided by the FAO (Gallai, 2008), while relevant for worldwide global estimations, is not representative of the Belgian conditions. This pollinator-dependence ratio is even probably no longer accurate for most of the recent European cultivars. At least for oilseed rape, such global pollinator-dependence ratio is to consider with circumspection (Melathopoulos et al., 2015), especially for recent cultivars and should be periodically assessed at the local scale.

We recorded production of 14.5 L nectar /ha/d by the studied cultivars. This production corresponds to 10-kg honey-equivalent per day per hectare. Over flowering period (40 days), the studied fields produced 400 kg honey-equivalent /ha/y. This potential production corresponds to the classical estimation for this crop, from 100 to 500 kg/ha/y (Janssens et al., 2006; Langridge and Goodman, 1975; Williams, 1980). So we confirm the high honey potential of this crop, widely used by beekeepers to produce abundant spring honey (Janssens et al., 2006; Kirk and Howes, 2012), even in Condroz. Thus, the scarcity of bees over oilseed rape fields should result from the combination of a low number of honeybee colonies compared to the cropped oilseed rape area, and a dilution effect of bee population over fields (Holzschuh et al., 2011). Despite the fact that we caught more wild bee species than *Apis mellifera* by pan traps in oilseed rape fields, only *Apis mellifera* was observed on flowers. As suggested by Cane et al. (2000), pan traps can be more attractive for non-foraging species. Especially our blue and white pan traps were of high contrast in the yellow oilseed rape fields. The number of pan trapped insects is inversely related to surrounding attractive flowers abundance, due to a contrast effect (Roulston et al., 2007). Insects the less attracted by oilseed rape were probably the most attracted by our pan traps. Thus, oilseed rape was highly attractive for *Apis mellifera* but not attractive for other species that only fly over the fields.

In Belgium, oilseed rape was the only main crop that could have the benefit of insect pollination. But we demonstrated that the Belgian oilseed rape production is not, or very poorly, dependent on insect pollination. Thus, none of the main Belgian crops is pollinator dependent. So, except for some local specific crops (i.e. strawberries, orchards), main Belgian crops have no direct need of insect pollination services. Nevertheless, oilseed rape provides huge spring flower resources for honeybees.

II. AGRI-ENVIRONMENT SCHEMES FOR POLLINATORS

Despite the low need of insect pollination service of Belgian agriculture, pollinating insects are far to be useless. These insects play a key role in biodiversity conservation and ecosystem functioning (Allen-Wardell et al.,

1998). They provide pollination services to numerous wild plants (Ollerton et al., 2011) and can also provide other ecosystem services as pest regulation and support of cultural values (Potts et al., 2016). Nevertheless, most of the pollinating insect species are negatively affected by farming intensification (Klein et al., 2007; Stanley and Stout, 2013). Even if not enough data exist to precisely quantify the decline of wild pollinator, pieces of evidence are here, and this decline is established and alarming (Gallai et al., 2009; Ollerton, 2017; Potts et al., 2010). Pollinator cause was recently added in several policy programs that included actions to mitigate pollinator decline or implemented plans to restore their habitats (Allen-Wardell et al., 1998; Defra, 2014; Delgoulet et al., 2017; Dicks et al., 2013). But evaluation and improvement of these measures are still to be provided.

In Belgian farmland, since 2005, the CAP has subsidised the establishment of ca 1300 km of managed-strips AES (Walot, 2016), including pollinator and bird flower-strips, likely to provide shelter and flower resources for insects. We studied these two types of flower-strip AES in Belgium. In both pollinator- and bird-strips we observed 13 sown and 65 spontaneous entomophilous plant species that provided summer flower-resources (pollen and nectar) to common pollinating insects. Despite this plant diversity, the flower resources were mainly provided by few species. *Centaurea jacea* and *Leucanthemum vulgare* provided nearly 70% of the pollen, and *Centaurea jacea*, *Cirsium vulgare* and *Cirsium arvense* provided nearly 60% of the nectar sugar. We observed insect visits on 42 plant species, but the three most visited species (*Centaurea jacea*, *Cirsium arvense* and *Lotus corniculatus*) represented more than 63% of the visited flower units. We mostly observed Hymenoptera (59%) and Diptera (34%) visiting flowers, as in hay-meadows (Forup and Memmott, 2005). Despite the 91 types of insects we observed visiting flower units, only three insect taxa (*Apis mellifera*, *Eristalis tenax* and *Bombus lapidarius* OTU) represented more than 53% of the flower visitors observed.

A. AES plant diversity, the complementarity of sown and spontaneous species

Plant communities we observed on AES strips were a mix of sown and un-sown spontaneous plant species. On pollinator-strip AES, which are flower-meadow-like strips, sown species provided 70.5% of the flower units, provided most of flower resources (94% of the pollen and 86% of the nectar sugar) and accounted for 86.4% of the flower units visited. Un-sown species were so less attractive than the sown ones even mainly (93.8%) from the same families. Nevertheless, flower resources were mainly provided by one plant family, Asteraceae. Asteraceae provided $88 \pm 7\%$ and 0% of the pollen produced by sown and spontaneous species respectively and $72 \pm 11\%$ and $61 \pm 29\%$ of nectar sugar produced by sown and spontaneous species respectively. At the opposite, in bird-strips, with a part of annually sown species, spontaneous species provided most of flower resources (62% of the pollen and 81% of the nectar sugar) and were the most visited by insects (76.9% of the visited flower units). Flower resources were provided mainly by both Asteraceae (all spontaneous, $38 \pm 31\%$ of the pollen and $52 \pm 28\%$ of the nectar sugar) and

Brassicaceae species (mostly sown, $50 \pm 37\%$ of the pollen and $32 \pm 32\%$ of the nectar sugar). Asteraceae species are known to provide abundant nectar (Baude et al., 2016; Hicks et al., 2016), but their pollen is often presented as a low-quality pollen for insects, especially for some bee species, with several essential amino acids missing (Forcone et al., 2011; Hanley et al., 2008; Vanderplanck et al., 2016). Thus, larvae cannot be fed only with Asteraceae pollen and imagoes have to collect pollens with high-quality pollens from other plant families (Di Pasquale et al., 2013; Eckhardt et al., 2014). Inversely, the Brassicaceae species provide high-quality pollen for insects (high nitrogen content and high digestibility, Eckhardt et al., 2014). So, even if they were not conceived with this objective, the bird-strip mix presented a flower-resource functional (nutritive) complementarity for insect feeding between sown and spontaneous plant species and a higher proportion of high-quality pollen than the pollinator-strip mix.

Two sown species, *Centaurea jacea* and *Lotus corniculatus*, present in pollinator flower-strips, are very commonly used in flower seed mixes for insects (Batáry et al., 2010; Bokenstrand et al., 2004; Haaland et al., 2011; Scheper, 2015). As in our records, these species were widely visited by insects. *Centaurea jacea* is often recorded as visited by abundant and diverse insects and *Lotus corniculatus* as mainly visited by honeybees and *Bombus* (Haaland et al., 2011; Hennig and Ghazoul, 2011; Korpela et al., 2013; Noordijk et al., 2009; Steffan-Dewenter and Tscharncke, 2000). Nevertheless, and as presented in previous studies (Bretagnolle and Gaba, 2015; Carvalheiro et al., 2011; Marshall et al., 2003), spontaneous species can also be very attractive to wild insects and provide them lots of resources (Carvell et al., 2007; Haaland et al., 2011). In the strips we observed, un-sown *Trifolium repens* that produced only 1% of the pollen available on strips, was highly visited and accounted for 25% of the collected pollen by *Bombus*. Most of the Fabaceae species as *Trifolium repens*, are known to produce high-quality pollen (without essential-amino-acids deficit) (Keller et al., 2005a; Pernal and Currie, 2001), and to be widely used by *Bombus* (Goulson et al., 2005; Kleijn and Raemakers, 2008). The low density of good-quality pollen providers could be a limiting factor to insect development (Kallioniemi et al., 2017; Keller et al., 2005b). High pollen quality plants, as Brassicaceae or Fabaceae species should be favoured in AES flower-strip mixes to increase the global nutritional value of pollen provided by such schemes (Carvell et al., 2007). Thus, pollinator-strip flower mix could be improved reducing the imbalance between Asteraceae and other flowering plant families.

Despite their contribution to the strip attractiveness, some of the spontaneous species, like *C. arvense*, are under legal restrictions and have to be destroyed (Pauly and Coppée, 2017; Vray et al., 2017). Nevertheless, in our strips, *Cirsium arvense* provided up to 20% of the pollen and 42% of the nectar per strip and accounting for 54% of the flower visits we observed on bird-strips where this species was present. The high attractiveness of this species for numerous insects was recorded in previous studies (Carvell et al., 2007, 2004; Haaland et al., 2011). Thus, to keep in bird-strips the numerous insects attracted by *Cirsium* species, sown plant mixes should be improved to provide

alternative resources to insects in strips where spontaneous *Cirsium* species would be removed.

We demonstrated that combining sown and spontaneous species can increase the nutritional interest of a flower mix for insects and provide some new avenues for improvement. Nevertheless, bird-strips, where spontaneous species were the most abundant, presented high variability of flower-resources quantities among sites and years and therefore a fluctuating capacity to feed insects. But flower resources constancy between years is important to provide reliable support to insect density and diversity (Ebeling et al., 2008; Roulston and Goodell, 2011). Thus, the perennial plant mix of pollinator-strips offered a higher constancy in flower resources provision among sites and years than the plant mix of bird-strips based on annual and spontaneous species. The constancy of flower resources provision from year to years is well adapted to the resource continuity required by insects (Rundlöf et al., 2014; Schellhorn et al., 2015). Perennial strips seem more adapted to provide stable level of flower resources among years.

Pollinator-strip and bird-strip entomophilous plant diversity were 25 ± 10 and 26 ± 7 species per strip respectively. This diversity was high compared to several English AES strips, even some sown with 'pollen and nectar' or 'wildflower' mixes, of which the maximal mean entomophilous plant diversity was 15.8 species per site (Pywell et al., 2006). The observed diversity was nevertheless close to the diversity in perennial English hay-meadows from 10 to >100 years-old (24 ± 5 entomophilous species, Forup and Memmott, 2005) or other perennial flower-strips (Bokenstrand et al., 2004; Campbell et al., 2017; Carvell et al., 2006; Engels et al., 1994; Haaland et al., 2011; Hicks et al., 2016). Even if, in these studies, sown flower-strip mixes can be more complex, containing from 20 to 52 species, only some of the sown species remain in the strips after several years of settlement (Bokenstrand et al., 2004, 32 sown species but an entomophilous plant species diversity of 22 ± 7 per strip after nine years). Thus, the number of species of the seed mixes, the strip management and the spontaneous species (from the seed bank of the soil or wind dispersion), seem adapted to obtain plant communities comparable to hay-meadows, known to be among the most-species-rich plant communities in farmland (Forup and Memmott, 2005). Therefore, the number of species in seed mixes should be retained. The addition of Fabaceae or Brassicaceae species or an alternative species to *Cirsium* species should added in the seed mixes instead of other species of the mixes.

B. Pollen and nectar, the limiting resource is not so obvious

On bird- and pollinator-strips, the dominant plant families were not the same. The flower unit densities and the amount of pollen and nectar provided were also different between the two types of flower-strips. Pollinator-strips provided more flower units than bird-strips (68 ± 25 , vs 35 ± 37 flower units/m²), more pollen (31 ± 52 vs 9 ± 13 µl/d/m²) but the same amount of nectar sugar (7 ± 3 vs 7 ± 5 mg/d/m²). The quantities of flower resources provided by flower-strips seem sufficient for the observed insects in summer (*Bombus* collected only

3% of pollen outside of the strips). But the flower resources spreading among seasons could be more problematic, especially because of a deep spring gap. Flower units were very scattered in strips until June and provided low amount of resources for early emerging insects. Thus, early emerging insect species lack flower resources to feed on and produce eggs (O'Rourke et al., 2014).

Flower resources availability (quantity and temporal distribution) is the main regulator of bee populations (Roulston and Goodell, 2011). Bee population on flower-strips is substantially linked to their ability to provide nectar and pollen (Knight et al., 2009). The population of other insects observed, as Diptera, are less sensitive to flower resources availability as their larvae are not fed with pollen and nectar (Biesmeijer et al., 2006). We therefore focusing on bee needs in the discussion about flower resource provision.

In the studied strips, we observed only polylectic bee species dominated by few very common species (*Apis mellifera*, *Bombus lapidarius* OTU). Such predominance of few widespread species is a recurrent observation on intensively managed farmland and AES in such landscape (Bommarco et al., 2012; De Palma et al., 2015; Kovács-Hostyánszki et al., 2016). Even if the observed strips were at least 3 km from apiaries, the high number of honeybees observed (15% of visitors, 2.5 bees/100m²/25min), reveals the attractiveness of the flower-strips at the landscape scale. Honeybees are sometimes described as very competitive for nectar resources and in position to seriously challenge to small wild species (Torné-Noguera et al., 2016). We observed *Apis mellifera* visiting 24 plant species and non-Apidae wild bees visited 12 plant species, including four species not visited by *Apis mellifera*. Moreover, we observed honeybees mainly on three plant species (*Centaurea jacea* (23 flower units/100m²), *Cirsium arvense* (6 flower units/100m²) and *Lotus corniculatus* (12 flower units/100m²)). But, except on *Lotus corniculatus* on which honeybees accounted for 80% of visitors, honeybees were not the main visitor on the two other species (achieving 8% of the flower visits). Thus, except on *Lotus corniculatus*, competition should be reduced among honeybees and other species for flower resources, that join observations of other studies (Butz Huryn, 1997; Roubik and Wolda, 2001; Steffan-Dewenter and Tschamntke, 2000). Therefore, the presence of honeybees on the flower-strips may not decrease their capacities to provide flower resources to wild species.

The energetic needs of flower-feeding insects are fueled almost exclusively by nectar (McCallum et al., 2013). One ha of bird-strip AES produced 60 ± 130 g sugar/ha/d over the year (April to September) and 234 ± 257 g sugar/ha/d at peak production (July). A pollinator-strip produced 64 ± 96 g sugar/ha/d over the year but 213 ± 132 g sugar/ha/d at peak production (July). This sugar production is low compared to other flower-strips (from 100 to 600 g sugar/ha/d in Hicks et al., 2016). Extrapolated to the full year (183 days from April to September), flowered strip produced ca 11 kg nectar sugar/ha/year. This production is low compared to mono-species patches of wild species (Baude et al. (2016), 261 species recorded with production ranging from 0 to 4733 kg nectar sugar/ha/year, with a flowering period from 29 to 364 days). This difference is due to the presence of Poaceae in the flower-strips (in the

seed mix or as a dedicated section) that reduces the part of the flower-strip dedicated to flower resource production (no Poaceae in Hicks et al., 2016). But Poaceae species, as in any meadow-like area, provide other services including shelter for insects and soil cover all long the year (Tscharntke and Greiler, 1995). Moreover, across the landscape, linear herbaceous elements were previously recorded as having a beneficial effect on pollinator populations (Lof and van der Werf, 2017).

