LIFE 4 POLLINATORS

INVOLVING PEOPLE TO PROTECT WILD BEES AND OTHER POLLINATORS IN THE MEDITERRANEAN

GUIDELINES

LIFE18 GIE/IT/000755

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About the project

LIFE 4 Pollinators – Involving people to protect wild bees and other pollinators in the Mediterranean

aims to improve the conservation of pollinator insects and entomophilous plants. Creation of a virtuous circle will lead to a progressive change in the anthropogenic practices that are currently threatening wild pollinators across the Mediterranean region.

LIFE 4 Pollinators run in four European countries: Italy, Greece, Spain and Slovenia, coordinated by the University of Bologna and co-financed by the LIFE Program of the European Union.

Find out more at:

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Foreword

Actions B4 and (part of) **B5** aimed at testing the suitability of the new indicator of wild bees (BEE) in the assessment of Rural Development Plan (RDP) measures. Part of the action also includes fostering protection measures for integrated agriculture, establishing rules for obtaining the bee-friendly ecolabel, and indicating best practices for the protection of pollinators.

The present deliverable (LIFE 4 Pollinators, PROJECT DELIVERABLE PRODUCT B4) contains a summary of the scientific effort behind the Pilot Project, the BEE indicator and guidelines for applying it (protocols for field sampling and calculations with the data collected; chapters 1-3). Results of replications are also reported (chapter 4). The following chapters are dedicated to protection measures for integrated agriculture (chapter 5), rules for obtaining the bee-friendly ecolabel (chapter 6) and best practices for the protection of pollination networks (chapter 7).

Table of Contents

1. The importance of indicators in evaluation RDP measures

The present output for Action B4 had a preparatory phase via action A2.1. The first activity was a review of existing CAP indicators carried out entirely by the CREA team. This work aimed to raise awareness of the importance of considering pollinators and especially wild bees in biodiversity assessments applied by competent authorities (Figure 1).

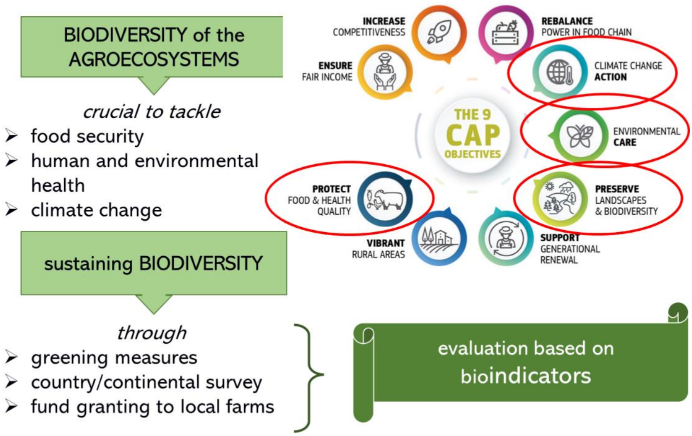


Figure 1: Evaluation of contexts (included the Sustainable Development Goals) where to apply a bioindicator.

The results of this preparatory work were presented at the 1st International Electronic Conference on Biological Diversity, Ecology and Evolution, 15–31 March 2021, and have been described in two scientific publications (Box 1). Extracts of these publications help clarify the importance of developing and applying a BEE indicator and illustrate our main results in the preparatory phase.

In the Proceeding Paper (2021, Biol. Life Sci. Forum) we highlighted that:

- *"…Pollinators are desirable candidates to contribute to indicators applied to monitor the trend of biodiversity loss. Their role in agroecosystems is recognized of crucial importance: they perform services in support of food production and indirectly inform on pollutants and environmental quality. Furthermore, the decline that pollinators are undergoing can precisely impact agriculture produce…"*
- *"…The biodiversity of the agroecosystems is becoming a crucial component in European legislation since it represents a key to tackle food security, human and environmental health, and climate change. A specific objective of the CAP…"*

> “…Greening measures have been implemented to counteract biodiversity loss, especially through fund granting. However, evaluating the resulting impact of these actions and, consequently, the financial effort linked to them, has not been successful so far…”

In the Scientific Paper (2021, Diversity):

