Poll-ole-GI: Rural Green Infrastructures for Pollinator Protection



Edition: Poll-ole-gi

COORDINATION: UBUCOMP - Universidad de Burgos

DESIGN, TYPESET & GRAPHICS: Un Cuarto Propio

ILLUSTRATIONS: Sara Martel Martín & Beatriz Martínez de la Hoz Un Cuarto Propio (UCP)

PRINTING: AMABAR (BURGOS)

FIRST EDITION: June 2019. Legal DEPOSIT: iterreg iddoe endoe

Citation: Project Poll-Ole-Gl (2019) Rural Green Infrastructures for Pollinator Protection. Technical Guide. https://www.pollolegi.eu

COORDINATION:



DE BURGOS



UBUCOMP UNIVERSIDAD DE BURGOS

PARTNER INSTITUTIONS:



Universidad Autónoma Social-Ecological



de Madrid



Systems Lab

BIOLOGICAL STUDIES CHIZÉ UNIVERSITY OF COIMBRA

CNRS - CENTER OF

CENTRE FOR FUNCTIONAL ECOLOGY

INRA NOUVELLE - AQUITAINE - POITIERS. U.E. APIS

UAM SOCIAL-ECOLOGICAL SYSTEMS LABORATORY

Poll Ole GI O ()

The southwestern European region or SUDOE covers large areas of the Iberian Peninsula and southern France, where oilseedgrowing plays an important role in the agricultural sector in terms of occupied area and economic impact. These crops, and sunflower and rapeseed in particular, share the need for entomophilous pollination, mainly performed by domestic bees (*Apis melifera*) and other wild bees. The continuous decline of pollinating insect populations, especially wildones, is a significant threat for these crops' profitability.

Different factors such as habitat alteration, intensive use of pesticides or climate change are behind the loss of one of the most important ecosystem services, pollination, which is crucial for the sustainability of agricultural production of more than 70% of SUDOE cultivated species. The Poll-Ole-GI Project promotes the creation of green infrastructure (GI) linked to oilseed crops in order to provide refuge and food resources for pollinators. The main goal is to guarantee the persistence of pollinators and pollination services for these crops, in spite of the upcoming threats such as climate change, environmental pollution, exotic species entry and pathogen actions. They all constitute a challenge in particular for the SUDOE region due to their special impact on this ecosystem.

In this Technical Guide, Poll-Ole-GI addresses key aspects such as the definition of Green Infrastructures (GI) including the most efficient floral mix composition, and an efficient way of monitoring the impact on the abundance and diversity of pollinators and also on the linked crops. Finally, a cost / benefit analysis of these measures in terms of crop yield and pollinator biodiversity will be assessed.

CONTENTS







Plants and Insect Pollinators one does not exist without the other







Plants and Insect Pollinators What is a pollinator? one does not exist without the other Crop pollinator dependence in study areas

Introduction





PLANTS AND INSECT POLLINATORS one does not exist without the other

crucial step in the reproduction for plants is pollination, namely the transport of pollen grains (carrying the male gametes), during the pollen release, to the stigma (the female part) during the receptive period. This process can be achieved by wind, water, animals or through self-pollination when a plant is able to deposit its pollen on its own stigmas. Most plants rely on different strategies for pollination and many depend on insects to ensure successful reproduction.

Indeed, insects are good candidates for pollination, as they are numerous and exceptionally diversified, can carry pollen successfully, fly and move quickly for their size and continuously explore floral resources. Consequently, studies to date indicate that up to 78% of all flowering plant species in temperate zones rely, totally or partially, on insects to achieve pollination¹.

If plants depend on pollinators to reproduce, pollinators depend on plants to feed and floral resources are fundamental to maintain insect populations. Insects are attracted by the colorful and fragrant flower; they perceive it as a signal for food. When they visit the flower to collect nectar or pollen, the pollen grains stick to their hairs and this pollen is moved to another plant as the insect continue its visits. Pollination is thus a mutualistic interaction that benefits both plants and insects.



Picture by Ingo Doerrie on Unsplash



A FRAGILE MUTUALISM

Despite their crucial role, pollinator populations are overall declining across the countries where they are being monitored. According to the first global assessment of pollinators from IPBES², in Europe, 37% of bees and 31% of butterflies are currently declining. That number is probably under estimated as it excludes 57% of the bee species for which the data is insufficient.

Moreover, a recent study suggests that this is an overall pattern for all insects, showing a 75% decline over 27 years in the total flying insect biomass in Germany³. Insect decline has been associated with numerous factors. Habitat loss, fragmentation and deterioration, pesticide use, and climate change are identified as the main factors responsible for the loss in abundance and diversity of insects. Insect decline in turn has significant consequences for the pollination of wild plants and crops. Limited pollination services will negatively impact the maintenance of wild plant populations and crop yield.

[1] Ollerton J., Winfree R., Tarrant S. (2011). How many flowering plants are pollinated by animals? Oikos 120: 321-326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

[2] IPBES (2016): Assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. S.G. Potts, V.L. Imperatriz-Fonseca, H.T. Ngo, J.C. et al. (Eds.). Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), Bonn, Germany. https://www.ipbes.net/assessment-reports/pollinators
[3] Hallmann C.A., Sorg M., Jongejans E., Siepel H., Hofland N., Schwan H., Heinz Schwan, Stenmans W., Müller A., Sumser H., Hörren T., Goulson D., de Kroon H. (2017) More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS ONE 12(10): e0185809.

Picture:; Wild pollinator. Andrés Velayos

WHAT IS A POLLINATOR?

pollinator is an organism that is able to move pollen from the anthers of a flower to the stigmas of the same or a different flower. Pollinators include a diverse group of animals. Although dominated by insects in most areas, in some other parts of the world, bats, birds, reptiles and primates can also contribute to plant reproduction.

In Europe, the most effective and best known pollinators are bees. Honeybees, bumble bees and wild bees (order Hymenoptera) all depend on floral resources for their growth, and their anatomy is well suited to pollen transportation and nectar harvesting. They have microscopically branched hair that is very effective at trapping pollen grains. In addition, the flight of bees accumulates an electrostatic charge that attracts and maintains pollen on the pollinator's body.