To appreciate the meaning of these sugar quantities in term of insect support, it would be interesting to estimate the number of bees that could be feed or produced with such amount of sugar. We find very poor data to estimate the sugar consumption by wild species, but many data are available for *Apis mellifera*. A mean of 59 mg sugar are needed to produce one honeybee worker (Rortais et al., 2005). Then it consumes 11 mg of sugar a day (Huang, 2010), during ca 33 days (Omholt and Amdam, 2004). So, 422 mg of sugar are needed by a bee. One ha of AES flower-strip provide enough sugar to produce and feed ca 26 000 bees-equivalent insects a year. This potential production of bees-equivalent per year is two to 11 times lower than strips studied by Hicks et al. (2016).

Nectar provides mainly carbohydrates, but insects also need other nutrients, including proteins, lipids and micronutrients, mainly provided by pollen. The pollen consumption is especially important for female bees for eggs production and larvae for their development (Woodard and Jha, 2017). Pollen limitation during larvae stages may have persistent adverse effects on adult bee physiology and performances (Bukovinszky et al., 2017; Wang et al., 2016). Using published estimates of the pollen quantities required for individual bee development (Dicks et al., 2015; Fewell and Winston, 1992; Müller et al., 2006), we can estimate the number of bees that can be produced per hectare of flower-strip AES.

Based on a pollen density of 1.2 kg/L (Harrington and Metzger, 1963; Sosnoskie et al., 2009), a bird-strip AES produces 109 ± 157 g pollen/ha/d over the year (April to September) and 287 ± 179 g pollen/ha/d at peak production (July). A pollinator-strip produces 366 ± 629 g pollen/ha/d over the year and 1248 ± 915 g pollen/ha/d at peak production (July). The peak pollen production of the pollinator-strips is 4.5 times lower than the production of pure-flower-strips (without Poaceae, Hicks et al., 2016). With the same calculation as for nectar, 183 days from April to September, the bird-strips produced ca 20 kg pollen/ha/y and pollinator-strips ca 67 kg pollen/ha/y.

For wild bees, based on the pollen volumes per brood cell of different species (Müller et al., 2006), and using the same pollen density as above, the mean pollen quantity needed to produce one bee is 43.4 mg. Thus, one ha of flower-strip could provide the pollen resources to produce 460,000 bees per year on bird-strips and 1,540,000 bees per year on pollinator-strips. The production of large social bees (*Apis mellifera*) require more pollen, around 156 mg pollen to produce one worker (Hrassnigg et al., 2005), but even with such large needs, the flower-strip pollen production allows a large production of bees each year (128,000 and 429,000 respectively). This potential for bee

individuals production is lower than other flower-strips that provided pollen to produce from 1 to 6.5 million bees/ha/y (Hicks et al., 2016).

The pollen resources are sufficient to produce five and 16 time more honeybee equivalent insect than nectar resources on bird- and pollinator-strips respectively. This imbalance is lower than on the flower-strip studied by Hicks et al. (2016) with imbalances from 15 to 24. But all the pollen produced cannot be collected by bees (at least some pollen is needed for pollination) and bees can present flower preferences and constancy that reduces the quantity of suitable pollen available (Filipiak et al., 2017; Vaudo et al., 2015). Thus, these potential numbers of bees produced from strip pollen are probably overestimated, so imbalances between nectar and pollen resources are probably lower. Moreover, these estimations are based on pollen diet containing nearly no Asteraceae species (Müller et al., 2006). But, even if we removed the Asteraceae pollen (up to 87% of the pollen available on strips), nectar seems remaining the limiting factor to host bees on flower-strip AES. The provision of pollen in sufficient quantity by strip flowers seems confirmed by the low collection of pollen from outside of the strips by *Bombus* (only 3% of the collected pollen). So, to increase the bee production potential of flower-strips, the nectar production quantity should be increased more than the pollen production.

Global flower resources production is important, but insects need food at precise periods, and provision gaps can be deleterious to populations (Roulston and Goodell, 2011; Woodard and Jha, 2017). The continuity of resource provision is yet crucial for flower-feeding insect communities, especially the wild ones (Aldridge et al., 2011; Schellhorn et al., 2015; Wratten et al., 2012). On bird-strips, we observed lower insect densities than on pollinator-strips. Compared to pollinator-strips, on bird-strips, the total pollen production was lower but still not limiting compared to nectar production. Moreover nectar was of better nutritional quality for insects. Bird-strips presented the weakest spring pollen production. This spring gap of flower-resources in April and May (no or very weak resources available on strips) was one of the main weakness of the studied AES strips that could limit the number of species they hosted. Even missed by studies starting observation in June (Campbell et al., 2017; Haaland and Bersier, 2010; Haenke et al., 2009), similar spring gaps and absence of bee visitor in spring was already observed in flower-strips (Carreck and Williams, 1997). Nevertheless, various insect species emerge in early spring from mid-April to May (Benton, 2006; Falk, 2015), as most of the Halictidae and *Bombus* species we observed. For all these flower-feeding insect species, early spring flower-resources are essential (Aldridge et al., 2011; Havens and Vitt, 2016; O'Rourke et al., 2014; Schellhorn et al., 2015; Wratten et al., 2012). Especially for the social insects as *Bombus* species, the spring resources condition the establishment of colonies by the new founders and the size of colonies during all the season. Continuity in population from years to years is also affected by low spring resources as queens produced by low strength colonies are less successful getting through the winter and found new colonies the following spring (Williams et al., 2012). The continuity in flower-resources provision from early

spring to autumn is a cornerstone of the pollinator support (Moquet et al., 2017; Schellhorn et al., 2015). Thus, incoming AES strips improvement should focus on the pollen production and its repartition among seasons. Flower mixes revisions should be implemented to elongate the flower-resources provision period, especially in early spring. Two options could be to consider.

First, 100% of pollen and nectar flower-resources early in spring (April) were provided by un-sown spontaneous species, particularly *Taraxacum* agg. (95 and 62% of pollen in April and May, 86 and 40% of nectar in April and May) despite their very low density (0.03 flower unit /m² in April, 0.6 in May). Even if we observed no insect visiting these species in April (flower unit density was probably too low), *Taraxacum* agg. is highly attractive for a broad diversity of insects and provide abundant nectar (Hicks et al., 2016; Kirk and Howes, 2012; Knuth, 1908; O'Rourke et al., 2014). The strip management with perennial grass section, and especially the pollinator-strip with a section mown twice a year could nevertheless be favourable to the development of this native species aggregate (Mølgaard, 1977). But these species did not spontaneously colonise the strips. As *Taraxacum* agg. are mostly meadow species (Mamarot, 2002; Rameau et al., 2012), the seed bank of intensively cropped fields and the surrounding environment probably contains too few seeds to allow their spontaneous presence at higher density in the strips. Increasing the density of *Taraxacum* agg. could be relevant, but seed production is currently technically not affordable (Pascal Colomb, ECOSEM, personal communication 2014). Nevertheless, other native species, not present on the strips, e.g. *Ranunculus* or *Lamium* species, both early flowering and attractive for insects (Baude et al., 2016; Kirk and Howes, 2012; Knuth, 1909), could be of interest to provide spring pollen to complete the nectar production of *T. agg.*. Such species could probably favourably be sown instead of poorly visited species like *Medicago lupulina* or *Achillea millefolium*.

The second option could be to use the AES strips as a measure to provide flower-resources complementary to spring flower-resources provided by other landscape elements. In Belgium, most of the early spring flower-resources for insects are provided by tree species such as *Acer*, *Prunus*, *Salix* or even *Quercus* species (Aupinel et al., 2001; Moquet et al., 2015; Somme et al., 2016). Such early flowering woody species are usually used in hedges (Pywell et al., 2011; Watson et al., 2011) or as isolated tree along fields (as the traditional 'tadpole' willows in Wallonia). Moreover, hedges, are usually pesticide-free (Osborne et al., 1991) and offer additional safety resources to insects, as nesting sites for Hymenoptera or host plants for Lepidoptera and Diptera larvae. So, promoting tree planting or re-creation of hedges in intensively farmed landscapes could be one of the most useful action to complete flower-strips AES. Thus, AES strips should be combined to other AES promoting trees or be set up close to already existing semi-natural elements like hedges or wooded areas providing early flower-resources (Eppink et al., 2012). Such AES combination is possible at the farm scale in Wallonia with the 'Agri-Environmental action plan" AES (MC10, Natagriwal, 2014). This AES involve an environmental diagnostic of the farm to improve the pertinence and the complementarity of possible AES combination. This

MC10 is the first step to combine several AES. Nevertheless, management at the landscape scale, including several actors, could be more pertinent than at the farm scale.

C. Quantity or diversity, it is not necessary to choose

The diversity of flower-resources available at a given time is not necessarily a clue to predict the insect diversity. The interaction networks highlighted the fact that most of the flower-insect interactions occurred among few species. The two most visited plant species (*Centaurea jacea* and *Cirsium arvense*, out of 41 visited species) received more than 50% of the insect visits and attracted the higher number of insect types (45 and 43 respectively out of 91). The two most frequently observed insect types, *Bombus lapidarius* OTU and *Apis mellifera*, realised 43% of the visits. We observed 68% of *B. lapidarius* OTU foraging on *Centaurea jacea*, and 60% of *Apis mellifera* foraging on *Lotus corniculatus*. The presence of one or two dominant plant species per *Bombus* OTU pollen diet (*Trifolium repens* in 74% of the pollen loads) confirmed this high fidelity.

The polylectic bee species we observed can forage on a broad diversity of flowers (Kirk and Howes, 2012). Nevertheless, some species, especially the social bees as *Bombus*, can forage on a small subset of the available plant species. As in Carvell et al. (2004) and Fussell and Corbet (1992) we recorded the majority of *Bombus* (54%) visiting a single species (*Centaurea jacea* in our case). These bees preferentially visit the species they expect to provide the higher density of suitable resources locally. They develop plant species fidelity (Grant, 1950; Spiesman et al., 2017) to increase their efficiency to collect nectar and pollen. This foraging strategy fits the theory of optimal foraging (Hodges, 1981; Pyke, 1980). The dominance of few plant species could thus be beneficial to these bee species, which could more efficiently collect flower-resources (no need to find a new area to forage (Goulson, 1999) or switch from flower type and learn how to exploit it). Collecting flower resources more efficiently allows worker bees to bring more resources to the nest and so to increase the food available for larvae. This increases the number and the size of new individuals (Spaethe and Weidenmuller, 2002; Sutcliffe and Plowright, 1988), reinforcing the colony strength. Nevertheless, even for such species, plant diversity could be beneficial as flowering periods could be combined to increase the length of flower-resources availability on strips (Fussell and Corbet, 1992). Such flowering period complementarity could thus increase the length of the maximal activity period of the flower-visitor insects (Havens and Vitt, 2016; Memmott et al., 2007).

The few non-Apidae wild bee we observed (9 species, 54 individuals) were all polylectic, but several Belgian species (Falk, 2015) are oligolectic and require specific plant families or species to collect pollen or nectar. Most of these species are rare. In areas where they are still present it can be of high interest to provide them the plants they need to feed on.

So, if the flower-strip AES have to be improved to favour the few most common polylectic bee species, the chosen plants could be reduced to few

widely visited species combined to provide nectar (i.e. *Centaurea jacea*) and pollen (i.e. *Lotus corniculatus* and *Trifolium repens*) and additional species for spring. If the flower-strip AES have to be improved to be more favourable to the less abundant but often more exigent species, the flower diversity should include preferences of local species (i.e. *Apiaceae* or *Cirsium* for some *Halictidae* species). Nevertheless, the competition for food from large common species (honeybee and bumblebees) can be strong, depleting the flower-resources for smaller wild species (Dohzono and Yokoyama, 2010; Herbertsson et al., 2016; Lindström et al., 2016a; Thomson, 2016). The presence of few dominant flower species dedicated to the common polylectic insects, could reduce the competition on the other flower species, more specifically chose for less competitive insect species (Pleasants, 1981; Steffan-Dewenter and Tscharntke, 2000). Thus, on flower-strips, it could be interesting to combine, in one mix or as specific strip sections, two sets of flower species.

One set of few but abundant plant species to provide pollen and nectar to the most abundant and common insect species that can develop a high fidelity.

Another set of less abundant and more diverse plant species to satisfy the needs of rarer local insect species.

D. Interaction diversity, and the interest of spotted site repartition

On each strip, we observed only 14 to 39 out of 91 insect types visiting flowers and 12 to 42 out of 78 flowering plant species. Each strip provided at least one insect type and one flowering species not observed in the other strips.

Compared to other environmental measures implemented in farmland, flower-strips could appear expensive and poorly efficient as hosting no rare species and applied on moderate areas, but the comparison is not so obvious. The current 1275 km of flower-strips (ca 1530 ha paid 1250€/ha/y) cost ca € 1.9 million per year (Natagriwal, 2016). So, for the 2003-2020 period of implementation, this scheme cost around € 34 million. As a comparison, in France, the annual cost of one ha of a protected area is € 28 (Labbé et al., 2013). Other AES, like 'natural meadows' (MB2, 200 €/ha/y) or 'high biological value meadow' (MC4, 450 €/ha/y, 6,500 ha, Natagriwal, 2014; Walot, 2016) provide compensations to farmers farming meadows in an environment-friendly way. These AES providing flower-hay-meadow areas, often include area under Natura 2000 program (Le Roi et al., 2010; Walot, 2016) targeting specific areas of high biological interest or particular species. The MB2 and MC4 focus on meadows conservation, so they include no creation of new semi-natural areas. The flower-strip aims are different as the flower-strip AES create new areas to host wild flora and fauna and to bring biodiversity in intensively farmed areas. That permitted to provide shelter to several insect species that would never benefit from specific protection plan. These strips were no spots of high biodiversity conservation. But, even if they do not help to save rare species, they can help common species to remain common and preserve functional ecosystems (Corbet, 1995).