- *“…we assessed past and recent indexes/indicators used for biodiversity assessments. Indicators have become a common tool to evaluate goals, especially at government level. The choice and targeting of indicators are constantly revised…”*
- *“…Good examples are the past and current indicators used to monitor biodiversity at the European level (CAP 2014–2020 and CAP post-2020), where FBI has been retained and HNV discarded. While overall international pressure can drive the selection of some indicators, others may be employed at the national level, according to national laws or national mitigation measures to be evaluated…”*
- *“…However, the indicator must consider the dimension of the employed variable: e.g., insects can be expected to interact with the environment very differently from birds. When considering pollinators, we found that some of them are included in past (HNV) or current (GBI, StN) indicators, or predicted in indicators yet to be defined…”*
- *“…Our analysis highlights two critical points: the background knowledge on the target and the efforts related to sampling and taxonomic identification. For pollinators, the situation is evolving fast. Public interest has increased sharply in recent decades: society is alarmed by pollinator decrease and interested in initiatives to understand the current situation and to sustain pollinator conservation…”*

BOX 1: References

PROCEEDING PAPER: Albertazzi, S., Monterastelli, E., Giovanetti, M., Flaminio, S., Zenga, E. L., Bortolotti, L., & Quaranta, M. (2021, March). An analysis of ecological indicators applied to agricultural ecosystems: what to retain to shape a future indicator for pollinators. Biol. Life Sci. Forum 2021, 2(1), 31.

SCIENTIFIC PAPER: Albertazzi, S., Monterastelli, E., Giovanetti, M., Zenga, E. L., Flaminio, S., Galloni, M., Quaranta, M., & Bortolotti, L. (2021). Biodiversity Evaluation: From Endorsed Indexes to Inclusion of a Pollinator Indicator. Diversity, 13(10), 477.

The results of our preparatory work emphasized the main issues to be tackled during development of an indicator, which are summarised in Figure 2.

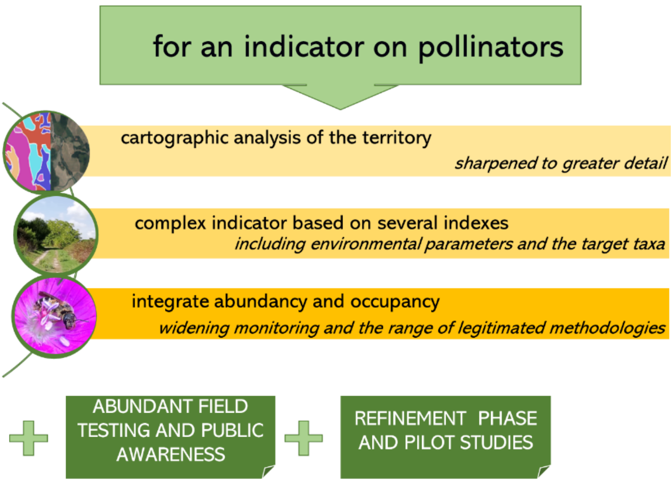


Figure 2 Conceptual results of the preparatory work.

2. Case-study of Emilia-Romagna for the development of a BEE indicator

2.1 Steps in the design of the BEE indicator

As emerged from our survey of the literature, the urgency of designing an indicator[1Glossary] based on pollinators is becoming critical. Since such an indicator is predicted to affect the assessment of RDP measures, we defined two criteria that the indicator must satisfy:

- it must assess the state of the pollinator community **at farm scale**;
- it must **discriminate** the effects of RDP actions implemented to favour pollinators.

We focused on wild bees because they are acknowledged to be the major pollinator group (Rader et al. 2016)[1]. The CREA research team in Bologna has long expertise on bees, encompassing their ecology and taxonomy; this expertise is determinant in pursuing the goal of developing the indicator.

First step – scientific monitoring: data was collected at farm level in collaboration with farmers' associations and farmers directly. Bee data at species level was associated with environmental and agricultural indices,[2Glossary] and with bee eco-functional traits[3Glossary]. The results were presented and discussed at national and international conferences and in a scientific article (submitted July 2024).

GLOSSARY

[1]Indicator: the "formula" to obtain the result (the indication of environmental quality); its value, obtained in a bee survey, needs to be related to the abundance of natural features in the farm and its surroundings.

[2]Index-Indices: elements related to a parameter known to influence the bee community.

[3]eco-functional traits: ecological or functional characteristics grouping bee species on the basis of a common characteristic or shared life trait.

[4]morpho-genus/genera: groups of bees resembling each other and belonging to close taxonomic groups.