Still, there are other groups of insects that, because of their different features, are important pollinators of certain plant species. Wasps and ants (Hymenoptera) contribute to a lesser extent in quantity but add to the diversity of pollinator insects.





By contrast, the flies (Diptera), syrphid (hoverflies) and non-syrphid groups, contribute largely to the pollination of small and open flowers, and is an important pollen vector for apiaceous group plants, such as Foeniculum or Daucus. Another group of fascinating pollinators are butterflies (Lepidoptera), especially adapted to collecting nectar in narrow tubular flowers. Moths, nocturnal Lepidoptera, also participate in the pollination of many plants, including those which are also pollinated during the day¹. Finally, insects of the order Coleoptera, such as Meligethes, feed on flowers and eventually carry pollen from flower to flower, although they are not considered as relevant pollinators. In fact, all pollinators are flowering insects but not all flowering insects are pollinators. This means that not all insects visiting flowers for pollen and nectar are good pollinators. They come to flowers to feed off pollen or nectar such as beetles which eat stamen and pollen but don't necessarily carry them to other flowers.



If all the insects mentioned above do not play the same role in the quantity of flowers pollinated, they all participate and have participated in the great diversity of plants that we have inherited today. In fact, a diverse pollinator community will contribute to the successful pollination of a plant community, including wild plants and crops and it is well documented that a diverse Illustration: Bee, UCP.

community of pollinators promotes stable and efficient crop yields.

[1] Knop E., Gerpe C., Ryser R., Hofmann F., Menz M.H.M., Trosch S., Ursenbacher S., Zoller L., Fontaine, C. (2018) Rush hours in flower visitors over a day-night cycle. Insect Conservation and Diversity 11, 267-275. https://doi.org/10.1111/icad.12277 Introduction

CROP POLLINATOR DEPENDANCE

in study areas

n Europe, 84% of crops depend on pollinators to attain profitable yield¹. The great majority of pollinator-dependent cultivated plants are fruit crops (such as apples, pears, or strawberries) and oleaginous crops (sunflower, rapeseed, flax)². These crops represent 35% of world agricultural production³.

Europe is the first producer of rapeseed in the world with 21.8 million of tons (6.7 million ha) and the third producer of sunflower with 9.5 million of tons (4.4 million ha). Hence, oleaginous crops are of great importance in European agriculture. Rapeseed (*Brassica napus* L.) and sunflower (H*elianthus annuus* L.) represent respectively the fifth and the third most important crops in Europe. Sunflower is an important crop in southern Europe. This crop grows in warm and dry weather whereas rapeseed is better adapted to temperate and cool regions. Indeed, 31% of the European sunflower seed area is cultivated in France and Spain (4th and 5th EU producers) and 22% of the total European rapeseed area is cultivated in France (2nd EU producer)⁴.

Consequently, oleaginous crops play an essential economical role in Europe. Biodiesel production depends in large part on rapeseed and sunflower, with 68% of the European biodiesel coming from these oleaginous crops. Moreover,

 Williams, I. H. (1994). The Dependence of Crop Production within the European Union on Pollination by Honey Bees. Intercept Limited, Andover, UK.
 Gallai N., Salles J-M., Settele J, Vaissière BE. (2009). Economic valuation of the vulne-

[2] Gallal N., Salles J-M., Settele J, Vaissiere BE. [2009]. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecological Economics 68: 810-821. https://doi.org/10.1016/j.ecolecon.2008.06.014



Picture: Experimental plot POLL-OLE-GI, Cuenca, UAM

sunflower and rapeseed are grown for their high-fat seeds and are used for human consumption and cosmetics. Europe is also the second producer of honey after China. (European Union: Agriculture and Rural Development). In 2017, the production of rapeseed and sunflower honey were respectively⁴ the second and third largest agricultural production in France⁵.

Both sunflower and rapeseed produce bright yellow flowers that are rich in nectar, providing an abundant and easily accessible resource attractive to many insect pollinators. These insects are all the more active in these cultures as it is currently difficult to find their food on an agricultural plain. Livestock and their associated grasslands are disappearing, hedgerows are being removed, the edges of fields are mowed too often or are nonexistent, and the diversity of crop weeds is depleted each year by the application of herbicides and nitrogen fertilizers.

Nowadays pollinators no longer benefit from farming practices and their populations keep dwindling. Yet, farmers still depend on insects for crop yield, as is clear for rapeseed and sunflower.

 [3] Klein A.M., Vaissière B.E., Cane J.H., Steffan-Dewenter I., Cunningham S.A., Kremen C., Tscharntke T. (2007) Importance of pollinators in changing landscapes for world crops. Proceedings Biological Sciences 274: 303-313. https://doi.org/10.1098/rspb.2006.3721
 [4] Agri-Food Data Portal (2017) Agri-Food Markets. https://agridata.ec.europa.eu/extensions/DataPortal/agricultural_markets.html
 [5] European Commission. DG Agriculture and Rural Development https://ec.europa.eu/agriculture/honey_en











Knowing pollinator's needs Honeybee key actor



Resources for honeybee and beekeepers

Pollinators' needs



KNOWING POLLINATORS' NEEDS

ollinators live in various biotopes such as meadows, hedges and bushes, moors, clearings, cultivated crops, fallow land etc.

Their behaviors stems from primary needs such as to feed, reproduce and ensure the survival of their offspring. Only adults are capable of searching for the most suitable site for themselves and their brood. They look for an area that provides either material supplies or refuges to ensure reproduction, build a nest and lay eggs, as well as enough available food to ensure the survival and development of their progeny. Sites explored for food may influence the pollinators' life cycle as their dietary requirements sometimes vary during different stages of their lifetime.

Most wild bee species live in solitary or occasionally in different social forms. A few species like honeybees are eusocial insects. For this particular bee, thousands of multigenerational individuals within a colony Illustration: Borago officinalis, UCP





cooperate, supporting one queen, which ensures colony cohesion and reproduction. Their nest is formed in a hive made by humans or, for feral ones, in trees or old walls. They are honey producers and colonies survive during the winter, so that the queen can live for several years.