The linear shape of flower-strips (from 6 to 21 m width) made them profoundly influenced by the surrounding environment. But yet, pollinators are known to be bioindicators of the state of the environment (Kevan, 1999). Indeed, we observed AES insect community variations between biogeographical regions (Condroz and Hesbaye). We recorded 40% more insect species in flower-strips from the less intensively farmed region presenting the highest surfaces of forest (13 ± 7 vs 4 ± 2 %) and meadows (38 ± 8 vs 15 ± 4 %). This tendency is known at the regional scale: the less intensively managed the area, the richer the flower-visitor-insect communities (Holzschuh et al., 2007; Sjödin et al., 2007; Tscharniske et al., 2005). This species richness variation was thus transferred at a lower scale, even on insect-favourable areas. So, contrary to Kleijn et al. (2004) we observed that AES effectiveness depends on landscape context.

All interactions pooled together, connectance (network complexity) was 0.1 (out of 1). Only 10% of the possible links among plants and insects were realised. This total connectance, was lower than on any single strip. Such tendency is typical of plant pollinator mutualistic webs of which the connectance decrease quickly with the increasing number of species in the web (Jordano, 1987). That suggest that several interactions are site specific and so local context dependant. Moreover, the total nestedness was low too (3.7 out of 100), lower than on any single strip (from 8.3 to 57.5). Each strip presented interaction networks more robust to perturbation than the total community network.

The total interactions among insects and flowers, all sites pooled together, were less random than on every single strip. Moreover, the total insect morphotypes diversity (91) was higher than on any strip (from 14 to 39). Each site provided some specific species and interactions that increased the global interest of the flower-strip AES. The interest of several small favourable habitat fragments across a large geographical area was previously observed as beneficial to increase insect species diversity (Peintinger et al., 2003; Tscharniske et al., 2002). Thus, the total interest of flower-strip AES is not to bring as many species as possible on each strip, but to accumulate numerous sites each one with its own added value. Flower-strip AES provided spotted environment-friendly areas within the agricultural landscape, going against the landscape simplification (Brosi et al., 2008; Persson and Smith, 2013). Contrary to large protected areas, distributed using sophisticated GIS models to balance human economic, and social needs (and biological value) (Brockington et al., 2008), flower-strips AES are implemented on voluntary bases by farmers. While protected areas focus conservation efforts on a reduced number of sites but large areas, flower-strips AES favoured the multiplication of sites, even small ($<250\text{m}^2$), with environment-friendly management. Moreover, while each protected area is expected to have a high biological value and acceptance, even if these areas often shelter only a small fraction of the species diversity of the region were implemented (Fuller et al., 2010), flower-strips AES only have means obligations. Flower-strips have no quantified biodiversity objectives (Le Roi et al., 2010). Despite this absence of quantified objectives and the small size of flower-strips, we demonstrated that

species richness of an environment-friendly scheme could result not from the high interest of each site, but from the combination of several sites of lower diversity.

Nevertheless, as observed comparing strip types and areas, the regional variation and the absence of difference in insect communities between types of AES strips could indicate that the strip management is maybe not the essential point to increase the flower-feeding insect diversity. The medium insect diversity on AES reflects the medium biological quality of the surrounding landscape, dominated by intensively cropped field (Ekroos et al., 2010; Kevan, 1999; Tscharntke et al., 2007). In their current implementation, the flower-strips AES are not enough to mitigate the intensive farming landscape degradation. But they can contribute to reduce it. AES strips could not be conceived as the only response to biodiversity decrease, but need to be integrated and combined with a more general landscape improvement policy (Gill et al., 2016; Meyer et al., 2017). The landscape improvement should favour a higher habitat diversity and especially those of particular interest for insects as semi-natural elements, hedges, meadows, flower-strips and legumes crops. Then, the improvement of AES strip management will make sense.

The current flower-strip AES are implemented according to a maximal area allowed by farm (not more than 9% of the cropped area), but without spatial distribution recommendations (except few restrictions, Le Roi et al., 2010). However, insects have moderate foraging areas, from few tens of meters for the smallest ones to few km for the larger ones (i.e. *Bombus*, *Apis*) (Beekman and Ratnieks, 2000; Gathmann and Tscharntke, 2002; Le Féon, 2010; Redhead et al., 2015). Whereas providing sufficient foraging resources within 100 ha blocks is efficient for large species as *Bombus*, it may not be the case for smaller wild bee species (Pywell et al., 2005). Thus, foraging resources should be distributed over the landscape (Brosi et al., 2008) according to the foraging distance capacity of the targeted species. Carvell et al. (2016) suggested that semi-natural elements should be available every 250 m in farmland to provide a maximal benefit to insects. Thus, to improve the landscape value for pollinators, providing sufficient density of semi-natural areas favourable to insects, flower-strip AES should be included in a more global management plan that not only focusses on how sites are managed or which area is provided but also on how sites are situated within the landscape. As explained by Opdam et al. (2006), “in human-dominated landscapes, biodiversity conservation needs a coherent large-scale spatial structure of ecosystems”. Other studies, as the UK ‘national pollinator strategy’ (Defra, 2014), are developing proposals to bring together farmers, foresters and other land managers to work together at a landscape scale. Planning insect-friendly management at a landscape scale and with several actors can increase the cohesion of scheme settlement, the complementarity and the continuity among landscape development plan to recreate large ecological networks (Forman and Collinge, 1997; Opdam et al., 2006).

Such global approach with 'ecological planning' should be the first step to improve the flower-strips implementation. This global reflection should increase flower-strips integration within the landscape, both their density and their connection with other semi-natural areas. Then, the flower mixes could be improved to provide a better resources complementarity within the habitat network.

III. CONSERVATION SCHEMES VS MASS FLOWERING CROPS, RESOURCE QUANTITIES ARE NOT THE ONLY CLUE

Although the large quantities of flower-resources provide by mass flowering crops, their interest for insects is reduced compared to flower-strips. Mass flowering crops offer monospecific flower-resources in abundance but for a short period. Oppositely, flowered strips provide various flower-resources at low density but for several months, that makes them more adapted to insects needs. *Brassica napus* fields produced 8 kg nectar sugar /ha/d for 40 days and flower-strips only 62 g nectar sugar /ha/d provided by 78 plant species during 183 days. *Brassica napus* flowers were visited by a single species (*A. mellifera*) while flower-strip flowers welcomed 91 types of insects able to provide several ecosystem services.

In Belgium, the main mass flowering crop is oilseed rape (FAOSTAT, 2016). This crop is cultivated on an area 10 times higher than those devoted to flower-strips. The oilseed rape cultivars we studied produced enough nectar to provide resources to 3 honeybee hives /ha to feed and produce a lot of honey (3 kg each). This sugar production was 36 times higher than the peak production of flower-strips. Nevertheless, this production of abundant flower-resources (Janssens et al., 2006; Kirk and Howes, 2012) has to be mitigated as the oilseed rape flowering period is ca 40 days. So, it is not suitable to feed insects all along the flying seasons, mostly from April-May to September.

In the literature we find that proximity to mass-flowering crops as *Brassica napus*, *Glycine max* or *Vicia faba*, could be an option to complement semi-natural areas, providing punctual flower-resources in huge quantities (Kallioniemi et al., 2017; Keller et al., 2005a; Nieto et al., 2014; Power, 2010; Schellhorn et al., 2015; Williams et al., 2012). The oilseed rape flowering period, in April-May, precedes the flower-strip blooming season. Thus, oilseed rape may provide early spring resources to early emerging insects as the *Bombus* species we observed nearby on flower-strips (Diekötter et al., 2010; Westphal et al., 2003). But, we observed only *A. mellifera* visiting flowers in the oilseed rape fields. In several other studies the diversity of foraging insects on oilseed rape was higher (Ali et al., 2011; Stanley and Stout, 2014; Stanley et al., 2013). But the low density of insects in the area combined with the dilution effect on large oilseed rape fields (Holzschuh et al., 2016; Schulp et al., 2014) probably contributed to made insects very sparse on flowers, reducing the probabilities of observations. Moreover, the presence of honeybee in oilseed rape fields is known to reduce the accessibility of flowers to other insect species (Lindström et al., 2016a). Thus, we confirmed the interest of oilseed for honeybees in spring (Calder, 1986; Williams, 1980), but

the capability of oilseed rape fields to provide early flower-resources to wild species appeared to be nil or at least very limited as wild insects seem to be not attracted by oilseed rape flowers. Moreover, most of the oilseed rape fields are under conventional farming and can act as insect traps due to the use of pesticides (Pohorecka et al., 2012; Williams, 1980). Last but not least, contrary to flower-strips settle for at least five years, crop rotations include oilseed rape fields. They could be very close to insect-friendly habitats (as hedges or flower-strips) one specific year and very far the next ones. This distance is not a problem for very mobile species as *Apis mellifera* that can fly up to few km but can be crippling for small wild species with a lower foraging area of few dozens of ha. Thus, oilseed rape fields are not a suitable landscape element to supplement flower-strips, as wild insects do not use the early spring oilseed rape flower-resource.

Among the recorded Syrphidae, 31 species have predatory larvae: *Eristalinus aeneus*, *Eristalinus sepulchralis*, *Eristalis abusivus*, *Eristalis arbustorum*, *Eristalis horticola*, *Eristalis intricarius*, *Eristalis nemorum*, *Eristalis similis*, *Eristalis tenax*, *Eupeodes corollae*, *Eupeodes latifasciatus*, *Eupeodes luniger*, *Eupeodes nielsenii*, *Eupeodes nitens*, *Melanostoma dubium*, *Melanostoma mellinum*, *Melanostoma scalare*, *Meliscaeva auricollis*, *Pipizella viduata*, *Platycheirus clypeatus*, *Platycheirus manicatus*, *Platycheirus melanopsis*, *Platycheirus nielsenii*, *Platycheirus podagratus*, *Platycheirus scambus*, *Platycheirus scutatus* s.l., *Platycheirus tarsalis*, *Scaeva selenitica*, *Sphaerophoria scripta*, *Syrphus ribesii* and *Syrphus vitripennis* (Speight et al., 2015). These species represented 353 of the 451 Syrphidae specimens we recorded. Thus 78% of the Syrphidae observed on flower-strips could potentially provide pest-management services to crops, as aphid regulation (Haaland et al., 2011; Haenke et al., 2009; Poláková et al., 2011; Speight et al., 2015). The AES strips may thus provide pest-management services to numerous crops on the surrounding, that did not oilseed rape fields.

Even if in the studied areas insect-pollination services were not requested by crops, measures dedicated to or effective as pollinating insect conservation could nevertheless welcome insects that can provide ecosystem services beneficial to the crops, as pest management (Kremen et al., 2007; Losey and Vaughan, 2006; Marshall and Moonen, 2002). These AES could, therefore, improve the crop qualities, reduce the needs of pesticides (Ferron and Deguine, 2005) and present a direct agronomic interest for farmers. This pest management services will increase the economic attractiveness of the AES, in addition to the subsidies, already paid to the farmer to compensate the costs of implementation and management of such areas (Del Corso et al., 2017).

IV. CONCLUSION

First, we demonstrated that in Belgium, the current oilseed rape cultivars are well adapted to a very low provision of insect pollination service. In fields, insects visited few as 1.1% of the flowers, but the pollen limitation was low (0.1), and the yields are among the highest in the world. Thus, none of the main Belgian crops needs insect pollination. Nevertheless,

oilseed rape is an important honey crop for honeybees but provided weak support to wild insect species in spring.

Secondly, we confirmed that flower-strips provided new areas to host common flower-foraging insects in intensively managed farmland. Even if each strip did not present a large diversity, a combination of several strips made this AES able to feed numerous insect species. These strips were no spots of high biodiversity conservation. But they help common species to remain common providing refuge areas in the intensively farmed landscape. Nevertheless, several modifications could be set about. The first step should be the drawing up of a global landscape reflection plan to optimise semi-natural elements and AES layout to increase their complementarity, especially needed to fill the spring flower resources gap of flower-strips, and connectance. Then the second step could be the improvement of the flower-strip sown plant mixes. But, as the most efficient flower resource improvement vary among insect species needs, flower mixes improvement should vary according to the way the AES aim will evolve. The seed mixes could thus be improved in different or combined ways according to the targeted insects to support. i) Increase the nectar production, to feed more honeybee hives per ha, but we demonstrated the low interest of this species for farmers. ii) Favour few dominant plant species and elongate the flowering period to favour *Bombus* colonies growth. iii) Increase or modify the flower-resources diversity and phenology, according to the targeted wild insect species to support. This last option seems the most interesting for cropped areas as several of the wild species (mainly hoverflies), poorly supported by current landscape, can provide interesting pest management services to crops.

Appendix

METHODS TO INVESTIGATE *BRASSICA NAPUS* L. POLLINATOR DEPENDENCY: A CRITICAL REVIEW

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Abstract

Insects contribute to the pollination of about 85% of the plant species worldwide. Among these plants, at least 1000 crop species depend on animal pollination. The world production of oilseed rape (*Brassica napus*) has considerably increased during the last half-century. With 35.7 million hectares cultivated and 65 million tons produced in 2014, oilseed rape represents 18% of the oleaginous production worldwide. Oilseed rape is regularly cited as one of the crop sufferings of pollinator decline. Nevertheless, the role of insect flower visitors in the yield of this crop is still under debate. The part of yield increase obtained from insect pollination compared to wind pollination has been surrounded by controversy, especially for modern cultivars. To synthesise which research approaches have been used to estimate pollinator dependency, we systematically reviewed research on this subject that was available on the Web of Science, Google Scholar, and Scopus in January 2016. Most of the 40 primary research studies were conducted in Europe (75%), on winter cultivars (77%) and monitored mainly honeybees (52%). In cases of experiments using caged plants, small plots of a few square meters or weighting seeds of unripe fruits, yields can be overestimated. We provided several recommendations for how to conduct a reliable study on oilseed rape pollination. Our main findings are the following: (1) Field observations provided more realistic and comparable results than caged plants. (2) Sowing densities, soil nutrient availability, as well as field and pest management, influenced the impact of insect pollination and subsequent yield. (3) Plot size conditioned pollinator behaviour as pollination was mainly influenced by insect density in <2 ha plots whereas insect diversity influenced pollination of >20 ha plots. (4) Standardised observation distances within a field should be used among studies (following FAO recommendations). (5) Only full ripe plants should be used to estimate yield. (5) Due to high compensation mechanisms, only data from entire plants, and per area unit, should be considered for yield

estimations. (6) As insect pollination dependency differed among cultivars, the same cultivar should be used for all the experiments.

Keywords

Oilseed rape, pollination, seed yield, insect, experimental design, canola

Highlights

- There is not yet a consensus about the interest of oilseed rape insect pollination
- Wind pollination should be more considered in oilseed rape pollination.
- Cadged experiments distort observations more than often considered.
- Field parameters are insufficiently described as drivers of pollinator dependency.
- We propose recommendations for further oilseed rape pollination studies.