The LIFE 4 Pollinators project aims to involve stakeholders linked directly to the environment where the indicator will be employed (farmers). The BEE indicator was designed on field data collected at local farms interested in the project. We selected monitoring sites to have different environmental and agricultural features. This phase was designed in collaboration with a local farmers' association (COLDIRETTI) and 5 out of 12 farms were selected. After the first year of monitoring, COLDIRETTI left the project, although three farms decided to continue, and another farmers' association, Confagricoltura took the place of COLDIRETTI. This brought two more farms into the monitoring in the second and third years.

Second step: a BEE indicator was designed on the basis of the monitoring information. Since it was important to have a tool suitable for use by a large number of

[1]Rader, R., Bartomeus, I., Tylianakis, J.M., Laliberté, E., 2014. The winners and losers of land use intensification: pollinator community disassembly is non-random and alters functionaldiversity. Diversity and Distributions 20, 908–917.

stakeholders, the CREA team produced a simplified version of the protocol and used simplified morpho-genus bee identification.[4Glossary] The BEE indicator was tested on farms in the countries involved in the project (Italy, Spain and Greece).

These two steps are described below in more detail.

2.2 The scientific monitoring: field protocol

BOX 2: Sampling in Brief

Method: hand-net capture of flying individuals, along fixed 50 m x 2 m transects at crop borders within farm boundaries. Individuals are kept alive in vials.

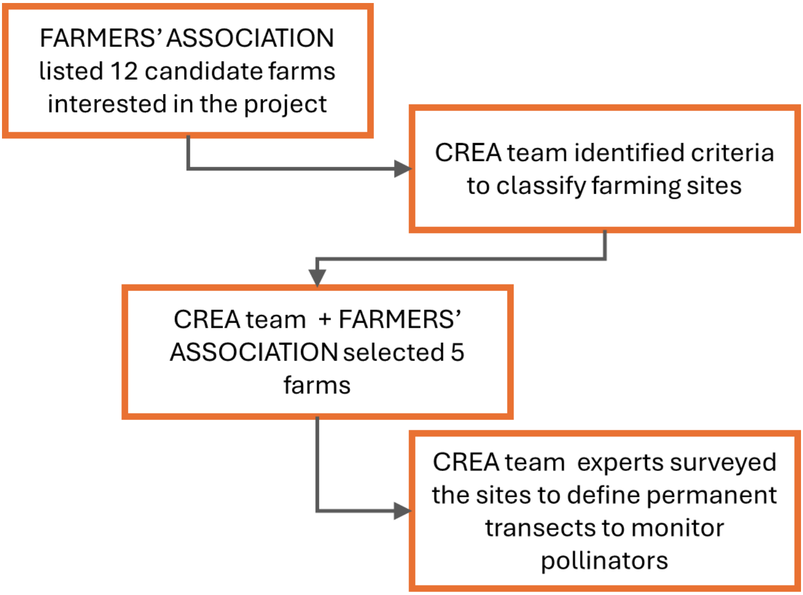
Time: twice a day, one day/month, 8 months/year for 2 years

Pollinators: wild bees, syrphid and bombylid flies, butterflies.

The CREA team had prior experience with a monitoring protocol for wild bees in a national project (BeeNet). That protocol was used as a baseline and adapted to the monitoring expected in the case-study of the LIFE 4 Pollinators project.

Key monitoring issues and the way we addressed them are summarised in BOX 2. The protocol was applied on volunteering farms, which were then selected on the basis of intrinsic characteristics: type (conventional or organic), altitude belt, farm size.

The farm selection workflow is illustrated below.



Details of the field protocol are reported in the following pages.

Field Protocol applied to the Scientific Monitoring

Sampling site

The sampling sites are selected *a priori* using cartographic data and information related to the objectives of the Life 4 Pollinators project. The areas monitored in each sampling site measure 200 m x 2 m. Since many farms have a variety of local habitats and biotopes, the sampling sites are divided into 4 transects, 50 m long (A, B, C, D) in order to intercept this diversity. Each transect is treated as an independent unit and is marked out in the field with red and white tape.

Timing

Sampling is conducted once a month at constant intervals by setting a specific sampling week which is maintained for the duration of the project. The estimated time for each survey is 15 minutes per transect, i.e. total time 1 hour. Monthly monitoring is carried out twice a day: morning and afternoon 10-11am and 15-16pm, respectively, allowing some flexibility within these intervals. The number of yearly replicas may vary in relation to pollinator activity depending on geographic location and altitude. For example, in Emilia-Romagna, pollinators are active for 8 months, sampling lasting from March to October inclusive. Sampling is repeated for three years.