Bumblebees are social insects as well, they live in small colonies

Picture: Hylaeus occupying a nest box, UAM



(several hundred workers). They have an annual life cycle, colonies are founded in spring or early summer and they produce honey for self-consumption, and only the next queens-to-be will survive the winter^{1, 2}. They build nests with vegetal materials provided from their immediate environment. Preferences of nesting sites may vary between species in term of type of habitat, location and position³. Wild bees often have a very short development cycle and their food needs are great and instant.

The wild bee community largely benefits from semi-natural environments with diversified plants that provide food through the entire season, which is needed by the successive species. They also use materials such as mud, leaves, resin and flowers to build cells where they will lay eggs and raise their brood depositing a mixture of nectar and pollen for the larvae development. The diversity of nesting behaviors is very great. Some of them settle their nest in the ground or in wood, with or without cover (in galleries hollowed in the soil or in dead plants) others may live in holes in walls, in grass strips, perennial crops, weeds².

Besides providing nesting sites, all these natural elements are also wildlife corridors connecting different resources and allowing pollinators to move from one site to another, switching to other floral food supplies. It has been suggested that bumblebee queens do not target areas with locally high floral resources to settle their nest⁴.

For their diet, pollinators generally look for two main products. One of them is a sweet substance called nectar which is produced by plants to attract and reward pollinators. It is produced by nectariferous glands within the flower, which are not always easily accessible. The nectar is the main energy supply in pollinators' diet. Insects eat it directly or after transformation to honey (honeybees). It contains sugar elements such as fructose, glucose, saccharose and, to a lesser extent, some amino-acids, lipids, terpenes, toxic compounds, antioxidants and minerals. The nectar amount and proportions of the different constituents may vary and will affect the attractiveness of the plant. These features can evolve greatly depending on different factors such as, the plant species or variety, the dynamic of nectar secretion (depending on time of blooming and pollinator exploitation), environmental factors such as temperature and humidity. the availability of water and nutrients^{5,6}. In some case, nectar foraging can be replaced by honeydew foraging to collect a sugar-rich sticky liquid generally secreted by aphids as they feed on plant sap.

The other collected product is pollen grains that are vital components for the development of larvae, the adult body constitution and their physiology, the immune defenses as well as the production of eggs. It contains proteins, lipids, glucides, vitamins, sterols, minerals and amino-acids. Each plant specie has a specific pollen composition so that the nutritional quality for the insects is variable.

For a generalist bee like the honeybee, a monofloral pollen diet, may cause a deficiency, weakening and increasing the sensibility to diseases and other threats coming from their environment. Thus, the bees need to have a diversity of plants available in their surrounding landscape during the rearing season^{7,8}.



Bees feeding on polyfloral pollen live longer than bees feeding in monofloral pollen. Moreover, pollen diversity and pollen quality also influence bee health and physiology⁸. Nectar and pollen, as well as water and propolis (vegetal resin), are the main supplies brought by the honeybee foragers into the colony for its development, reproduction and overwintering. What is important for bee nutrition is getting a varied and steady diet during their cycle. Not surprisingly, the proximity of wooded areas provides more favorable conditions for bee colonies in early spring⁹.



Illustration: Xylocopa nest, UCP

[1] Tasei J.N., Aupinel P. (2008) Nutritive value of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers (Bombus terrestris, Hymenoptera : Apidae). Apidologie 39: 397-409. https://doi.org/10.1051/apido:2008017

[2] Pouvreau A. (2004) Les Insectes Pollinisateurs. Delachaux et Niestlé, Paris, FR.

[3] Goulson D. (2003) Bumblebees: Their Behavior and Ecology. Oxford University Press, Oxford, UK.

[4] O'Connor S., Park K.J., Goulson D. (2017) Location of bumblebee nests is predicted by counts of nest-searching queens, Ecological Entomology, 42, 731-736. https://doi.org/10.1111/een.12440

[5] Bruneau E. (2012) Nectaires et Nectar, Fiche Technique, Flore et Miellées, http://www.cari.be/abco/2012/ [6] Cerruti N., Allier F. (2018) Ch13. La variété d'une plante cultivée peut-elle influencer l'activité de butinage de l'abeille domestique et sa production de miel? Le cas du tournesol. In Decourtye, A. (Dir.) Les Abeilles des Ouvrières Agricoles à Protéger. Éditions France Agricole, p.150-161.

[7] Di Pasquale G. (2014) Influence of pollen diet on the honeybee health, in Apis mellifera L. PhD Thesis. Université d'Avignon.

[8] Di Pasquale G., Alaux C., Le Conte Y., Odoux J-F., Pioz M., Vaissière B.E., Belzunces L.P., Decourtye A. (2016) Variations in the Availability of Pollen Resources Affect Honey Bee Health. PLoS ONE 11(9): https://doi.org/10.1371/journal.pone.0162818

[9] Odoux J-F., Aupinel P., Gateff S., Requier F., Henry M., Bretagnolle V. (2014) ECOBEE: a tool for long-term bee colony monitoring at landscape scale in West European intensive agrosystems. Journal of Apicultural Research 53: 57-66. https://doi.org/10.3896/IBRA.1.53.1.05





HONEYBEES

first actors in rapeseed and sunflower pollination

he European continent hosts around 2000 bee species 10 % of the worldwide bee diversity^{1,2}. Among this great diversity, our honeybee, *Apis mellifera* is just one species.

While wild bees forage more frequently on wild plant species and focus on few genera of plants (oligolectic), honeybees and bumblebees are called generalist pollinators (polylectic) because they forage on a large variety of flowers to collect either nectar or pollen. Cultivated crops seem to attract more generalist pollinators than specialists^{3,4,5} with bees and bumblebees being considered as efficient pollinators.