I. INTRODUCTION

Oilseed rape, *Brassica napus* L. *oleifera* (known as Canola in North America), is widely cultivated worldwide (Clément, 1981; Clive, 2010; Coste, 1937; FAOSTAT, 2016; Streeter et al., 2011) (Fig. 1). The worldwide oilseed rape production has considerably increased during the last half century (Fig. 2). With 35.7 million hectares cultivated and 65 million tons produced in 2014 (FAOSTAT, 2016) oilseed rape represents 18% of the oleaginous production worldwide. It is the second oily crop after soya (71%) and prior sunflower (11%) (APPO, 2015). In Europe, oilseed rape is the first oleaginous crop and represents 74% (24.4 million metric tons) of the oleaginous production a long way ahead before sunflower (21%) and soya (5%) (APPO, 2015). The expansion of the oilseed rape crop is nevertheless recent. The massive oil quality improvement lead by the French National Institute for Agronomical Research (INRA) since 1976 has launched the increase of the oilseed rape production from 3.6 in 1961 to 65 million metric tons in 2014 (FAOSTAT, 2016, Fig. 2). Since the 2000's, the oilseed rape production is increasing because of the policy support of biofuel production that was multiplied by six times worldwide (up to 1901 thousand barrels per day) and by 14 times in EU (EIA, 2016). Biofuel of rapeseed oil origin represented 79% of the production in EU and 13% in the USA (Ajanovic, 2011).

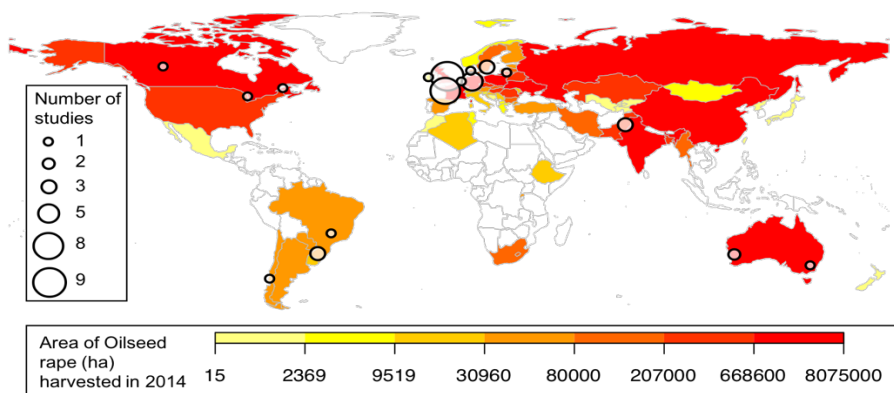


Figure 18. Oilseed rape harvested area worldwide in 2014 (data from FAOSTAT, 2016), and repartition of the studied papers across 14 countries.

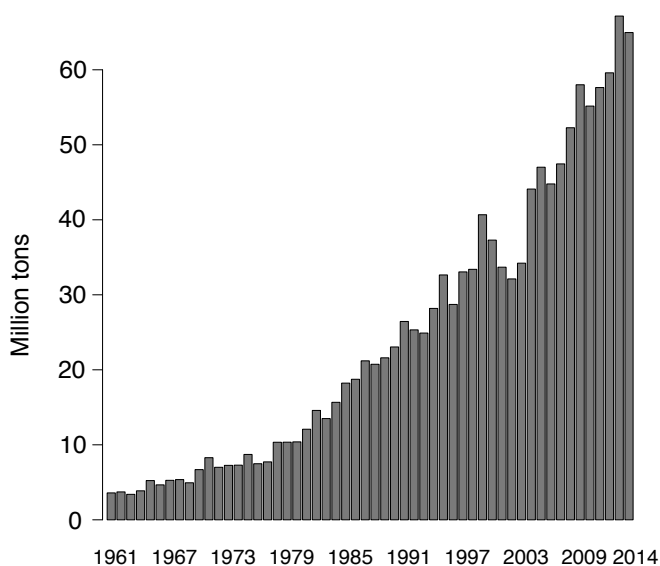


Figure 19. The evolution of oilseed rape production worldwide (in metric tons) from 1961 to 2014 is the expression of Rape oil importance for current society. Data from FAOSTAT, 2016.

The seed yield (e.g. the weight of seeds produced per area) is the main quantitative gauge of agricultural production. The seed quality is the other criterion used to determine the economic seed value. For oilseed rape, the most important qualitative measure is seed oil content (Delourme et al., 2006; Pua and Douglas, 2004).

Insect pollination is an essential ecosystem service allowing the fruit and seed production of entomophilous plants and increasing both the yield quality and quantity of many crops. Worldwide, this service is estimated to provide more than €150 billion benefits a year (Gallai et al., 2009). Thus, a good understanding of the importance of insects as pollination vectors of *Brassica napus* could be an essential tool to improve seed yield and quality.

Brassica napus is known to profit from different ways of pollination (Delaplane and Mayer, 2000; Knuth, 1908). Spontaneous self-pollination (within a single flower, without pollinating vector), self- and cross-pollination (within a single plant or among plants respectively) are possible. Different vectors such as the wind (both to provoke plant movements and to move the airborne pollen) or insect visitors can carry the pollen. Insects seem to be particularly efficient oilseed rape pollinators. Oilseed rape is frequently cited in the literature as one of the emblematic crops that will suffer from pollinators decline (Carrington, 2013; Corbet et al., 1992; European Commission, 2015a; Garibaldi et al., 2016, 2009, Potts et al., 2016, 2014). *Brassica napus* is indeed often recorded as largely visited by insects, especially honeybees (Delaplane and Mayer, 2000; Delbrassinne and Rasmont, 1988; Halinski et al., 2015; Kirk and Howes, 2012; Westphal et al., 2003; Witter et al., 2014), that Abrol (2007) described as “marvellously coevolved pollen transferring devices” for that species. *Brassica napus* provides about 25 to 91 million flowers per hectare per day (P.O. and R.H. respectively, pers. obs.) during the few weeks of flowering time. Flowers have the characteristics for insect pollination. They are easily accessible open bright-yellow flowers (petals from 11 to 18 mm, Lambinon and Verloove 2012). They produce important quantities of sugar-rich nectar and have sticky pollen (Blochtein et al., 2014). These flowers have the characteristics for insect pollination and are not well designed for wind pollination (Cresswell et al., 2004) (Fig. 3). As a result, rape is considered as the most important honey crop since the Second World War (Calder, 1986; Delaplane and Mayer, 2000; Kirk and Howes, 2012; Westcott and Nelson, 2001). Nevertheless, there is still no consensus on the exact impact of insect pollination on *Brassica napus* yield.

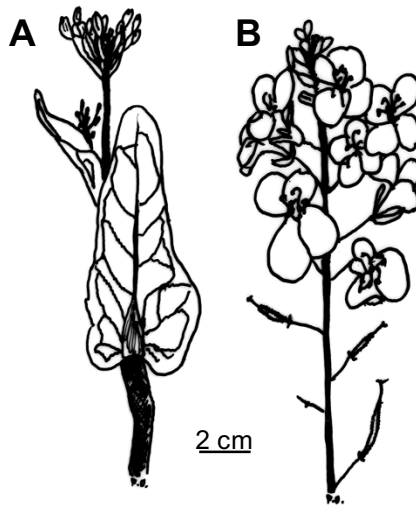


Figure 20. Oilseed rape inflorescence at BBCH (Federal Biological Research Centre for Agriculture and Forestry, 2001) stages 57 (A) and 65 (B) (drawing by P.O.).

Since the 70's, the role of insect pollinators in *Brassica napus* yields is under debate (Free, 1993; Langridge and Goodman, 1982; McGregor, 2009; Mesquida and Renard, 1981; Williams and Free, 1978a). Until these days, we do not know with certainty the exact degree of benefit obtained from insect pollination compared to wind pollination, especially for the most recent cultivars (Hayter and Cresswell, 2006; Jauker and Wolters, 2008; Klein et al., 2003; Morandin and Winston, 2005; Pierre and Renard, 2010). The methods used were variable across studies and so are the conclusions they present. In the FAO estimation of world crops insect dependency (Gallai, 2008) *Brassica napus* was referred as only moderately dependent on insect pollination (dependency ration of 0.25), but with a large variability across references (from 0.1 to 0.4). Some studies concluded to a null effect of insect pollination on rape yields (Langridge and Goodman, 1982; Mesquida et al., 1988) whereas other studies showed a high insect pollination effect that increases yields by five times (Munawar et al., 2009). On consequence, it is unclear how the insect pollination could increase the yield of *Brassica napus*. This potential increase is especially questioning as "in spite of the entomophilous structure of the rape flower, which is very attractive to nectar- and pollen-gathering bees, the crop gave good yields without insect pollination" (Free and Nuttall, 1968).

The importance of *Brassica napus* insect pollination vary according to study and parameter considered. Field constraints and protocol chose to study insect (and wind) mediated pollination impacted plant physiology, the efficiency of pollinator vectors and seed yield establishment (Fig. 4). We propose therefore a methodological review based on the literature related to insect pollinators and oilseed rape production, to investigate and confront the

different methods or conditions used. We aimed to provide thought on the way to assess pollinator importance for *Brassica napus*, and we addressed the following questions: i) Which pollinators were considered and at which density? ii) How were the different rape cultivars used across studies? iii) How was the wind pollination taken into consideration? iv) How were the field and plant parameters considered and comparable across studies and how they could interact with the yield observations?

We hypothesised that effects of insect pollination on *B. napus* yield, at field level, were often overestimated due to protocols used and that impacts of wind pollination was underestimated.

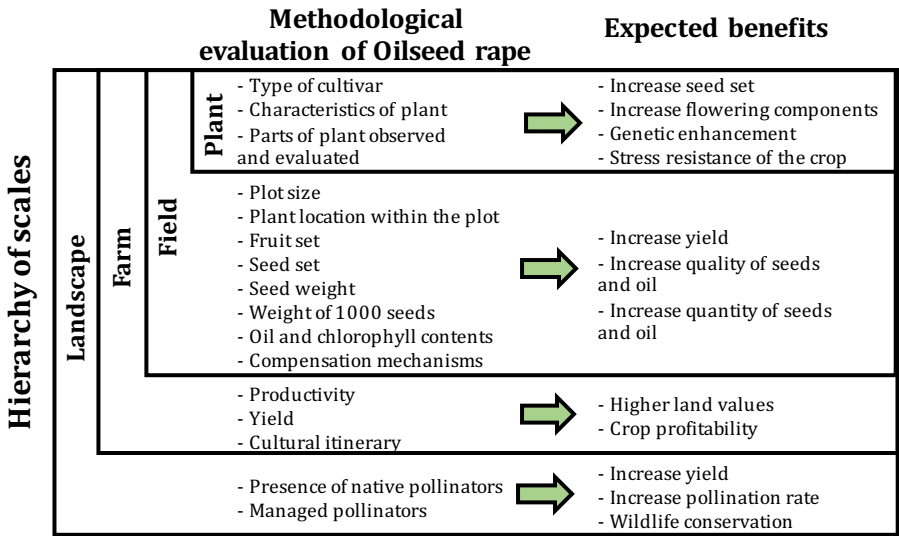


Figure 21. Possible parameters to consider to assess Oilseed rape pollination, according to studied scale and aims. Larger the scale, numerous the parameters.

II. MATERIAL AND METHODS

We based our analysis on scientific papers found via Google Scholar, Scopus and Web of Science using the search sequence “*Brassica napus*” AND “pollination” AND “yield”. We focused on international papers strictly about *Brassica napus* species and pollination (no pest studies). Then, we dismissed the articles referring only to gene flow or crossing. Finally, we obtained a corpus of 40 relevant papers (hereinafter called “the corpus”), world spread (Fig. 1 and Supplementary Table S2), dating from 1956 to 2015. We focused on oilseed rape production, not on the particular hybrid seed production.

We investigated the methods used across the different papers of the corpus, comparing how the field observations were designed, which yield parameters were followed and how the pollination vectors were assessed. We focused on three main elements. i) the plant material studied, i.e. type of cultivar, part of

the plant observed and evaluated. ii) the field conditions, i.e. plot size and plant location within the plot. And iii) the parameters used to assess the seed production, i.e. seed set, seed weight, fruit set and weight of 1000 seeds. We described experimental parameters of insect observation or management, i.e. wild vs managed insects and visitation rate measures.

III. RESULTS AND DISCUSSION

A. *Brassica napus* cultivars, drivers of diversity

Crop cultivar, corresponding to the genetic specificity of the plants, is one of the factors determining the yield potential, seed quality and stress resistance of the plants. It is so essential to consider this parameter when studying the impact of insect pollination on seed yield. The *Brassica napus* cultivars have considerably evolved during the last 30 years thanks to the genetic selection. In 2015 the European Agricultural species catalogue contained 1,331 current cultivars, and 953 cultivars that were not used anymore (European Commission, 2015b). Moreover, numerous new cultivars appear each year. Only in France, around 30 new rape cultivars are registered each year (Pierre and Renard, 2010). The very vast majority (86%) of cropped oilseed rape cultivars were winter oilseed rape cultivars; the spring oilseed rape was less cultivated (only 187 current cultivars on the 1,331 European ones, European Commission, 2015b).

The breeding of oilseed rape has increased in the late 80's when the "0" (simple zero) cultivar group (erucic-acid-free) and the "00" (double zero) cultivar group (erucic-acid-free and low glucosinolate content) were created. During the next decade, the cultivars of the line cultivar group were the only ones sold. Meanwhile, the 90's breeders have been interested in the hybrids to increase yields (Diepenbrock, 2000; Westcott and Nelson, 2001). Firstly, the hybrid cultivars were male sterile and therefore cultivated in association with one or two lines of non-hybrid cultivars. These were the CHL (Composite Hybrid-Line(s)) or CHH (Composite Hybrid-Hybrid) groups. Then the first restored (full fertile) hybrid cultivars were created in the early 2000's (Pierre and Renard, 2010). Nowadays the most cultivated cultivars belong to the restored hybrid cultivar group (Christine Cartrysse *pers. comm.*). The imbalance between spring and winter cultivars was found in the studies. Ten studies were performed on spring oilseed rape, whereas 31 were based on winter oilseed rape.