Field Activity

Sampling Site Code

Sampling sites are indicated by unique identification codes (sampling site codes) consisting of: - a 2-letter acronym for the local/political/administrative unit (e.g. Emilia-Romagna Region: ER) - 2 letters for the initials of the farm (e.g. La Martina: LM)	Specimen codes consist of the sampling site code plus: - the daily run (M: morning; P: afternoon) - transect letter (A, B, C, D) - Sequential number of catch.

General information regarding the surrounding area

Station data, to be recorded on the "sampling site classification" field sheet when the sampling site is identified during the first sampling trip, includes:

Under "sampling site":

- GPS coordinates at the centre of the core area containing the sampling site, specifying the reference system
- Locality (Municipality and Province)
- Farm where sampling takes place
- Altitude a.s.l. (m)

Under "transect":

- Identification letter (A, B, C, D)
- GPS coordinates at transect end points

Monthly surveys

Field activity should preferably be carried out by at least two surveyors, for safety reasons and to ensure correct and complete data collection. The information to be repeated at each sampling is described below.

NOTE: Sampling should only be done when weather conditions are suitable for pollinator flight: bright dry days, minimum temperature 15°C, no or light wind. Weather conditions should therefore be checked in advance. Weather conditions at the morning and afternoon samplings must be recorded with suitable instruments.

Sampling field sheet

- Sampling site code
- Name of surveyor responsible for entomological sampling
- Date (dd/mm/yy)
- Period: morning = M, afternoon = P
- Start/end time of each transect
- Start/end sampling temperature (at least 15°C)
- Start/end wind speed (less than or equal to Beaufort level 3, 12-16 km/h)
- Estimated cloud cover (<50% is necessary for sampling)
- Plants: record plant species on which indivduals are collected and obtain photographic documentation to confirm identification.
- Insects: number and record specimens consecutively with transect code

Sampling with hand-net

- Walk each transect (A,B,C,D) at a steady pace, taking an effective time of 15 minutes (i.e. stop timer when placing insect in vial and recording information).
- The transect is walked once per observation run in a single direction, which is maintained constant throughout the project.
- Sample only APOIDEA (wild bees), DIPTERA (hoverflies and Bombilidae) and LEPIDOPTERA (Rhopaloceran).
- It is NOT necessary to capture Apis mellifera, other Hymenoptera, beetles or non-rhopaloceran lepidoptera. In case of doubt (e.g. Crabronidae), capture the specimen to avoid misidentification.
- Place each specimen in a separate vial with cork occupying 1/4 of vial volume and two drops of ethyl acetate (99.2%). Containers must have only one label.
- Place specimens in a cooler to prevent spoilage.
- On the field sheet record the flowers visited at the time of capture of each specimen.

Label

The field label, one per container, can be a piece of paper tape on which the following information is indicated:

- Harvest date (dd/mm/yy)
- Sampling site code (as noted above)
- Observation run (morning = M or afternoon = P)
- Letter of transect (A, B, C, D)
- Sequential number of each catch

Specimen Preparation

Conservation of specimens

Prepare specimens for identification within 2-3 days of capture, otherwise store them at -10°C (even in a domestic freezer, if there are no other options). All specimens collected with ethyl acetate can be prepared dry, as follows.

Preparation of specimens

- Use the data entry file prepared at the beginning of the work to create the entomological tag by entering all the capture data. Pin the specimen with an entomological pin of appropriate diameter for the size of the specimen (0, 1, 2 or micropin), taking care to pin the upper right part of the thorax.
- Place the specimen on a preparation surface or an appropriate drying rack (for Lepidoptera) with the tag nearby.
- Arrange the individual parts of the insect's body using other pins or paper bands to highlight characters useful for determination.
- To enable handling of the specimen later without damaging it, make the insect "climb" along the pin at the right height (1/3 of the pin) when preparing it.
- Leave specimen to dry for about a week, then remove it from the drying rack, label it and place it in an entomological box.
- Store the box in a dry dark place.

Database

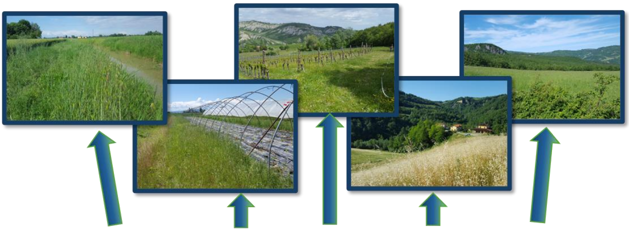
All the data recorded on the field sheets must be entered on database sheets for further analysis. Do this soon after the field survey to avoid losing/forgetting information. After insect preparation, determine specimens to species level; if this cannot be done by the surveyors, seek advice from specialists. A final database with all the specimens identified can be shared for analytical purposes.