In rapeseed and sunflower crops, honeybees are the most frequent pollinators and their food collection is substantial^{6.7}. In these crops, considering all pollinators, respectively 53% to 95% and 85% to 98,2% are honeybees, followed by bumblebees, some wild bees and finally some diptera including hoverflies and other insects foraging for nectar and/or pollen^{8.9}. Conversely, spontaneous flora,

[1] Nieto A., Roberts S.P.M., Kemp J. et al. (2014) European Red List of bees. Luxembourg: Publication Office of the European Union. https://bit.ly/2vbDjm7
[2] Rasmont P., Devalez J., Pauly A., Michez D., Radchenko V.G. (2017) Addition to the checklist of IUCN European wild bees (Hymenoptera: Apoidea). Annales de la Société Entomologique de France 53: 17-32. https://doi.org/10.1080/00379271.2017.1307696
[3] Rollin O., Bretagnolle V., Decourtye A., Aptel J., Michel N., Vaissière B.E., Henry M., (2013) Differences of floral resource use between honey bees and wild bees in an intensive farming system. Agriculture, Ecosysems & Environment 179: 78-86. https://doi.org/10.1016/j.agee.2013.07.007

[4] Holzschuh, A., M. Dainese, González-Varo J.P. et al. (2016) Mass-flowering crops dilute pollinator abundance in agricultural landscapes across Europe. Ecology Letters 19: 1228-1236. https://doi.org/10.1111/ele.12657

[5] Rollin, O., Benelli G., Stefano B., Decourtye A., Wratten S.D., Canale A., Desneux N.



Picture: Hylaeus (Prosopis) variegatus on sunflower. Óscar Aguado

attracting wild bees in particular, participate strongly in the ecosystem equilibrium through eco-systemic services regulation.

In agricultural landscapes, rapeseed and sunflower provide honey flows attracting beekeepers, some of them work directly with farmers to provide pollination services and take advantage of honey production. Consequently, the oleaginous crops benefit both stakeholders. One enhances crop yield and the other benefits from the availability of floral resources to harvest honey. Several studies show the benefits of honeybee pollination on yield and seed quality of sunflower and rapeseed^{9,10}. Nonetheless, pollination by honeybees does not replace wild bee pollination, it is a complementary help to cultivated crops¹¹. Pollinator diversity is more relevant than pollinators density to achieve high yields in most entomophile crops¹².

(2016) Weed-insect pollinator networks as bio-indicators of ecological sustainability in agriculture. A review. Agronomy for Sustainable Development 36: 22 pp. https://doi.org/10.1111/ ele.12657

[6] Requier, F., Odoux J-F., Tamic T., Moreau N., Henry M., Decourtye A., Bretagnolle V. (2015) Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. Ecological Applications 25: 881-890. https://doi.org/10.1890/14-1011.1
[7] Odoux J-F., Feuillet D., Aupinel P., Loublier Y., Tasei J.N., Mateescu C. (2012) Territorial biodiversity and consequences on physico-chemical characteristics of pollen collected by honey bee colonies. Apidologie 43, 561-575. https://doi.org/10.1007/s13592-012-0125-1
[8] Carvalheiro, L. G., Biesmeijer J.C., Benadi G. et al. (2014). The potential for indirect effects between co-flowering plants via shared pollinators depends on resource abundance, accessibility and relatedness. Ecology Letters 17: 1389-1399. https://doi.org/10.1111/lel.12342
[9] Fougeroux A., Leylavergne S., Guillemard V., Geist O., Gary P., Cenier C., Caumes-Sudre E., Senechal C., Vaissière B. (2017) Effet de l'activité des insectes pollinisateurs sur la pollinisation et le rendement de tournesol de consommation, OCL 2017, 24(6), D603, EDP Science. https://doi.org/10.1051/ocl/2017050

[10] Kleijn D., Winfree R., Bartomeus I. et al. (2015) Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. Nature Communications 6: 7414. DOI: https://doi.org/10.1038/ncomms8414

[11] Perrot T., Gaba S., Roncoroni M., Gautier J-L., Bretagnolle V. (2018) Bees increase oilseed rape yield under real field conditions. Agriculture, Ecosystems & Environment 266: 39-48. https://doi.org/10.1016/j.agee.2018.07.020

[12] Garibaldi L.A., Steffan-Dewenter I., Winfree R. et al. (2014). "Wild pollinators enhance fruit set of crops regardless of honey bee abundance." Science 339: 1608-1611. https://doi.org/10.1126/science.1230200

What is important for pollinators?

RESOURCES FOR HONEYBEES AND BEEKEEPERS

Availability of resources and risks

he mass blooming of rapeseed and sunflower crops produce a large density of floral resources. These two crops are easy for bees to forage and provide large quantities of nectar and pollen to pollinators during critical periods of their development. However, on a landscape scale, the flowering runs for a short period of time, from April-May (2 to 5 weeks) for rapeseed and from July-August (2 to 3 weeks) for sunflower. Consequently, during the intercrops period, there is a scarcity of resources in the landscape. Honeybees are affected by this resource shortage and colonies are more sensitive to diseases, virus and pesticide loads.

Requier et al.¹ have shown a carryover effect of a severe pollen shortage period on the survival of the offspring even after overwintering. So, floral scarcity between crops can cause significant declines in honeybee colonies with serious economic consequences for beekeepers².

When crop floral resources are not available, bee communities depend on resources from wild plants in semi-natural areas such as, woods and bushes, meadows, weeds and horticultural plants³. However, the availability and the continuance of floral resources is strongly affected by the different factors largely discussed nowadays. The reduction of semi-natural habitats and their fragmentation, the exposure to pesticides. monocultures and uniform landscapes with severe loss of biodiversity, the changing climate conditions, are among the main factors which brings together a broad consensus among scientists⁴.

Today, solutions have to be proposed to protect the biodiversity of pollinators and preserve their natural habitats to ensure the sustainability of pollination ecosystem services, beneficial to beekeeping activity, agriculture, and biodiversity.