No single pair of cultivars would show the same biological characteristics (Blochtein et al., 2014) nor attractiveness and response to pollinators (Marini et al., 2015). These differences were mainly due to the quality and the quantity of nectar produced (Blažytė-Čereškienė et al., 2010; Kevan et al., 1991; Pierre et al., 1999) and the cultivar earliness. The nectar volume available had a direct impact on the pollinator behaviour (Hayter and Cresswell, 2006; von Frisch, 1951) and varied significantly among cultivars, from 0.2 to 6 µl/flower per day (Mesquida et al., 1991; Pierre et al., 1999). The differences in nectar total sugar concentration were huge among cultivars as it ranged from 8.25 to

66.56 g/100 g of nectar. Moreover, a variable genotype effect on the nectar composition (sucrose/glucose/fructose) appeared across the season. Finally, the pollination characteristics, i.e. the quantity of pollen production (Hayter and Cresswell, 2006), self-incompatibility, and predispositions to self and cross-fertilisation (from 20 to 42%) also varied sharply among cultivars (Olsson, 1960).

The vast number of rape cultivars that appear across the different studies corresponds to the quick evolution of breeding. It is evident that various groups of cultivars were cropped among studies. Nevertheless, for most cultivars, we lack the information about the cultivar group it belongs to. Throughout the corpus, this is mentioned only for few cultivars (seed yield was affected by insect pollination for line cultivar, but not for the hybrid ones, Marini et al., 2015). This information was also often missing in the species catalogues. Therefore, it is only the year of the study that can help to find the cultivar group the study is probably about.

Another noticeable trait in oilseed rape cultivars that must be taken into consideration while studying the pollinator effect is the presence or absence of cytoplasmic male sterility. Any cultivar of this group does not produce any pollen and is therefore strictly dependent on pollen transfer from a male fertile cultivar.

Within the ten spring oilseed rape studies, seven were performed only on full fertile spring oilseed rape cultivars (Bartomeus et al., 2014; Becker et al., 1992; Blažytė-Čereškienė et al., 2010; Bommarco et al., 2012b; Jauker et al., 2012; Jauker and Wolters, 2008; Kevan and Eisikowitch, 1990; Sabbahi et al., 2005), while one team only worked with both male fertile and male sterile cultivars (Jauker et al., 2012).

Only two teams (Stanley et al., 2013; Williams et al., 1986) compared a full fertile spring cultivar with two full fertile winter cultivars in the same study.

Within the 31 winter oilseed rape papers, 23 examined only full fertile cultivars, and six used both full fertile and male sterile cultivars (Diepenbrock, 2000; Mesquida and Renard, 1982, 1981, Pierre et al., 2009, 2002).

Across all papers we explored, one cultivar, 'Jet Neuf', was studied in different countries, by different teams, and during several years (Delbrassinne and Rasmont, 1988; Lerin and Rivault, 1982; Mesquida et al., 1988; Williams, 1984; Williams et al., 1987). We found five cultivars cited in two papers each. The fact that teams used the same cultivar during two (Manning and Boland, 2000; Manning and Wallis, 2005) or three (Mesquida and Renard, 1982, 1981) consecutive years, or used a same set of plots for different studies (Bartomeus et al., 2014; Bommarco et al., 2012b), explains these double occurrences. Another case appeared in Brazil where a network for oilseed rape pollination was fomented by the government and the FAO (Blochtein et al., 2014; Halinski et al., 2015; Witter et al., 2014).

The comparison of studies that used different cultivars could be inaccurate due to pronounced evolution and variability in biological characteristics among cultivars.

As the diversity and specificity of cultivars should respond to spatial and temporal parameters, it makes sense for each study to use the currently available and/or locally most cultivated cultivar(s). Using the same cultivar among treatments is highly recommended. We also recommend providing information about studying cultivar: type of cultivar (spring/winter, hybrid or not) and easily measurable data as plant size, flowering length, nectar secretion per 24h/flower and global sugar concentration.

B. Insect monitoring

a) Monitored species

Two approaches exist to assess pollination mediated by insects: monitoring insects naturally present on fields or using managed insects. On *Brassica napus*, as on many crops, the most frequently used kept species is the honeybee *Apis mellifera*. This species was used in 90% of the studies working with managed pollinator insects (Fig. 5, Table 2 and Table 3). Only two studies used managed pollinators that were not *Apis mellifera*. The other non-honeybee species were considered in comparison or/and in association with honeybees. The results of these papers shown a variable impact of *Apis mellifera* on rape yield, from non-significant (Lerin and Rivault, 1982) to a yield multiplied by five (Munawar et al., 2009). The origin of the differences of honeybee dependency of *Brassica napus* seed yield is questioned. Recording only the insect presence or visitation rate when studying insect is not sufficient to appreciate the pollinators' quality. More precise, even time consuming, observations of insect flower visitation behaviour should provide useful data to evaluate pollinators' quality and estimate the efficiency of pollinators in yield improvement. *Apis mellifera* is known to often be a nectar robber on *Brassica* plants (Willmer, 2011). Robber honeybees collect nectar using an illegitimate flower visitation with avoiding the stamens or bypassing or piercing the petals. The insect does not touch the reproductive parts of the flower and has not any pollination role. Legitimate visits, where the honeybee touch stamens and stigmas, can represent as few as 12% of the total flowers visited (Delbrassinne and Rasmont, 1988).

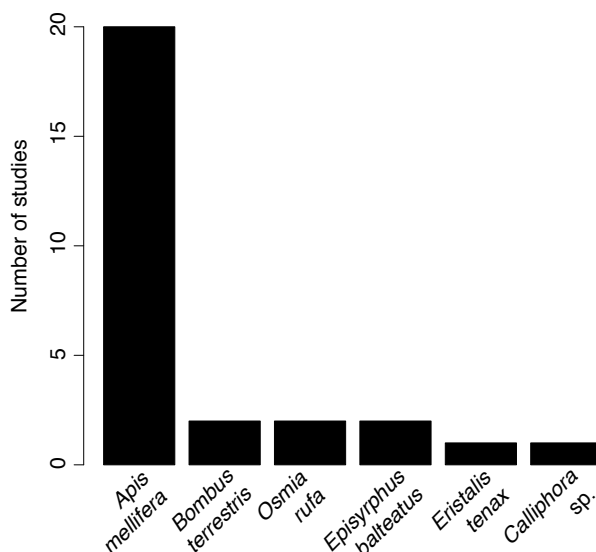


Figure 22. Number of studies using managed pollinator species (N=22)

Several other insect species, as solitary bees, bumblebees or hoverflies species, are also good crops' pollinators (Kremen et al., 2007, 2002; Rader et al., 2015). Solitary bees (such as *Andrena* spp. and *Osmia* spp.) were the most efficient bee-pollinators of oilseed rape with 71.3% of visits having the potential to result in pollen transfer to the stigma. This pollen transfer was much higher than the 34.0% observed for honeybees or the 35.1% for bumblebees (Woodcock et al., 2013). Nevertheless, oilseed rape pollination by non-honeybee managed species was poorly studied (five studies). For instance, Lerin and Rivault (1982) worked with *Bombus terrestris* and did not found any significant yield increase. Mesquida et al. (1988) compared the pollination efficiency by *Apis mellifera*, *Bombus terrestris* and *Calliphora* sp. without detecting any significant increase in yield. More recently, Jauker and Wolters (2008) identified a seed yield increase (+15 to 25%) when the hoverfly *Episyphus balteatus* pollinated *Brassica napus* (Table 2).

Wild non-honeybee species could also be efficient like the wild bees *Plebeia emerina* and *Tetragonisca fiebrigi*, (Witter et al., 2015). The pollination effectiveness nevertheless varied among species and field conditions (Witter et al., 2015).

Using several insect species together (Garibaldi et al., 2013; Klein et al., 2003) including non-bee species (Rader et al., 2015), increases pollination quality and fruit set. Nevertheless, few papers (two) considered the rape pollination by a mix of different managed insect species. *Apis mellifera* and *Osmia rufa* (Steffan-Dewenter 2003) or *Apis mellifera*, *Osmia rufa*, *Episyphus balteatus* and *Eristalis tenax* (Jauker et al. 2012), were the other studied species, but with contrasting results depending on the cultivar, the number of insects used and protocols.

The rape pollination studies monitoring non-honeybee insects or insect mixes remain still rare even they can provide alternatives. Pollination effectiveness comparisons among insect species used in combination are difficult, especially due to critical size of honeybee population ($> 40,000$ individuals per hive). The effectiveness of a small number of non-honeybee insects was often compared to the efficiency of a larger honeybee population. To express the pollination efficiency according to the ratio 'number of flowers to pollinated/number of insects', could help minimise this population-size effect.

b) Field observations

1. Insect density estimations

The insect-pollination effect is variable across the studies, mainly due to differences in managed pollinator densities. Studies monitoring honeybees on field reported low bee densities on *Brassica napus* flowers. Westcott and Nelson (2001) observed 1,100 to 1,200 bees per hectare per day on male sterile flowers (1.1 to 1.2 bees/100 m²/h) and from 14,900 to 24,900 on full fertile flowers (15 to 25 bees/100 m²/h). Sabbahi et al. (2005) recorded from nine to 360 honeybees/100 m²/h depending on the hive density around the field (from zero to three). On the contrary, most of the studies with managed caged insects established very high insect density, up to 400,000 times higher than densities observed in open fields (Table 2, Fig. 6.B). However, some cages were managed with reasonable insect densities such as only 1.6 to 9.8 times higher than in the field (Mesquida and Renard, 1981).

2. Recording methods

On fields, unmanaged pollinators were also studied. Across the different studies, several technics of insect observations were used: transect walks, sweeping-net catchings, pan traps, and fixed-time observations on small plots or a single plant. Each method has its advantages and weakness (Table 2). Moreover, the performances of these insect monitoring and sampling methods did not correspond to the same sampling effort and not provided the same record efficiency among species (Gibson et al., 2011; Musser et al., 2007; Titmarsh et al., 1991; Westphal et al., 2008). These differences were especially evident when technics were put into practice with protocol variations as colour (Saunders and Luck, 2013; Vrdoljak and Samways, 2012), surface area, duration or period of the day (Herrera, 1990).

For valid comparisons, monitoring samplings need to be conducted with simple, replicable and standardised methods (Gotelli and Colwell, 2001; Roulston et al., 2007; Westphal et al., 2008). For the transect observations, both the size of transects (from 4 to 300 m²) and the observation duration (often estimated from 5 to 15 minutes, when timed) varied among studies. Using a mean plant density of 60 /m² and a standard walking time of five minutes per 100 m of walked transect, we can extrapolate these observations to an insect visitation rate per 100 m² per hour. Nevertheless, comparisons of insect visitation rates based on un-standardised protocols have to be considered with caution, even if brought back to the same unit.

Table 23. Advantages of different insect observation methods used in studied papers.

Advantages	Pan traps ¹	Transect walks ²	Sweeping catching ³	Fixed time observations ⁴
Insect diversity	+++	-	++	-
Plant-insect interactions	---	+++	---	+++
Insect behaviour	---	++	---	+++
Small insect sampling	+++	-	+++	-
Method objectivity	+++	---	--	---
Surface area consideration	---	+++	-	+++
Time consideration	+++	--	---	+++
Full day sampling	+++	---	---	---
Number of papers where used	2	6	2	5

¹ (Halinski et al., 2015; Stanley et al., 2013)

² (Blažytė-Čereškienė et al., 2010; Bommarco et al., 2012b; Brunel et al., 1994; Delbrassinne and Rasmont, 1988; Free and Ferguson, 1980; Sabbahi et al., 2005)

³ (Morandin and Winston, 2005; Witter et al., 2014)

⁴ (Ali et al., 2011; Langridge and Goodman, 1982; Marini et al., 2015; Mesquida and Renard, 1981; Stanley et al., 2013)

Pan traps, very efficient to monitoring insect diversity present on the site, did not provide any visitation information. For insect visitation observation on *B. napus*, we recommend using 20 minutes' observations of one m² with a known number of open flowers, at different periods of the day during favourable weather conditions (Willmer and Stone, 2004). This method allows insect visitation and behaviour records, calculation of visitation rate, and minimise disruption induced by walking transects (Westphal et al., 2008).

3. Plot type and insect observations

Observations made on fields with unmanaged insects reveal rather little insect densities and thus reduced flower visitation rates (Fig. 6.A). In Mesquida et al. (1988), bee density never exceeded seven per m² in field crops. Blažytė-Čereškienė et al. (2010) recorded from 10 to 30 bee visitations per 100 m² on five minutes walked transect count (so 120 to 360 bee visitations/100 m²/h). Bartomeus et al. (2014) observed 117 flower-visits/100 m² per hour. Stanley et al. (2013) observed 0.019 insect visitation per plant in five minutes, including only 0.004 honeybee visitation (extrapolated to 1368 insect visitations/100 m²/h including 288 honeybee visitations). Morandin and Winston (2005) recorded from five to 18 bees per 100 m² of sweeping transect (extrapolated to 60 to 216 insects/100 m²/h). Bartomeus et al. (2015) recorded

from four to 12.6 visits per square meter per hour (400 to 1260 insects/100 m²/h).

Studies conducted on medium sized plots (100-150 m²) present conditions between the real field experiments and small plot experiments. This medium size plot had high insect densities (Fig. 6.B). In France, a mean of 290 flower-visits per 100 m² of walked transect, including 130 honeybee flower-visits were observed (Brunel et al., 1994) (extrapolated to 3480 insects/100 m²/h including 1560 honeybees). In Pakistan, an extremely high rate of 0.742 insect-visits per plant per minute was recorded (Ali et al., 2011) (extrapolated to 267,120 insect-visits/100 m²/h). These results seem to reflect more on the attractiveness of medium sized patches of ilseed rape in experimental station rather than the oilseed rape pollination patterns observed in large-size fields.