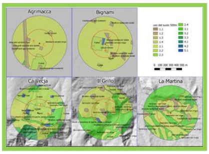


2.3 Scientific monitoring: data and results

In 2021 we collected 766 specimens of wild bees (Apoidea), 313 flies (Syrphidae and Bombylidae) and 203 butterflies (Ropalocera). All specimens were determined to species level by expert taxonomists (Apoidea were identified by experts at the CREA Laboratory of Entomology) or sent to specialists.

We verified the ecological information for each of the five farms (Figure 3).





Farm	Agrimacca	Bignami	Cà Vecja	Il Grillo	La Martina
Bees	**125**	**196**	**132**	**202**	**104**
Species	**41**	**51**	**44**	**70**	**58**
Altitude mean	19,4	25,6	206,7	211,5	701,7
Slope mean	0,3	0,3	15,5	17,3	12,2
Solar radiation	4488	4518	4254	4252	4979
L.U. parcels	11	12	33	25	25
Complexity	0,18	0,17	0,90	0,61	0,81
Simpson index	0,31	0,23	0,79	0,66	0,63

Figure 3 Data collection in the 5 farms during the first year of monitoring.

We focused our analysis on wild bees. We associated information related to *eco-functional traits*, found in a restricted-access database and in the "Checklist of Italian Wild Bees" (Comba 2019[2]) with bee-records of the first year. We retrieved data about species parasitism, sociality, nesting behaviour, lecty, seasonal occurrence, voltinism and size from the database. Other data was completed by CREA experts.

We retrieved data on species distributions in Italy from the checklist and considered environmental-agronomic parameters that could affect the bee community. A long process of tests and statistical analysis of indexes was carried out to verify the influence of the various factors on the bee communities recorded.

Ecological components under assessment identified for LIFE4P:

- Cleptoparasites: parasites species observed/expected cleptoparasites
- Nesting location: underground/total
- Phenology: index of seasonal occurence
- Lecty: oligolectic species/total
- Sociality: social species/total, its meaning differs between taxa (Halictini, Bombi, Xylocopini)
- Size and home range: small home range/total, from intertegular distance
- Areal: species with narrow distribution (endemism)/total
- Red list status: threatened/totale
- eveness=Shannon/log sp
- All indexes should score between 0 and 1

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[2] Comba, M., 2019. Hymenoptera: Apoidea: Anthophila of Italy. Bibliographic checklist of Italian wild bees with notes on taxonomy, biology, and distribution [WWW Document]. URL https://digilander.libero.it/mario.comba/ (accessed 3.1.22)

The results were shared and discussed in the following contexts:

EURBEE9-9th European Congress of Apidology, Belgrade, Serbia, 20-22 September 2022

(Book of Abstracts, p. 327). Title: "Adapting monitoring methods to enlarge stakeholders' participation in pollinator protection initiatives".

XIX Convegno Nazionale AISASP, Milano, Italia, 30 August - 01 September 2023

(Book of Abstracts, p. 41). Title: "Panoramica sulla biologia e l'ecologia degli Halictini sociali e solitari nell'agroecosistema della Provincia di Bologna [*Overview of the biology and ecology of social and solitary halictini in the agroecosystem of the Province of Bologna*]"

(Book of Abstracts, p. 23). Title: "Prospettive sull'uso dei tratti funzionali delle api selvatiche in un indicatore di biodiversità in ambito agricolo [Perspectives on the application of functional traits of wild bees for a biodiversity indicator in the agricultural context]"

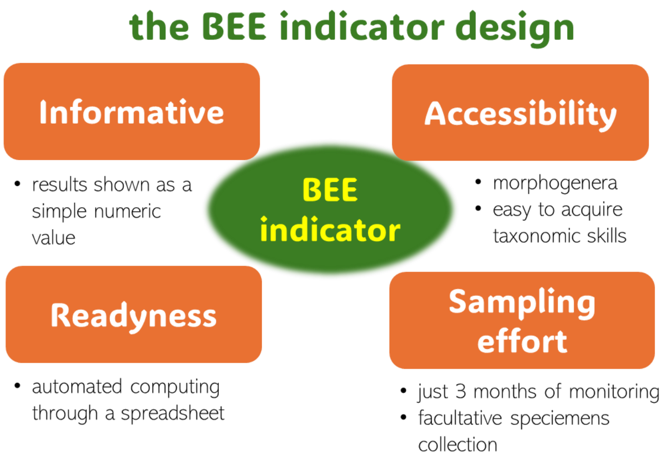
Finally, a scientific manuscript was submitted to the journal "*Agricultural Systems*" in 2023 and is currently under review. Summary of the main results:

- ✓ a group of environmental and agricultural variables reflect **altitude** gradient
- ✓ farm context is influenced by **landscape** (structure and composition) and the **methods** used by local farmers
- ✓ **social bee** abundance decreased from plain to mountain
- ✓ three bee functional traits (**phenology**, **distribution** in Italy and **rarity**) are closely linked to agricultural management system rather than to agri-environmental landscape
- ✓ a **better understanding** of ecosystem types in relation to their pollinators is **needed.**

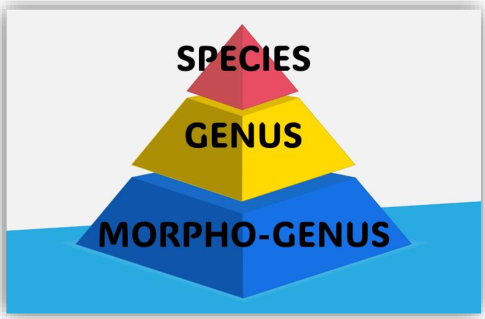
3. The BEE indicator: elaboration and protocol

3.1 The tool

Our aim was to develop a tool suitable for use as a bioindicator to monitor environmental quality and its improvement after application of RDP measures. Once the main drivers influencing wild bees had been outlined by analysis of the monitoring results, we developed the BEE indicator. The box shows the key topics addressed.



As a first step, we considered the need to simplify the field protocol in order to reduce field work costs and surveyor expertise. Calculation of the indicator also needed to be self-evident. In simplifying the protocol, we decided to ignore species level and determine bees at morpho-genus level.[4 Glossary] This dispensed with the need for detailed taxonomic skills (though preliminary training of personnel is needed) and enables the indicator to be applied by farmers, government authorities and regional administrators.



To identify the bees, we used The Diagnostic Tables of Wild Bee Morphogenera of Italy (Quaranta et al., 2019)[3] ,conceived as an evolving tool and

[3] Quaranta M., Felice E., Agujari M., Andreani A., Angiolini M., Bartaccini A., Flaminio S., Galardi M., Lenzi R., Marchi M., Sagona S., Tafi E., Felicioli A., 2019 - Diagnostic Keys of wild bee morphogenera. Print: Tipografia Musiani, Bologna.

freely available online for educational purposes. In the table, genera and species are grouped in 25 morpho-genera, excluding the morpho-genus Apis (which contains a single species, *Apis mellifera*). We added a morpho-genus, Melecta, comprising the genera *Melecta* and *Thyreus,* and updated the nomenclature, including genera in the latest European checklist (Ghisbain et al. 2023).[4] Similarities and differences with respect to the scientific monitoring procedure are summarised in BOX3.

To assess wild bee richness, the surveyor assigns specimens captured along the four transects to their morpho-genera. The BEE indicator is then calculated with the formula. For more information, see FIELD PROTOCOL below.

BOX 3: SAMPLING IN BRIEF

SAMPLING METHOD: hand-net capture of flying individuals along fixed 50 m x 2 m transects at crop borders within farm boundaries; insects are identified on-site at the end of monitoring and not collected.

SAMPLING TIME: twice a day, one day/month, 3 months/year for 1 year

SAMPLING POLLINATORS: restricted to wild bees which are released after identification.

3.2 The calculation

Values of the BEE indicator need to be combined with previously estimated naturality. A statistical model was used to predict the value of the BEE indicator based on a range of naturality and to create a table of results. The table allows the operator to verify the value of the observation in relation to the percentage of natural features in the environment.