[1] Requier F, Odoux J.F., Henry M., Bretagnolle V. (2017) The carry-over effects of pollen shortage decrease the survival of honeybee colonies in farmlands. Journal of Applied Ecology. 54: 1161-1170. https://doi.org/10.1111/1365-2664.12836
[2] Testu P. (2018) Menace de diminution des surfaces de tournesol et de colza. Réseau Biodiversité pour les Abeilles, Communiqué de Presse, 29 août 2018, 2 p. https://www.reseau-biodiversite-abeilles.fr/index.php/press-release/menace-de-diminution-surfaces-de-tournesol-de-colza/
[3] Requier, F., Odoux J-F., Tamic T., Moreau N., Henry M., Decourtye A., Bretagnolle V. (2015) Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. Ecological Applications 25: 881-890. https://doi.org/10.1890/14-1011.1
[4] Simon-Delso N., Amaral-Rogers V., Belzunces L.P. et al. (2015) Systemic insecticides Ineonicotinoids and fipronil]: trends, uses, mode of action and metabolites. Environmental Science and Pollution Research International 22: 5-34.

https://doi.org/10.1007/s11356-014-3470-y











The Consortium

Case study: LTSER Plaine et Val de Sèvre (France)



Case studies: Burgos and Cuenca (Spain)

What can be done..?



THE CONSORTIUM

transnational multidisciplinary team was established based on strong capacities about pollinators monitoring and assessment amd risk evaluation in the SUDOE Region.

Core partners are UBUCOMP, University of Burgos -ES (Coordinator of the Project), the Social - Ecological Systems Lab -Universidad Autónoma de Madrid - ES, Centre for Functional Ecology, University of Coimbra - PT, Centre for Biological Studies of Chizé - CNRS - FR and INRA Nouvelle-Aquitaine-Poitiers - U.E. APIS - FR.

These partners are supported by wide social networks in every country, involving small farmers and beekeepers, environmental groups, researchers, policy makers, etc. bringing together different perspectives on pollinators.





UC - CENTRE FOR FUNCTIONAL ECOLOGY





What can be done to improve pollination

<u>i</u>



CASE STUDY: LTSER Plaine et Val de Sèvre (France)



Experimental sites in LTSER Plaine et Val de Sèvre case study (France)

Sunflower fields
Rapeseed fields
LTSER



POLL-OLE-GI IN ACTION

LTSER (FRANCE)

1

2

3

Site selection LTSER (450 km²) in western France

Inputs (fertilizers and persticides) reduction:

Inputs reductions on field margins in oleaginous crop real conditions. (The same experiment is implemented on cereal crops.)

Number of experimented fields:

In 2018 : 13 sunflower fields and 12 rapeseed fields. In 2019 : 15 sunflower fields and 11 rapeseed fields.





What can be done to improve pollination



CASE STUDY: LTSER Plaine et Val de Sèvre (France)



I

EXPERIMENT DESIGN



SPECIFICATIONS:

2 areas are delimited in the margins of the field.

In one area (in red on the design) the farmer reduces herbicide inputs or mechanical weeding by half. In the other area (in I yellow), the farmer applies only half of nitrogen inputs. l

I The distribution of the 2 areas builds an experimental design of 8 zones (Z) where insects, weeds and crops are monitored I through different protocols.



What can be done to improve pollination



CASE STUDY: LTSER Plaine et Val de Sèvre (France)



RUNNING THE EXPERIMENT:



WEEDS MONITORING:

Botanical surveys No-crops areas observations Weeds samples weigh evaluation



7 CROPS MONITORING: Yields samples Monthly, or fortnighly growth surveys Pollination exclusion

Nectar harvesting



33

What can be done to improve pollination

02

CASE STUDY: LTSER Plaine et Val de Sèvre (France)



MAIN RESULTS:



[1] Perrot T., Gaba S., Roncoroni M., Gautier J-L., Bretagnolle V. (2018) Bees increase oilseed rape yield under real field conditions. Agriculture, Ecosystems & Environment 266: 39-48. https://doi.org/10.1016/j.agee.2018.07.020

[2] Catarino R., Gaba S., Bretagnolle V. (2019) Experimental and empirical evidence shows that reducing weed control in winter cereal fields is a viable strategy for farmers. Scientific Reports Scientific Reports 9, 9004. https://doi.org/10.1038/s41598-019-45315-8





"Herbicides (Figs. B and D) and insecticides (Figs. A and C) in oilseed rapefields do not significantly increase yieds (Figs. A and B), while they significantly reduce gross margins (Figs. C and D) of farmers" What can be done to improve pollination

Ĩ



CASE STUDIES: Burgos and Cuenca (Spain)



Experimental sites in Burgos

10km 🛏 🗕



Experimental sites in Cuenca

500m ------



POLL-OLE-GI IN ACTION

BURGOS SITE

Site selection



CUENCA SITE

Site selection 11 plots across 5 municipalities

Green Infrastructure implementation:

Adjacent plots for sunflower crop 1.250 m² annual flowers mix

Control: 11 crops next to semi-natural environment and 11 control plots.

Installation of two nest boxes per Green Infrastructure

Running the experiment Pollinators monitoring, melliferours flowering, sunflower production estimation, quality of seeds

11 plots across 6 municipalities

Green Infrastructure implementation Adjacent plots for sunflower crop 2.500 m² annual flowers mix

Control: 11 crops next to fallow lands and others next to natural vegetation

Installation of two nest boxes

per Green Infrastructure

Running the experiment

Pollinators monitoring, melliferous flowering, sunflower production estimation, quality of seeds

What can be done to improve pollination



CASE STUDIES: Burgos and Cuenca (Spain)



GREEN INFRASTRUCTURES IMPLEMENTATION



SITE SELECTION:

I

6 experimental dryland farming areas (min. 1ha) in which sunflower is introduced in rotation with cereals. A field adjacent to the selected crop will have sunflower the following year.

11 Green Infrastructures (EGI) were implemented at each case study. Next to these plots, are located others nearby to natural vegetation (NGI) and others with no natural vegetation nearby (NON) for control.

I

I

I

A plot of 10 km around the Green Infrastructures has been analyzed in terms of crop species and crop management, within a 5 to 10 year period.



FLORAL MIX DETERMINATION

Selection criteria:

- Annual local species
- Food providers for pollinators
- Phenology for continuous flowering
- Taxonomy diversity
- Seeds availability

Floral mix was adapted from the mixture "Operación Polinizador" (Syngenta) to the particular conditions of Burgos and Cuenca by Social-Ecosystem Lab (UAM) and tested in 3x3 m microplots by UBUCOMP (UBU).