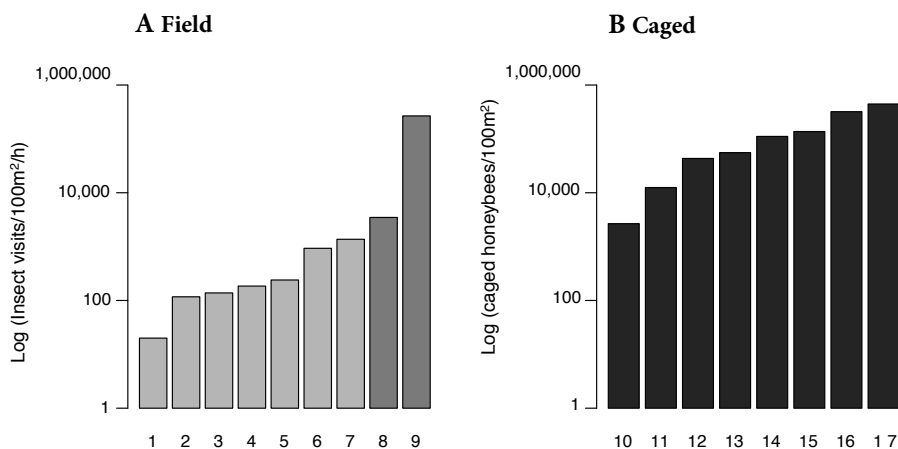


Figure 23. Difference of pollinator density among field vs enclosed observations: **A.** Median number of insect visitations standardized per 100 m² and per hour, on large field studies (light grey) and medium sized plots (grey) **B.** Median number of honeybees enclosed, standardized per 100 m², in different caged experiments (black). Numbers refer to following studies: 1.(Westcott and Nelson, 2001) 2.(Morandin and Winston, 2005) 3.(Blažytė-Čereškienė et al., 2010) 4.(Sabbahi et al., 2005) 5.(Bartomeus et al., 2014) 6.(Bartomeus et al., 2015) 7.(Stanley et al., 2013) 8.(Brunel et al., 1994) 9.(Ali et al., 2011) 10.(Jauker et al., 2012) 11.(Munawar et al., 2009) 12.(Steffan-Dewenter, 2003) 13.(Mesquida and Renard, 1981) 14.(Pierre et al., 2009, 2002) 15.(Williams et al., 1987) 16.(Adegas and Nogueira Couto, 1992) 17.(Free and Nuttall, 1968; Mesquida et al., 1988).

Experiments using cages to observe a potential effect of insects on the rape pollination have not all concluded to the same results (Table 3). Furthermore, nearly half of the studies proved a null or negative pollinator effect on yields (six studies versus 13). The dependence to pollinators was reported as cultivar dependent (Hudewenz et al., 2014; Williams, 1978). Global conclusions are maybe vain. Indeed, the only three studies within the corpus working with the same cultivar (Jet Neuf) have all proved no insect pollinator

Table 24. Insects used in caged experiments on rape pollination.

Number of <i>Apis mellifera</i> used (personal estimations in brackets)	Other insects	Caged area (m ²)	Conclusion on insect pollination	References
one colony (around 40,000 workers)	-	9	No effect	(Mesquida et al., 1988)*
-	two <i>Bombus</i> queens (founder)	9	No effect	
-	several hundreds of <i>Calliphora</i> flies	9	No effect	
one colony (around 40,000 workers)	-	12.5	Increase in seed quantity but not quality	(Adegas and Nogueira Couto, 1992)
one colony (around 40,000 workers) with access to both the cage and the outside	-	9	No significant effect on seed production	(Free and Nuttall, 1968)
one small colony (around 10,000 workers)	-	7.3	Mitigated bee effect	(Williams et al., 1987)
one small colony (around 10,000 workers)	-	9	Negligible effect on whole fertile plants	(Pierre et al., 2009, 2002)
one small hive (around 5,000 workers), no queen	-	9	No bee effect	(Mesquida and Renard, 1981)
500 workers	-	4 (5 plants)	Positive effect on seed production	(Munawar et al., 2009)
250g (around 3,250 workers)	10 <i>Osmia</i>	7.5	Positive effect on seed production	(Steffan-Dewenter, 2003)
-	0 to 100 <i>Osmia</i>	7.5		
Honeybees outside of the cages, 17 hives for 2.7 ha	-	4	No any insect effect	(Langridge and Goodman, 1982)
approximately 200	-	7.5	Positive bee effects on seed production	(Jauker et al., 2012)
-	1 to 36 <i>Osmia rufa</i>	7.5		
-	1 to 96 <i>Eristalis tenax</i> and <i>Episyrphus balteatus</i> with different ratios	7.5		
-	12 & 25 <i>Episyrphus balteatus</i>	4	No correlation between seed production and insect density	(Jauker and Wolters, 2008)
-	one <i>Bombus</i> queen (founder)	4 (16 plants)	Effect on plant morphology but not on yield	(Lerin and Rivault, 1982)
na	-	na	Increase the seed quality	(Kevan and Eisikowitch, 1990)

* Studies using the same rape cultivar: Jet Neuf

effect. From these three studies, Mesquida et al. (1988) did not find any significant effect for honeybees, bumblebees, and *Calliphora* flies. Williams et al. (1987) concluded that honeybees had no or a negative effect on yield, and Lerin and Rivault (1982) showed an effect of bumblebees on plant morphology (shorter branches and flowering time), but no significant effect on yield for this cultivar.

Pollination experiments with enclosed managed insect are designed to understand specific plant-insect interactions and high-density effects but are probably not suitable for providing data useful for agronomic perspectives. Such pollinator densities, up to 1100 beehives per hectare (extrapolated from Free and Nuttall, 1968 and Mesquida et al., 1988), are unrealistic. Nevertheless, it is hard to work under cages with realistic honeybee densities due to the size of an individual colony. Studies with low-density non-honeybee insects seem closer to real field conditions. Overall, high or ultra-high insect densities are not needed to study pollinator effect on yields. Working with moderate insect densities (up to 2-3,000 insect visits per 100 m²/h) seems more realistic and will allow easier field-level extrapolation of the results. Furthermore, high insect densities do not appear to have a positive effect of insect pollination on seed yield, maybe because flowers were damaged by over-visitation. For instance, pollination could be equally effective with a low solitary bee density (2.5 to 4.8 insects/m²) rather than high honeybee density (26.7 insects/m²) (Jauker et al., 2012). With hoverflies, the rapeseed yield was also not correlated to pollinator densities (Jauker et al., 2012; Jauker and Wolters, 2008). Pollinator density alone should be not a stable parameter enough to explain rape yield without considering other parameters.

4. Techniques used to manage insects impacted yield estimations

The technique used to manage insects influenced the observations and can lead to a less favourable environment for the plants (Free, 1993; Olsson, 1960). Mesh bags and cages made of fine mesh (up to 2.5 mm) are common tools used in entomophilous pollination studies (Kearns and Inouye, 1993; Shivanna and Tandon, 2014; and Table 3). The objectives of their use are double: protecting plants from insects locally present and/or keeping managed insects on plants, but allowing access to airborne pollen. Mesh cages or bags moderately change microclimatic conditions (temperature, Kearns and Inouye, 1993) or flowering duration (Marini et al., 2015). Their use can be straightforward and unambiguous in the case of 100% entomophilous flowers or spontaneous self-pollination as no pollen have to come from outside of the bags. In plant species that are probably partly wind pollinated such as rape, the use of mesh is more problematic because it may alter the wind velocity (Table 3). Yield decreases by 11 and 51% were recorded in plots caged with one mm and 2.5 mm mesh respectively compared to the yield in open plots in the same field (Jauker and Wolters, 2008). The mesh size is so an important factor influencing the negative effect on wind pollination but the consequences of changes in airborne pollen kinetics it can induce may not be easy to evaluate (Mesquida et al., 1988).

It established that *Brassica napus* wind pollination could be of importance (Free, 1993; McCartney and Lacey, 1991; Williams, 1984). The meshes bags

or cage parameters, especially the mesh size can significantly modify the wind pollination quality (Gonzalez et al., 1998). The meshes can change the wind, light and humidity conditions inside the cages, reducing the wind-mediated pollination rate (Olsson, 1960). Nevertheless, only very few studies taken the impact of meshes on wind pollination of oilseed rape into consideration (Table 4). It is hard to separate the effects caused by the absence of insects from those caused by the changes in physical conditions due to the presence of cages or bags (Mesquida et al., 1988; Williams et al., 1987).

A negative cage effect on yield (even with enclosed pollinators) was measured. *Brassica napus* yield decreased by 30% to 50% under a cage in comparison to un-caged field plots (Mesquida et al., 1988; Sabbahi et al., 2005). The cage effect seems to be so strong that it is not possible to compensate its effects on pollen kinetics even by adding lots of bees or fans inside. For instance, the yield of uncaged plots and the yield of caged plots with bees (one hive (around 40,000 bees) per 12.5 m² under a cage) were of 207% and 103% higher respectively, compared to the yield of caged plots without insects (Adegas and Nogueira Couto, 1992). The mesh not only reduced insect visitation of crops but also distorts other critical pollination parameters, especially when the mesh is fine (one mm).

Observation conducted on real fields, even if the part of unmanaged-parameters increases, are more realistic and should be preferred when possible. Ideal observations would be performed on the field with a local increase of pollinator populations. Pollinator densities would be estimated after pollinator observations. Control plots of low insect visitation could be obtained using insect repellent molecules sprayed on few plants (Free, 1993; Naumann et al., 1994; Solomon and Hooker, 1989), even if protocols are still to be built.

When cages are needed we recommend therefore to use as large mesh size as possible (>2.5 mm) to avoid insect flux and to reduce wind flow as little as it might be. We recommend the measurement of airborne pollen quantity both inside and outside of the cages is recommended.

Table 25. Bagged or caged experiments: mesh sizes and effects taken into account in different studies.

Mesh size	Main bagging or caging objectives	Secondary mesh effects recorded	Reference
2.5 mm	- keeping managed insects out	- no effect on airborne pollen flow	(Langridge and Goodman, 1982)
2 mm	- no pollinator access - keeping managed insects inside	- wind speed decreases by 90% for wind of 2.75 km/h - wind speed decreases by 60% for wind of 4.50 km/h	(Mesquida et al. 1988 ; Mesquida and Renard 1981)

Mesh size	Main bagging or caging objectives	Secondary mesh effects recorded	Reference
2 mm	- no pollinator access	- nothing mentioned	(Mesquida and Renard, 1982)
2 mm	- no pollinator access - keeping managed insects inside/outside - allowing pollen flow	- wind speed decreases	(Pierre et al., 2009, 2002)
1 mm	- total insect exclusion	- maybe an effect on temperature and solar radiation	(Durán et al., 2010)
2.5 mm	- partial insect exclusion	- maybe an effect on temperature and solar radiation	
1.2 mm	- no pollinator access	- nothing mentioned	(Stanley et al., 2013)
1 mm	- no pollinator access	- minor changes to micro-climatic conditions	(Marini et al., 2015)
1 mm	- no pollinator access	- nothing mentioned	(Bommarco et al., 2012b)
1 mm	- no pollinator access - keeping managed insects inside	- nothing mentioned	(Jauker et al., 2012; Jauker and Wolters, 2008)
<1 mm	- no pollinator access - keeping managed insects inside	- nothing mentioned	(Steffan-Dewenter, 2003)
0.7 mm (14 meshes/cm)	- keeping managed insects inside/outside	- wind speed decreases - a potential greenhouse effect	(Lerin and Rivault, 1982)*
Only the small insects (e.g. thrips and midges) could pass	- no pollinator access - keeping managed insects inside	- nothing mentioned	(Williams et al., 1987)*
na	- no pollinator access - keeping managed insects inside	- nothing mentioned	(Adegas and Nogueira Couto, 1992; Kevan and Eisikowitch, 1990; Munawar et al., 2009)
na	- no pollinator access	- nothing mentioned	(Blochtein et al., 2014; Free and Nuttall, 1968; Sabbahi et al., 2005; Shakeel and Inayatullah, 2013; Witter et al., 2014)
Butter paper	- no pollinator access	- nothing mentioned	(Ali et al., 2011)

* Studies using the same rape cultivar: Jet Neuf

c) Pest pressure interacts with pollinators

The activity of adults and larvae of pollen beetle, mainly *Brassicogethes aeneus* and *Brassicogethes viridescens* (Meligethinae), could reduce both pollinator visitation rate and plant fitness (Alford, 2003; Bartomeus et al., 2015; Krupnick et al., 1999; Krupnick and Weis, 1999; Veromann et al., 2006). Meanwhile, they were recorded as efficient pollinators in rape (Alonso, 2004; Gómez, 2003; Ramsay et al., 2003) especially heavily infested fields (Bartomeus et al., 2015). Even if the negative effect of *Brassicogethes* spp. damages could be negligible at the plant level thanks to compensation capabilities of rapeseed (Williams pers. comm.; Diepenbrock, 2000; Williams and Free, 1979), the impact can be recorded as high for studies focused on flower level (due to flower losses).

The small size of pollen beetles makes them hard to keep out of study plants, even using fine-mesh cages or bags (Shivanna and Tandon, 2014). Therefore, we recommend the use of classical yellow water traps (Sedivy and Vasak, 2002), eventually with additional medium size wire netting to prevent trapping of bigger insects, to estimate and compare levels of pollen beetle infestation among plots.

C. Wind pollination

Several studies investigated the effect of wind pollination on yield under real field conditions, with different methods. *Brassica napus* flowers have pollen grains covered with sticky pollenkitt, small stigma and petals with a negative effect on airflow (Cresswell et al., 2004; Eisikowitch, 1981). Nevertheless, pollen can be released into the air by checked plants (Delaplane and Mayer, 2000) and the airborne pollen flow tends to remain rather high in rape fields and can result in high pollen deposition (Hayter and Cresswell, 2006) and fruit set (Pierre et al., 2009, 2002; Williams, 1984). Indeed, wind pollination is confirmed up to few km around pollen source (Timmons et al., 1995) and even under cages where the wind and therefore pollen flow were reduced (Mesquida and Renard, 1982).

The wind was not only an efficient pollination vector as it carried the pollen from plant to plant, but also as it induced the direct mechanical contact between flowers and provoked pollen falls within flowers, from stamens to stigma. Under greenhouse conditions, the wind effect can be simulated by shaking the plants twice a day (Eisikowitch, 1981; Williams et al., 1986) and equals pollination by hand. Wind pollination can also be insect-mediated as insects visiting flowers increased the quantity of airborne pollen (Delaplane and Mayer, 2000; Hayter and Cresswell, 2006; Pierre et al., 2009). We found one study (Mesquida et al., 1988) where the wind effect in cages was locally simulated using electric fans.

Wind pollination is a vector of oilseed rape pollination. Nevertheless, as presented in section 3.2.3, several techniques used to study insect pollination (mesh bags or cages) reduce the wind, and airborne pollen flows around the studied plants. Insect pollination was so studied in or compared to a wind-

perturbed environment. These wind perturbations were poorly considered (Table 3) and, in fact, pooled with the insect effect. To assess the role on insect pollination under real field conditions, we recommend paying a particular attention to the experimental design to minimise as much as possible the factors impacting the airflow both on the plot and the plant levels.