the BEE indicator calculation

calculated by counting the number of different bee morpho-genera collected along the 4 transects:

$$BEE = (n_A+n_B+n_C+n_D)/4$$

where n_i is the number of morpho-genera collected in three months, and *A, B, C* and *D* are the 4 transects

the BEE indicator values across naturality ranges

Percentage of natural features	BEE Class -2	BEE Class -1	BEE Class 0	BEE Class +1	BEE Class +2
0 – 10%	< 3.75	3,75 - 4.50	4.75 - 5.50	5.75 - 6.25	> 6.25
10 – 20 %	< 4.50	4.50 - 5.25	5.50 - 6.50	6.75 - 7.50	> 7.50
20 – 30%	< 5.25	5.25 - 6.25	6.50 - 7.50	7.75 - 8.50	> 8.50
30 – 40%	< 5.75	5.75 – 7.00	7.25 - 8.50	8.75 - 9.75	> 9.75
40 – 50%	< 6.50	6.50 – 7.75	8.00 - 9.50	09.75 – 10.75	> 10.75
50 - 60%	< 7.25	7.25 - 8.50	8.75 - 10.50	10.75– 12	> 12

[4] Ghisbain, G., Rosa, P., Bogusch, P., Flaminio, S., Divelec, R.L., Dorchin, A., Kasparek, M., Kuhlmann, M., Litman, J., Mignot, M., Müller, A., Praz, C., Radchenko, V.G., Rasmont, P., Risch, S., Roberts, S.P.M., Smit, J., Wood, T.J., Michez, D., Reverté, S., 2023. The new annotated checklist of the wild bees of Europe (Hymenoptera: Anthophila). Zootaxa 5327, 1–147.

FIELD PROTOCOL FOR THE BEE INDICATOR

Location

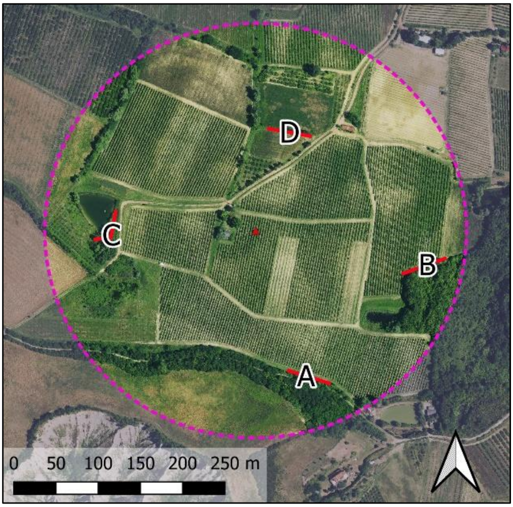
In this phase, the sampler selects a monitoring area on the farm that includes four transects. The following process can be performed on physical maps and/or with various types of GIS software (e.g. QGIS, ArcGIS). With the help of maps and the farmer, a monitoring area is identified at the centre of the farm.

The monitoring area:

- consists of a circle with a radius of 250 m (see figure right)
- is located in the central part of the farm (to minimize the influence of nearby crops)
- must include the most representative crops and habitats on the farm

The four transects (A, B, C, D) must**:**

- be 50 m long and 2 m wide (not necessarily in a straight line)
- fall within the circular monitoring area
- be separated by at least 100 m
- be outside cultivated fields.



Circular selection inside farm boundaries and location of 4 walking transects (A, B, C, D).

Deciding the transect. Field edges are optimal, as are similar features where agricultural habitat diversity is maximized: ditches, roadsides, forest edges and pond edges. Shaded locations should be avoided. Landscape elements can be identified using aerial photos and verified by field observations. The position of the transect must remain unchanged throughout the monitoring period.

Naturality

The second phase consists in attributing a naturality value to farm landscape. The percentage of the **area which is natural** is considered an **indicator of the naturality** of the surrounding environment. Areas not strongly influenced by human activity are the most suitable for hosting a diverse bee fauna: "natural" areas often host food resources throughout the season of bee activity. Besides offering food resources, uncultivated land, scrub and forest edges are habitats rich in bee nesting sites. All areas without buildings, crops or water bodies are considered natural areas. **Two fundamental criteria** that distinguish natural areas from cultivated ones are: **lack of tillage and lack of pesticides**. For example, forest, shrubland, meadows, wasteland and urban parks are considered natural areas, whereas wheat fields, olive groves and vineyards are not.

Sampling

The third and most important phase of the process takes place over a three-month monitoring period. Sampling is based on catch-and-release, using a hand net. Bees are identified in the field at morpho-genus level.

Sampling must be **repeated for three consecutive months between spring and summer**, to coincide with the main flowering season under local climatic conditions. In warm temperate climates, the most suitable periods to detect the peak of bee diversity are typically the months of May, June and July. In Mediterranean climates, the monitoring period can be brought forward by one (or two) months, to avoid periods of drought. Sampling must only be carried out under suitable atmospheric conditions:

- temperature between 15°C and 35°C
- cloud cover less than 50%.
- absence of rain and wind speed less than 15 km/h (<4 Beaufort scale).