In Burgos' plots, compost addition (10 t/ha, half of the plot)







Borago officinalis



帚

Calendula arvensis



春春春春春

Coriandrum sativum



春春春春

Salvia pratensis



春春春春 Melilotus officinalis



春春春春

Diplotaxis erucoides



春春春春 Echium plantagineum

Nigella damascena

帚



silene vulgaris

杳

Sinapis alba







FLORAL MIX EVOLUTION

| Plants | Family | 2017 | 2018 |
|-----------------------|------------------------|------------------|------|
| Borago officinalis | Boraginaceae | 15% | 20% |
| Calendula arvensis | Ateraceae | 15% | 20% |
| Coriandrum sativum | Apiaceae | 15% | 20% |
| Medicago sativa | Fabaceae (Leguminosae) | 10% | 20% |
| Melilotus officinalis | Fabaceae (Leguminosae) | 10% | 0% |
| Sinapsis alba | Cruciferae | 10% | 5% |
| Vicia sativa | Fabaceae (Leguminosae) | 10% | 0% |
| Echium plantagineum | Boraginaceae | <mark>5%</mark> | 0% |
| Salvia pratensis | Lamiaceae (Labiatae) | 5% | 0% |
| Nigella damascena | Ranunculaceae | <mark>3</mark> % | 0% |
| Diplotaxis erucoides | Brassicaceae | 1% | 15% |
| Silene vulgaris | Caryophyllaceae | 1% | 0% |



Time of flowering

| 春 | ND |
|-------|-----------|
| 春春春 | Medium |
| 春春春春 | Good |
| 春春春春春 | Excellent |

Meliferous potential

What can be done to improve pollination



CASE STUDIES: BURGOS AND CUENCA (ES)



RUNNING THE EXPERIMENT:



Picture: Experimental plot in Burgos (UBU)



NON - NO VEGETATION NEARBY

NGI - NATURAL GREEN INFRASTRUCTURE

EGI - ENHANCED GREEN INFRASTRUCTURE

- Nest boxes
- Pan-traps





CASE STUDIES: BURGOS AND CUENCA (ES)



MAIN RESULTS:



5

FLORAL MIX RESULTS:

 The green infrastructures have peaks of flowering that benefits the abundance of wild bees.

I

2.Semi-natural habitats provide food and refuge to a large number of wild bee species

3. There are no differences in the presence of bees inside the sunflower fields at the increasing distances from the EGI.

SOWED AND WEED SPECIES ABUNDANCE



47



MAIN RESULTS



BEES ABUNDANCE AND DIVERSITY

CASE STUDIES: BURGOS AND CUENCA (ES)

1. The main pollinator of sunflower intensive crop is the honeybee.

2. The visitation rate of wild bees is influenced by the distance to Green Infrastructures.

3. Wild bees are not the main pollinators, but they contribute to increasing the agroecosystems' resilience.



DIVERSITY OF WILD BEE SPECIES FOUNDED



What can be done to improve pollination



CASE STUDIES: BURGOS AND CUENCA (ES)



BEES VISITATION RATE ACROSS THE DIFFERENT SYSTEMS



NON - No Vegetation Nearby EGI - Enhanced Green Infrastructure

NGI - Natural Green Infrastructure

I

I



I

I

I

h

VISITATION RATES AND ABUNDANCE:

1. Visit rates of wild bees to sunflower heads was also significantly higher in fields adjacent to Green Infrastructure I

2. Abundance of wild bees was significantly higher in sunflower fields adjacent to Green Infrastructure than in fields located adjacent to seminatural areas.



Wild bee abundance (number of individuals captured)

What can be done to improve pollination



CASE STUDIES: BURGOS AND CUENCA (ES)



MAIN RESULTS:





I

I

L

I





SOCIO-ECONOMIC IMPACT:

A cost-benefit analysis per hectare was performed taking into account the current investment and maintenance costs of Green Infrastructures, and the benefits derived from the increased seed set observed in the sunflower fields with Green Infrastructures compared to those located close to natural habitats,.

The Net Present Value analysis (applying a 5% discount rate) revealed that Green Infrastructure implementation becomes profitable between the third and the fifth year after installation, depending on the different scenarios of subsidy; being more efficient in the long term under the scenario with an indirect agroenvironmental annual subsidy.











Influence of agricultural

management practices on bees

The ApisRAM Model for pesticide Risk Assessment

Bee Model



INFLUENCE OF AGRICULTURAL

management practices on bees

ollinators are affected by a cluster of stressors such as climate change, predators, pests and diseases, landscape intensification and shortage of resources and also by crop management practices.

Among the later, the massive use of pesticides in intensive agriculture has a significant negative impact on insects, contributing also to the loss of wild and managed pollinators, including honeybees.

Among those compounds, neonicotinoids have given rise to deep concerns among the beekeeping and scientific communities during these last decades¹. These systemic insecticides exhibit very high toxicity and are highly specific and selective to invertebrates as neurotoxic agents². After foliar spraying, soil application (granules) or seed treatment (dressed seeds), and due to their high solubility in water, their active ingredients spread into the whole plant through the vascular system, being found not only on foliar tissues but also on nectar and pollen. Their metabolites have been found in honey and pollen, and are potentially toxic for bees³. Even at sub-lethal doses they can induce locomotor deficit⁴, learning impairment⁵, reduction of colony performance⁶, and foraging efficiency⁷.

In 2018, the European Food Safety Authority (EFSA) evaluated the existing data from various studies and assessed the risks towards bees and decided on the ban of three of the most toxic neonicotinoids (imidacloprid, thiamethoxam and clothianidin) from all outdoor uses in the European Union⁸. Nonetheless two other neonicotinoids are still allowed in the market and are widely used (thiacloprid and acetamiprid).

In pilot areas of Poll-Ole-GI, management practices were compiled for common crops (Burgos-Spain, LTSER-France) and pollinator exposure to pesticides was evaluated, with special attention to neonicotinoids. In France, samples of nectar and pollen were also collected directly from sunflower and rapeseed crops, as well as from honey bee hives during their flowering period for pesticide residue analysis. To complement this assessment, honey, bees and bee bread samples were also analysed.