D. Compensation mechanisms hide pollination deficits

Compensation is a complex mechanism of positive plant response to stress, according to environmental parameters. For seed yield, *Brassica napus* has effective compensation mechanisms (Clarke and Simpson, 1978; Diepenbrock, 2000; Lerin and Rivault, 1982; Mesquida et al., 1988; Mesquida and Renard, 1981; Williams and Free, 1979). The plant pollination by bees reduced the flowering period by few weeks, reduced the stem elongation and reduced the number of branches (Lerin and Rivault, 1982; Mesquida and Renard, 1981; Williams et al., 1987). Plants were so more compact, with more advanced fruit growth, homogenization of seed size and maturity stages (Mesquida and Renard, 1981; Williams et al., 1987) that improved the harvest quality. Conversely, in low sowing densities (Angadi et al., 2003; Lerin and Rivault, 1982) or inappropriate conditions for pollination (Mesquida and Renard, 1981; Williams, 1978; Williams et al., 1987), branches were longer and more numerous. When flowers were badly pollinated or damaged by pests, their number increased to compensate the loss (Angadi et al., 2003; Diepenbrock, 2000; Marini et al., 2015; Williams and Free, 1979). A small seed number can result in heavier seeds (Adegas and Nogueira Couto, 1992; Mesquida et al., 1988; Williams, 1978). Nevertheless, the extent to which the plant can compensate the loss varies according to the plant section stressed or damaged (Diepenbrock, 2000).

To appreciate pollination impacts on global seed yield we recommend considering plants in their whole. Additional information on plant morphology and development under each condition could add interesting ways of discussion about compensation mechanisms that could buffer several effects at the plot level.

E. Methods used to determine seed production

The key criterion to estimate the effect of plant-pollinator interactions is the seed yield and its different components (i.e. fruit set, seed set, seed weight): farmers are paid according to the weight of seed they produced, and specific penalties could occur for low quality or not dry seeds. Providing yield estimations per hectare is so the most useful information for both farmers and seed companies. The accuracy of these estimates was sometimes discussed and “the reliability of yield analysis depends on how the yield parameters have been gathered or selected and whether a single plant or the entire crop is considered” (Diepenbrock, 2000). The multiple compensation mechanisms occurring in *Brassica napus* increased these differences.

a) Differences in plant parts sampled influence pollination dependency conclusions

Insect pollination effects are known to differ according to the plant section (Mesquida et al., 1988), and the branch position considered (Clarke, 1979). Conclusions of studies are so dependent of the ways the plants were sampled for estimating seed production. The most productive flowers are situated on the lower half of the main raceme: this part of the plant -where the assimilate availability is the greatest- has the highest fruit set, seed set and seed weight (Clarke, 1979; Diepenbrock, 2000; Tayo and Morgan, 1975). The main raceme provides, therefore, most of the seed production of the plant (from 81 to 72% in Lerin and Rivault, 1982). Analysing the pollinator effect on the flower production on the main raceme seems to be the most interesting, but there is no real consensus. However, as the bottom of this plant section is already the most productive part, it may be there that the potential of insect pollination benefit is the lowest: the yield of this plant section was the most constant among treatments (Diepenbrock, 2000). As an example, insect pollination increased the seed weight by 11% considering only the first 25 flowers of the main raceme and by only 4% considering the first 50 flowers of the main raceme (Mesquida et al., 1988). Moreover, on the same plant, insect pollination can increase the weight of seeds produced by the main raceme and decreasing the production of the branches (Lerin and Rivault, 1982).

In the corpus, the plant sections and the flowers studied (number and position) differed considerably among studies. Connections are weak among studies that sampled as few as three (Morandin and Winston 2005, seed set linked to bee density) to six (Stanley et al. 2013, pollinator exclusion resulted in a decrease of yield) random flowers and studies using the first 20 (Williams, 1978,) or the first 50 (Mesquida and Renard, 1981,) flowers of the main raceme, while others considered the whole main raceme (Mesquida and Renard, 1981, 1982; Pierre et al., 2009).

For most of the corpus' papers, comparisons are simplest as the whole plants were taken into consideration to evaluate the global seed yields and the different yield parameters (Bartomeus et al., 2014; Bommarco et al., 2012b; Durán et al., 2010; Free and Nuttall, 1968; Lerin and Rivault, 1982; Manning and Boland, 2000; Manning and Wallis, 2005; Williams et al., 1986; Witter et al., 2014). Nevertheless, in some studies, a subsample of fruits was used not for the global yield, but to appreciate the other parameters (Marini et al., 2015; Mesquida et al., 1988; Munawar et al., 2009; Sabbahi et al., 2005; Shakeel and Inayatullah, 2013; Williams et al., 1987).

Studying flowers from different parts of the plant (i.e. from various parts of a branch and different branches) is essential to understand how the yield is formed within a plant and how different factors modify the seed production. Nevertheless, we cannot extrapolate the observed effects on the flowers of the main raceme or any other plant section to the whole plant (Marini et al., 2015), and even less to the field level (Fig. 7). Global seed yield estimations should consider data from the whole plant.

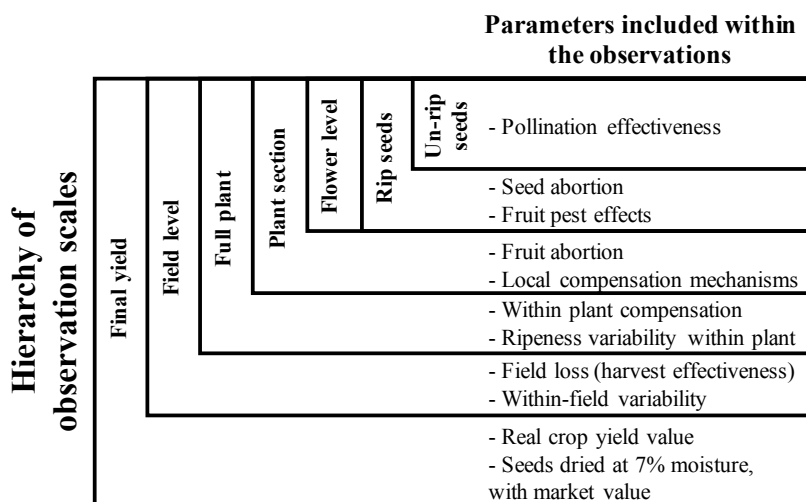


Figure 24. Possible observations scales and yield parameters includes. Larger the scale, numerous the parameters.

b) Variability in growth stages considered not reflect harvest reality

From the fertilisation to seed maturity, the *Brassica napus* seed quality and composition evolved following three phases (Norton and Harris, 1975). During the third one, there is a reduction of water and chlorophyll seed content. Moreover, abortion of seeds occurs from two weeks after the end of flowering to maturity (Clarke, 1979). The seed number can be reduced by nearly three times (from 19 to seven, Norton and Harris, 1975). Comparing the seed production based on data collected at different stages could induce a weak estimation of the impact of pollinators on (final) seed yield.

In the corpus' papers, the time of harvest differed considerably among studies. oilseed rape seeds were collected from only 12 days after pollination (Morandin and Winston, 2005), to six or eight weeks after pollination. Twelve days after pollination correspond to an un-mature stage, before the main seed abortion occurring during weeks three to five (Norton and Harris, 1975). Oppositely, six or eight weeks after pollination corresponds to a stage where the fruits had already reached maturity (Stanley et al., 2013).

Ripe seed samplings are not enough to guaranty samples homogeneity. The results concerning seed production and yield are not easily comparable, because of a different water content of the material studied. In most of the studies, freshly harvested plants were evaluated without indicating the seed moisture content. In five studies the plants were dried before being evaluated. Different procedures were followed to dry the plants: i) 25-30°C during four weeks (Steffan-Dewenter, 2003, no pollinator effect), ii) 25°C for 12 h (Jauker et al., 2012, positive pollinator effect on yield), iii) one week in a greenhouse then 24 h at 65°C (Marini et al., 2015, pollinator increased seed yields when no nitrogen fertilization). Another method consisted first to dry the plants

outside, then in a warm room for two weeks (Williams et al., 1987, insect pollination did not increase seed oils content, impact on yield is year dependent) or at room temperature without specifying the time (Stanley et al., 2013). In short, missing information about differences in ripeness level or seed water content makes it impossible to assess the pollinator effects on seed production weight and quality, especially as differences of maturity could occur among treatments (Mesquida and Renard, 1981).

We recommend basing seed production calculations on ripe seeds only. To study the seed yield, we recommend harvesting whole mature plants and drying the seeds if necessary to reach a water content corresponding to optimal storage and standard seed market quality moisture from 7 to 9% (Canola Council of Canada, 2014; Kasprzycka et al., 2010; United Oilseeds, 2009).

c) Yield observation parameters conditioned the results

The observed parameters to assess seed production were variable across studies (Table 5), usually from a low to a higher scale estimation. In some studies, the approach was reverse (Free and Nuttall, 1968) or corrected using a mathematical model to compare the levels of seed production among plants, according to the number of fruits, the plant height and the number of branches (Manning and Boland, 2000).

The seed weight production per area, as well as the seed quality, are the results that seem to make the most sense from an agronomist's or an economist's point of view. The other yield components (fruit set, the weight of thousand seeds, seed size, seed number), not correlated with the global seed yield (Durán et al., 2010), do not allow any extrapolation to the field level. For example, a higher fruit set on the main raceme leads to a lower number of ramifications and fruits (Lerin and Rivault, 1982; Mesquida et al., 1988). A higher number of fruits per plant was partially compensated by a low number of seeds per fruit according to some studies (Manning and Wallis, 2005; Sabbahi et al., 2005; Steffan-Dewenter, 2003), whereas others did not find any such compensation (Jauker and Wolters, 2008; Shakeel and Inayatullah, 2013). The higher seed size could mitigate the lower seed number and vice versa (Durán et al., 2010; Marini et al., 2015). Nevertheless, these relations were not linear, and plots with numerous big seeds versus plots with rare small seeds were also observed (Bommarco et al., 2012b; Jauker et al., 2012).

In addition to seed weight, the seed quality is the other criterion used to determine the seed value (price). For a high seed quality, high oil content and low chlorophyll content are expected. On spring *Brassica napus* cultivars, insect pollination led to higher oil yield and lower chlorophyll seed content, increasing the market value by 20% (Bartomeus et al., 2014; Bommarco et al., 2012b). On the contrary, studies on winter cultivars report no or a negative effect of bee pollination on seed oil content (Adegas and Nogueira Couto, 1992; Langridge and Goodman, 1982; Williams et al., 1987).

Table 26. Observed yield parameters

Seeds	Size ¹	
	Quality	- Germination rate² - Oil content³ - Oil and chlorophyll contents⁴ - (Water content)
	Weight	- Of individual seeds⁵ - Per fruit⁶ - Per plant⁷ - Per thousand seeds⁸ - Per plot⁹
		
Fruits ¹⁰		
	- Per plant - Fruit set	

¹(Manning and Boland, 2000)²(Kevan and Eisikowitch, 1990; Langridge and Goodman, 1982)³(Adegas and Nogueira Couto, 1992; Langridge and Goodman, 1982; Williams et al., 1987)⁴(Bartomeus et al., 2014; Bommarco et al., 2012b)⁵(Adegas and Nogueira Couto, 1992; Lerin and Rivault, 1982; Williams et al., 1987)⁶(Blažytė-Čereškienė et al., 2010; Blochtein et al., 2014; Manning and Boland, 2000; Stanley et al., 2013)⁷(Bartomeus et al., 2014; Blochtein et al., 2014; Mesquida et al., 1988; Steffan-Dewenter, 2003; Williams, 1978; Williams et al., 1987; Witter et al., 2014)⁸(Durán et al., 2010; Mesquida et al., 1988; Mesquida and Renard, 1981; Sabbahi et al., 2005; Shakeel and Inayatullah, 2013)⁹(Free and Nuttall, 1968; Halinski et al., 2017 in prep.; Marini et al., 2015; Mesquida and Renard, 1982; Mesquida et al., 1988; Williams et al., 1986)¹⁰(Blochtein et al., 2014; Jauker et al., 2012; Mesquida et al., 1988; Pierre et al., 2009, 2002; Williams et al., 1986)

Results obtained with highly various methods and from different parts of plants are impossible to be extrapolated to the whole plant and even less to the parcel level or among cultivars. The different approaches or the use of some parameters only made the comparative analysis very hard. To estimate the impact of pollination on seed yield under realistic field conditions and among cultivars, we recommend measuring the total seed weight produced per unit area. We also recommend measuring seed quality parameters (at least oil and water contents) as they are useful complementary parameters to estimate the economic profit of rape production improvement.

F. Field parameters

a) Plot size and shape and sampling patterns

The size, shape and location of the study plot directly affected the result of the plant (Otypková and Chytrý, 2006), yield (Arny, 1921; Arny and Hayes, 1918), insect (Garibaldi et al., 2016; Olsson, 1960; Prasifka et al., 2005) and wind observations (Klein et al., 2006). Field size conditioned insect visits of the crop, and the insect movements and dispersion over the field: bigger the

insects, dipper in the field they can visit the flowers (Le Féon, 2010). Insect visitors insects were more diverse and numerous at the field edges and less varied and rarest in the field centre (Brunel et al., 1994). Insect density and diversity determined pollination efficiency (quantity and quality of pollen deposit, level of cross-pollination). Location in the field were parameters that influence the relative importance of the wind versus insect pollination, especially for oilseed rape (Olsson, 1960). In fields smaller than two ha the pollination quality was linked to the pollinator density, whereas in fields larger than 20 ha the pollination quality was influenced by the pollinator diversity (Garibaldi et al., 2016). On consequence observations in small plots cannot be extrapolated to larger fields.

In our studied corpus, field sizes were highly different from one study to another, as well as within studies, extending from only a few square meters (Adegas and Nogueira Couto, 1992; Steffan-Dewenter, 2003) to 293 ha (Manning and Wallis, 2005). This size parameter is nearly never discussed but could help to understand some observations.

The number of replicates varied from one single field (Manning and Boland, 2000; Mesquida and Renard, 1982; Williams, 1984) to ten fields (Bommarco et al., 2012b; Free and Ferguson, 1980; Stanley et al., 2013). A higher number of fields could result in a more diverse field size considered (i.e. from 1 to 40 ha, Bommarco et al., 2012) and mixing several effects linked to the field size. Conclusions from low replicate level studies could be more accurate rather than conclusions from replicated studies mixing fields sizes.

Independently of the plot size, the location of the plants studied within the plot can also strongly impact the results (Arny, 1921). Light and wind conditions, water and nutrient availability, as well as accessibility for pests and pollinators, varied from the plot edge to its centre (Cromartie, 1975). Different field areas should be considered from edge to centre gradient to appreciate the pollinator effects over a full field.

The FAO published standardised protocols to study pollinator effects on crop yields (Vaissière et al., 2011). The FAO recommended conducting observations at fixed distances from the field edge (25, 175 and 325 m for large fields (>450 m) or 5, 15, 35 and 45 m for small fields). For tricky shaped fields, the FAO recommended using the full field as an experimental plot. The implementation of these protocols would help to compare results among studies. Among the published studies, several protocols were used as studying plants at a given distance from the edge, the whole plot, the centre of the field or plot to avoid edge effect, or random plants in the plot (Fig. 8). The lack of comparisons among the different areas of the same field makes hazardous comparisons among studies working with various field patterns.