In Burgos (Spain), hive samples such as pollen from pollen traps, beebread, nectar, honeybees and honey were collected during the 2018 beekeeping season. No neonicotinoid residues were found in any of the samples screened. However, several other substances that are harmful to honeybees were found: acrinathrin, carbendazim, coumaphos and also metabolites of amitraz used to treat Varroa mites on honeybee colonies.

Percentage of neonicotinoids or other persticide residues in different samples of plant, pollen and nectar.





02 THE ApisRAM MODEL for pesticide Risk Assessment

oney bees' colony health and development are affected by multiple environmental, biological and chemical stressors and there is a general assumption that there is not a single factor responsible for the unusual colony losses⁹. For honey bees, pesticide risk assessment tests take into consideration colony survival and development (measuring effects on larvae and behavior) and substances are approved if the exposure is negligible or if it has acceptable acute or chronic effects¹⁰, creating a strong first tier assessment. However, when assessing effects under field conditions (higher tier) and taking into account multiple exposures or combined stressors, there are several methodological caveats in the existing field methods. These do not encompass all features of colony development, and some methodologies (e.g. colony activity, forager's mortality, in-colony brood assessment) do not provide accurate data for robust statistical analysis.

Therefore, to tackle the issue of assessing effects under a multiple stressor environment, two main issues arise:

- Create efficient methodologies and protocols to gather reliable data;
- 2. Create strong models that take into consideration the multiple exposures and effects on colony dynamics.

Therefore, to create a strong statistical analysis that can bring together several stressors, their interaction and their effect on the colonies. EFSA proposed the development of a mechanistic model the ApisRAM model – that is currently under construction by the modelling team of Aahrus University¹¹. The "base model" is composed of a honeybee colony model, including foraging behavior, the colony and in-hive products. Then, it will be placed into a complex landscape which includes the "Resource Providing Unit" and the "Environmental Drivers" modules.

Finally, the multiple factors and stressors on colony health will be included, such as Pesticides, Biological Agents and Beekeeping Practices modules. The final development of a strong model for honeybee risk assessment entails high levels of complexity, requiring a long time to be developed, with



Overview of the inputs and outputs of the model considering the collected data for post-model validation. The first stage includes the several factors that can influence the colony dynamics. The second stage includes the data on exposure and effects on bees

continuous evaluation at several stages and validation with different types of data. Therefore, to help unravel the multiple stressor approach by contributing to model validation and improvement, the beekeeping season of 2018 at Albillos (Burgos, Spain) was used to gather data on the several factors that can effect colony dynamics, ultimately to be used for post-model validation, namely on detailed data on landscape composition and landuse management and cropping practices, flower resources availability and their use by honeybees, colony development and other in-hive measurements (including signs of pests and diseases).

ApisRAM will be a tool to be used in a nearby future not only to assess pesticide risks to honeybees, but also as a tool to model colony health status and honey production taking into account different landscape features, namely the availability of flower resources in space in time, and the different environmental factors influencing them. So, it can be used as a management tool to better plan and manage agricultural landscapes.





[1] Chagnon M., Kreutzweiser D., Mitchell E.A., Morrissey C.A., Noome D.A., Van der Sluijs J.P. (2015) Risks of large-scale use of systemic insecticides to ecosystem functioning and services. Environmental Science and Pollution Research 22: 119-134. https://doi.org/10.1007/s11356-014-3277-x

[2] Pisa L.W., Amaral-Rogers V., Belzunces L.P. et al.(2015) Effects of neonicotinoids and fipronil on non-target invertebrates. Environmental Science and Pollution Research 22: 68-102. https://doi.org/10.1007/s11356-014-3471-x

[3] Sánchez-Hernández L., Hernández-Domínguez D., Martín M.T., Nozal M.J., Higes M., Yagüe, J.L.B. (2016) Residues of neonicotinoids and their metabolites in honey and pollen from sunflower and maize seed dressing crops. Journal of Chromatography A 1428: 220-227. https://doi.org/10.1016/j.chroma.2015.10.066

[4] Charreton M., Decourtye A., Henry M., Rodet G., Sandoz J.C., Charnet P., Collet C. (2015) A locomotor deficit induced by sublethal doses of pyrethroid and neonicotinoid insecticides in the honeybee Apis mellifera. PLos ONE 10(12): e0144879. https://doi.org/10.1371/journal.pone.0144879

[5] Decourtye A., Lacassie E., Pham-Delegue M.H. (2003) Learning performances of honeybees (Apis mellifera L) are differentially affected by imidacloprid according to the season. Pest Management Science 59: 269-278. https://doi.org/10.1002/ps.631

[6] Goulson D. (2013) An overview of the environmental risks posed by neonicotinoid insecticides. Journal of Applied Ecology 50: 977-987. https://doi.org/10.1111/1365-2664.12111

[7] Tan K., Chen W.W., Dong S.H., Liu X.W., Wang Y.C., Nieh J.C. (2014) Imidacloprid atters foraging and decreases bee avoidance of predators. PLos ONE 9(7): e102725. https://doi.org/10.1371/journal.pone.0102725

[8] EFSA (2018). Evaluation of the data on clothianidin, imidacloprid and thiamethoxam for the updated risk assessment to bees for seed treatments and granules in the EU. EFSA supporting publication 2018:EN-1378. 31pp. https://doi.org/doi:10.2903/sp.efsa.2018.EN-1378

[9] Steinhauer N., Kulhanek K., Antunez K., Human H., Chantawannakul P., Chauzat M.P. (2018 Drivers of colony losses. Current Opinion in Insect Science 26: 142-148. https://doi.org/10.1016/j.cois.2018.02.004 [10] Rortais A., Arnold G., Dorne J.L. et al. (2017). Risk assessment of pesticides and other stressors in bees: principles, data gaps and perspectives from the European Food Safety Authority. Science of the Total Environment, 587-588, 524-537. https://doi.org/10.1016/j.scitotenv.2016.09.127

[11] EFSA (European Food Safety Authority) (2016). A mechanistic model to assess risks to honeybee colonies from exposure to pesticides under different scenarios of combined stressors and factors. EFSA supporting publication, EN-1069. 116 pp. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/sp.efsa.2016.EN-1069

Illustration: Bees' colony. UCP





Conclusions



POLL-OLE-GI conclusions

CONCLUSIONS

ollination is a key ecosystem service providing human populations with cultural, social, nutritional, ecological and economic values. Pollinator load can be seen as a common good. Pollinator conservation is a multi-scaled issue, that needs to be tackled with a multidisciplinary systemic approach.

In intensive farmlands, farmers are key stakeholders for the maintenance of healthy habitats and food for pollinators: green infrastructures (GIs) development depends on the recognition by farmers of the necessity and the benefits of such semi-natural elements in the landscape. In this Technical Guide, many different pollinator-friendly actions were listed, depending on target (food, or breeding sites for pollinators) but also on context (farming systems) and landscapes. Though they are mainly designed to be implemented by farmers, some can be implemented by other stakeholders. including citizens.

Rehabilitation of hedgerows, limitation of soil-work, improved grassland and field margin management, pesticide (herbicides, fungicides, insecticides) and fertilizer reduction and GIs, are a nonexhaustive list of practices contributing to the preservation, rehabilitation and development of wild flowers, healthy crops and pollinators (wild or domestic). They are tightly intertwined within the agricultural landscape and imply friendly practices as crucial elements especially for insect-pollination dependent crops like oleaginous crops. More broadly, GIs act as reservoirs and promotors of functional biodiversity, likely to improve the productivity of crops by the provision of supporting and indirect services such as pest population regulation, soil protection, pollination and water quality.

The different management options and GIs presented in the Technical Guide encompass actions that are of no cost, or at least low or very low cost. Some projects led in the different Poll-Ole-Gi study sites have demonstrated that some GIs or practices can even decrease farmers' expenses while favoring pollinators and their resources or habitats, such as the reduction of inputs or mechanical soil work (see Technical Actions). Indeed, decreasing farmers' expenses may thus compensate for a possible yield drop due to loss of cultivated areas or wild plant competition (see Policy Guide).

GIs development will also have a positive impact on beekeepers/farmers relationships and will help in restoring the complementarity of those rural economic activities. Farmers managing pollinators' vital resource features may in turn benefit from a rich and abundant pollinator load, honeybees and wild bees being complementary for an efficient pollination service.

Finally, GIs development can have broader positive cultural and socio-economic impacts with the preservation of landscapes and patrimonial ecological features.

Illustration: Flower mix, UCP



THE POLL-OLE-GI TEAM:

COORDINATION:

UNIVERSIDAD DE BURGOS, UBUCOMP:

Carlos Rad (crad@ubu.es), Evan A.N. Marks, Milagros Navarro, Javier López Robles, Sonia Martel, Nieves González-Delgado, Basilio Ramos, Felisa Abajo, Daniel Pérez-Alonso, Altamira Arcos, Luis Marcos, Sandra Gutierrez, Sandra Curiel, Paula Sánchez-Zulueta, Noelia Sáinz-Alonso, Eneko Iriarte, Alexandra Casado, Mathilde Auffret.

Grupo de Investigación en Compostaje UBUCOMP - Universidad de Burgos.Facultad de Ciencias, Pl. Misael Bañuelos s/n 09001 Burgos (Spain)

PARTNERS:

UNIVERSIDAD AUTÓNOMA DE MADRID, SOCIAL-ECOLOGICAL SYSTEMS LABORATORY

José Antonio González Nóvoa (jose.gonzalez@uam.es), Violeta Hevia Martín, Jorge Ortega Marcos, César Agustín López Santiago, Francisco Martín Azcárate, Paloma Alcorlo Pagés, Libertad Chapinal Cervantes, Aura Pérez Morín.

C/ Darwin, 2. Facultad de Ciencias-Edificio Biológicas, Departamento Interuniversitario de Ecología. Universidad Autónoma de Madrid. Campus de Cantoblanco, 28049 Madrid (Spain)

INRA NOUVELLE - AQUITAINE - POITIERS. U.E. APIS:

Pierrick Aupinel (pierrick.aupinel@inra.fr), Jean-François Odoux, Pauline Arneodo, Clovis Toullet, Melanie Chabirand, Nathalie Lemaire, Daniel Raboteau, Thierry Tamic, Emilie Cadet, Colombe Chevallereau, Dimitry Wintermantel.

Centre INRA Poitou-Charentes, Station de Magneraud. Saint-Pierre-d'Amilly 17700, Charente-Maritime (France)

UNIVERSIDADE DE COIMBRA:

José Paulo Sousa (jps@zoo.uc.pt), Sílvia Castro, João Loureiro, Lucie Mota, Nuno Capela, Artur Sarmento, Joana Alves, António Silva, Rúben Mina, Henrique Azevedo Pereira, Catarina Siopa, Liliana Almeida, Fernanda Garcia, Mariana Castro, Hugo Gaspar, Rafael Carvalho., Mari Gigauri, Caio Domingues. Faculdade de Ciências e Tecnologia, Departamento de Ciências da Vida. Calçada Martim de Freitas, 3000-456 Coimbra (Portugal)

CNRS - CENTRE D'ETUDES BIOLOGIQUES DE CHIZÉ:

Vincent Bretagnolle (vincent.BRETAGNOLLE@cebc.cnrs.fr), Sabrina Gaba, Sylvie Houte, Marylin Roncoroni, Jean-Luc Gautier, Thomas Perrot, Rui Catarino, Fabien Vialloux, Yohana Marescot, Karine Besse. Centre d'Etudes Biologiques de Chizé. CNRS UMR 7372 - Université de La Rochelle, 405 Route de La Canauderie. 79360 Villiers-en-Bois (France)

ACKNOWLEDGEMENTS:

ASOCIACIÓN DE DINAMIZACIÓN COMUNITARIA ADEMÁS DE TI, LA PARRILLA FUNDACIÓN OXÍGENO SYNGENTA CONSEJERÍA DE AGRICULTURA Y GANADERÍA, JCYL LUIS OSCAR AGUADO. ANDRENA S.L. RÉGION NOUVELLE AQUITAINE MINISTÈRE DE L'AGRICULTURE



www.pollolegi.eu