We recommend, within a study, to use several fields of comparable size and shape and to localise observations using the same pattern among plots, ideally following the FAO recommendations of distances from field edges (Vaissière et al., 2011) to homogenise field plots location among studies.

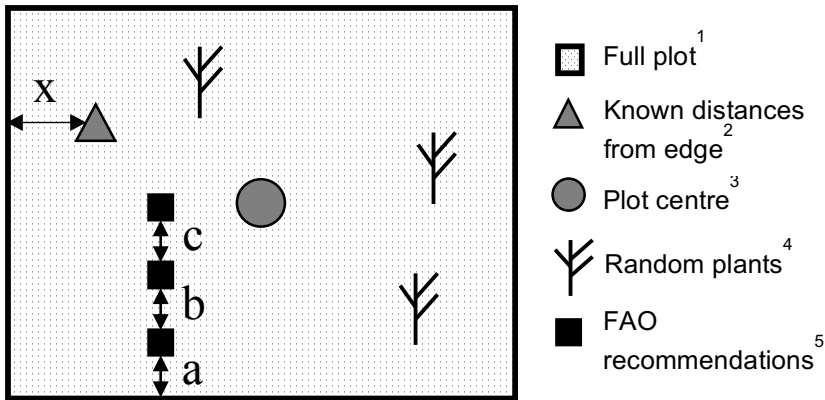


Figure 25. Plant sampling design within plots or fields varies among studies

¹(Adegas and Nogueira Couto, 1992; Bommarco et al., 2012b; Jauker and Wolters, 2008; Lerin and Rivault, 1982; Munawar et al., 2009; Shakeel and Inayatullah, 2013)

²(Manning and Boland, 2000; Manning and Wallis, 2005; Morandin and Winston, 2005; Stanley et al., 2013; Witter et al., 2014)

³(Bartomeus et al., 2014; Becker et al., 1992; Blažytė-Čereškienė et al., 2010; Durán et al., 2010; Marini et al., 2015)

⁴(Ali et al., 2011; Jauker et al., 2012; Mesquida et al., 1988; Mesquida and Renard, 1981; Pierre et al., 2002; Sabbahi et al., 2005; Steffan-Dewenter, 2003; Williams et al., 1987)

⁵(Vaissière et al., 2011)

b) Chemicals used affected insects' behaviour

In the considered studies, seed coatings and treatment of the crops during several consecutive years (to have information about persistent compounds) were often not analysed. Even the use of chemicals during the studied blossom period could remain un-discussed. No information was provided to appreciate the chemical effect on pollinator behaviour among studies. Nevertheless, the chemicals used for seed coating and phytosanitary treatments of the crops, both during the study year and the previous ones (persistent compounds), influenced insect pollination health, behaviour, diversity and insect density on the crop (Henry et al., 2012; Thompson, 2003; Woodcock et al., 2016). The type of active molecules contained in the previously used chemicals significantly influenced the bee visitation rate on oilseed rape flowers (Kazda et al., 2015). Comparing results on insect pollination regardless of treatment with pesticides can thus distort the conclusions.

We warmly recommend investigating at least the chemical used on the studied crop, and if possible on the previous crops too.

c) Soil characteristics affected insects' behaviour

The soil characteristics (i.e. depth, type, nutrient availability), the fertilisation and previous crops in the crop rotation are rarely specified and considered in pollination studies (Cardoza et al., 2012; Marini et al., 2015). These parameters affect plant development, flower production and floral resources (both pollen and nectar qualities and quantities), insect behaviour (both pollinator and pest), disease pressures and seed production in terms of quality and quantity (Cardoza et al., 2012; Hokkanen, 2000; Marini et al., 2015; Ozer, 2003; Rathke et al., 2006, 2005; Sieling and Christen, 1997). For some rape cultivars the nitrogen fertilization (from zero to 200 kg/ha) increased the seed yields from 49 to 94% (Mahli et al., 2007; Rathke et al., 2006), what is more than the range of rape pollination dependency provided by the FAO (10 to 40%) (Gallai, 2008). Nutrient availability fluctuation both among and within fields (Cambardella et al., 1994; Miao et al., 2006) could affect the yield and disrupt or cancel observations of pollinator effect (Marini et al., 2015) among plots of a different pollination treatment even of one study. The seed yield gains linked to insect pollination were directly dependent on the level of nitrogen fertilisation: in plots without nitrogen input, the gain was high whereas it was insignificant when the nitrogen input was high (170 kg/ha) (Marini et al., 2015).

Several studies were conducted under partly or entirely controlled conditions. For instance, some experiments were conducted outside but with potted plants to manage the soil parameters and resource competition (Lerin and Rivault, 1982). Others were carried out in greenhouses (Eisikowitch, 1981; Williams, 1978; Williams et al., 1986) to control additional parameters as wind and water availability. These technics increase repeatability and comparisons both within and among studies, but such studies are not the most common on *Brassica napus* and are far from realistic field conditions.

Doing soil analysis, at least for nitrogen availability, obtaining the fertilisation management, the field management history (both crops and chemicals) and working with as homogenous as possible plots are recommended.

d) Studies distribution among climatic zones

The majority of the studies we considered in our corpus (70%) took place in European countries, and few were done in America, Asia, and Oceania. In each of the countries including Australia, Belgium, Brazil, Canada, Chile, Denmark, France, Germany, Ireland, Italy, Lithuania, Pakistan, Sweden, and the UK, the agronomic, environmental and climatic conditions differed. According to the climate zone defined in Peel et al. (2007), 67% of the studies were conducted under temperate climate (Cfb, Cfa and Cwb) (including 3 studies done in zones with dry summer (Csa)), 26% under cold climate (Dfb) and 7% in an arid climate (BWh) (Supplementary Table S2 and Fig. 9). The climatic parameters and the latitude (Ollerton and Cranmer, 2002) strongly impact the diversity of flower-visiting insects (Kleijn and van Langevelde, 2006) as well as the plant growth, phenology, and physiology. *Brassica napus* development, and thus the seed yield, is much affected by the quality of vernalization, the sum of effective temperatures, the highest temperatures,

the day-length, the duration of the growing season (de Koning and van Diepen, 1992) and the water stress (Champolivier and Merrien, 1996).

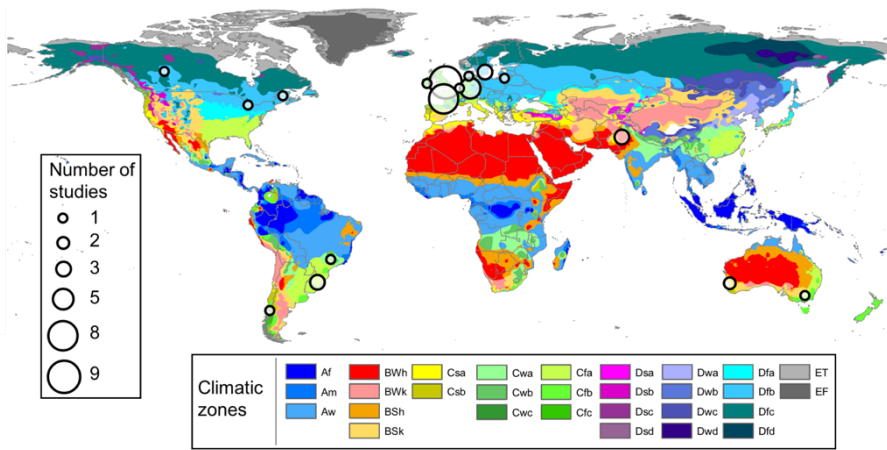


Figure 26. Number of studies per country and per different climate zones according to the Köppen-Geiger climate classification. See Table S2 for climate details and studies references. Map adapted from Peel et al. (2007).

The *B. napus* flowering period extends from 20 to 45 days in Europe (Bommarco et al., 2012b; Delbrassinne and Rasmont, 1988; Free and Ferguson, 1980; Lerin and Rivault, 1982; Stanley et al., 2013; Williams, 1984; Williams et al., 1987) and in North America (McGregor, 2009; Morandin and Winston, 2005), whereas in Australia it can last from 56 to 69 days (Manning and Boland, 2000; Manning and Wallis, 2005) and from 19 to 82 days depending on the cultivar in Brazil (EMBRAPA, 2016; Tomm et al., 2009). It can be interesting to investigate if variation in flowering time among studies reflected on a difference in pollination potential among sites or a difference in pollination receptivity among cultivars.

The response of *Brassica napus* to insect pollination probably vary with a similar pattern of the European South-North gradient observed by Leonhardt et al. (2013). From the cold North to the warm Southern Mediterranean European countries, both crop dependency on insect pollination, bee diversity, and stability of pollination services increased. The higher pollination rate and seed yield impact were observed in the hotter and dryer location, in Pakistan (Ali et al., 2011; Munawar et al., 2009; Shakeel and Inayatullah, 2013).

Water availability, temperature, insect species, soil characteristics and several other parameters substantially vary with geographical localisation and climate. These parameters will affect the plant physiology (leave indexes, chlorophyll efficiency, flowering period), the potential stress conditions, the pest, and pollinator nature and behaviour etc. The meteorological context will also influence the farmer to choose the sown cultivar: spring vs winter and early- vs late- flowering cultivar. But none weather conditions nor temperatures nor precipitations were specified in the analysed papers.

e) Factors interactions

All the studies of the corpus globally only confirm the role of a scale effect. On *Brassica napus*, insect pollination has a physiological sense on the flower level, increasing the single flower productivity. In contrast, there is no such effect on the plant level (Lerin and Rivault, 1982) (cf. compensations mechanisms) and even less on the plot level, where pollination quality does not seem to be a limiting factor for yield. Pooling together the main factors determining the yield (pollinator visits, pest attack, soil pH and nitrogen fertility) for two close cultivars, explained only 20% of the yield variance. The random factors, associated with unmeasured parameters explained 55% of these differences (Bartomeus et al., 2015). Seed yield is strongly affected by several factors such as genetic determination (varying across cultivars, Pavlista et al., 2011; Sana et al., 2003), resource limitations or availability, mainly N and water, (Pavlista et al., 2011; Rathke et al., 2006), and growing conditions. The main growing conditions impacting yield are soil pH (Bartomeus et al., 2015), sowing density, agro-climatic conditions (Angadi et al., 2003; de Koning and van Diepen, 1992; Zajac et al., 2013) and pest pressure (Bartomeus et al., 2015; Brown et al., 1999). Without taking into consideration the effect of these numerous parameters, it is hard to isolate the seed yield part only link to insect pollination from the influence of other parameters.

Therefore, we suggest paying particular attention to the homogeneity of at least the main soil characteristics, pest pressure, cultivar and cropping parameters among plots of a study. Publishing such data could help further comparisons among studies.

IV. CONCLUSION

Besides insect pollination effect, the yield of *Brassica napus* is impacted by other numerous factors. The main impacting factors are i) the genetic potential of the cultivars and their capabilities to compensate damage, ii) the agro-climatic conditions, i.e. wind, soil quality, water and nutrient availability, iii) the plant health (infestation by pests and diseases) and last, the use of pesticides and other plant health products. However, these factors were rarely taken into account while evaluating the impact of pollinating insects on seed yields. That makes difficult the observation of clear links between rape pollination by insects and yields increase. The comparisons of conclusions from different studies are only approximate due to several unmentioned field parameters. For further research, we suggest that local and regional scale studies are needed to understand the impacts of insect-mediated pollination on *Brassica napus* pollination. For such observation, we suggest the following recommendations.

Plots will be delimited in as homogenous as possible real fields and located following the FAO standardised protocols. The same cultivar(s) of oilseed rape should be used across treatments and the field management history and soil characteristics should be obtained and provided with data to help further comparisons among studies. When cages are needed we recommend using mesh size as large as possible (>2.5 mm) to avoid insect flux and to allow

wind flow as much as possible. The measurement of airborne pollen quantity both inside and outside of the cages will be used to assess the cage effect.

Ideal observations would be conducted on the field with a local increase in pollinator populations and visiting rate observations to estimate pollinator density after the event. Control plots of low insect visitation could be obtained using insect repellent molecules. Ultra-high insect densities are not needed to study pollinator effect on yields. Working with moderately high insect densities (up to 2-3,000 insect-visitations per 100 m²/h) seems more realistic and will allow easier field-level extrapolation of the results. In addition to pollinator observations, we recommend the use of classical yellow water traps to estimate and compare levels of pollen beetle infestation among plots.

The total seed weight produced per area unit will be the primary data considered to measure seed yield. Seed quality parameters are good complementary parameters to estimate the economic profit of oilseed rape production improvement. The seed quality parameters should be measured on seeds from whole ripe plants. If not dry enough, these seeds should be dried to reach a water content corresponding to optimal storage and standard seed market quality moisture, from 7 to 9%. We recommend for future studies including additional information on plant morphology and development (e.g. flowering time, the number of flowers and branches per plant, fruit abortion...) under each condition for improving the knowledge about compensation mechanism and their effects at the plot level.

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Since 2005, flower-strip Agri-Environment Schemes (AES) are subsidised in Wallonia to create insect friendly areas in fields. Flower-strips can benefit pollinator insects, which can be needed by entomophilous crops as *Brassica napus*. But insect diversity and abundance hosted by Walloon flower-strips and flower resources (pollen and nectar) they provide are not well known. And the exact insect pollination *Brassica napus* needs are still under debate.

In this thesis, we investigated flower-visiting insect diversity and abundance in both *B. napus* fields and flower-strips. We assessed the dependency of new *B. napus* cultivars to insect pollination and the flower resources provided by flower-strips. We particularly focused on insect-flower interactions networks to understand which insect and plant species interacted.

Apis mellifera was the only flower visitor in *B. napus* fields, visiting only 1% of the flowers. But the pollination deficit was low (0.1), studied cultivars were poorly impacted by insect pollination.

In flower-strips we observed 85 morphotypes of flower visitors, dominated by few common polylectic species (*Bombus lapidarius* OTU, *Apis mellifera* and *Eristalis tenax*). We found insects visiting 40 of the 78 sown and spontaneous plant species flowering on flower strips. *Centaurea jacea* (sown) provided most of the flower resources (31% of nectar, 53% of pollen) and received 44% of the insect visits, and Fabaceae species provided 52% of *Bombus* pollen diet. Nevertheless, flower-strips provided very few flower-resources in spring, and most of the insect species were poorly abundant.

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