Sampling is repeated **once a month, twice in the same day**: in the morning (8-12am) and afternoon (12am-5pm). Each transect must be walked **for precisely 15 minutes** (at each capture, timing is interrupted: a stopwatch is therefore useful). The sampler chooses times of day based on local weather conditions.
Once the transect has been walked, **bees are identified at morpho-genus level** and **the data is recorded on the field sheet**. The diagnostic guide for identification of morpho-genera is freely available online. Once identified, the insects are released.
Those that cannot be identified in the field can be can be stunned with ethyl acetate, preserved and labeled (date, location, transect). Such samples can subsequently be prepared and identified with the help of a stereomicroscope and the specialist literature, or by consulting a specialist.

Conducting the sampling. The objective of monitoring is to collect all individuals encountered along the transect in the established time, using a hand net. Transects are walked once, in a single direction (the direction must maintained during replications) in 15 minutes. The sampler must therefore be equipped with a stopwatch to stop timing during sample collection and handling. Insects are collected while visiting flowers or in flight. Honey bees are not sampled. The path along the transect should be walked at a constant regular pace, paying equal attention to all flowering plants. Captured bees are placed in transparent tubes and stored in a portable refrigerator until the end of the transect is reached. After identification in the field, they are released.

Field sheet for the **BEE indicator**

Farm name ___________________ Date _________

Sample name _________________ Morning / Afternoon _________________

At start: Temperature______ Cloud cover ________________

At the end: Temperature______ Cloud cover ________________

Time (hr:mins)

Transect A _____ Transect B _____ Transect C _____ Transect D _____ End _____

n°	Transect (A,B,C,D)	Morfo-genus	Captured (yes/no)

n°	Transect (A,B,C,D)	Morfo-genus	Captured (yes/no)

4. Replications in Slovenia, Spain and Italy

Replications were carried out in Slovenia, Spain and Italy (Figure 4) to verify the feasibility of the BEE indicator and the above procedure.

Figure 4 Location of replication sites in the countries participating in the project

Briefly, replications were carried out by:

- applying the non-specialist version of the BEE indicator protocol (pages 16-18);
- selecting an organic and a conventional farm at a site/s in each country, after cartographic and survey by local staff;
- repeating the monitoring for 3 consecutive months, once in the morning and once in the afternoon;
- visual identification of wild bees in the field to morpho-genus level;
- recruiting samplers from among volunteers, students and CREA employees.

The replications concerned eight farms: two in the Balearic Islands, two in Slovenia, and four in Italy in two distant regions, Apulia (Puglia) and Veneto. Apulia was chosen as a proxy for Greece, where unforeseen circumstances prevented us from monitoring.

We prepared a document for each site (Farm Report) to share with farmers. The document explains the aim of monitoring, the results obtained on the farm, the value of the BEE indicator obtained and how to score it against the naturality of the farm. We also added suggestions on how to improve conditions in order to foster and conserve pollinators.

Below is an example of a Farm Report. Farm names have been deleted for privacy reasons.

4.1 Farm Report: Acknowledgement to Farmers

To [Farm Name], Thank you for agreeing to sample wild pollinators.

Thank you for contributing to Life 4 Pollinators!

The main objective of the project is to involve as many people as possible (farmers, local government, students and citizens) in protecting wild bees and other pollinators in the Mediterranean region. LIFE 4 Pollinators is co-financed by the European LIFE fund, coordinated by the University of Bologna Alma Mater Studiorum, and involves partners from Italy, Greece, Spain, Slovenia.

The activities conducted by the CREA team (CREA Research Centre for Agriculture and Environment) consist in studying the **effects of agricultural methods on the biodiversity of wild bees**. The study involved monitoring the presence and variety of bees at a number of farms, including [*Farm Name*]. Some farms were asked to participate for different periods (2 or 3 years or a few months) depending on the type of data needed and its importance for the project. The Farm Report records data relating to your farm and provides materials and indications developed by the project LIFE 4 Pollinators.

On a monthly basis, the CREA team sampled pollinating insects with a hand net along pre-established transects that included uncultivated fields, roads, ditches and field edges, chosen on the basis of the ecological variables of your farm. We classified all the bees captured by taxonomic group and assessed the state of Apoidea biodiversity on your farm.

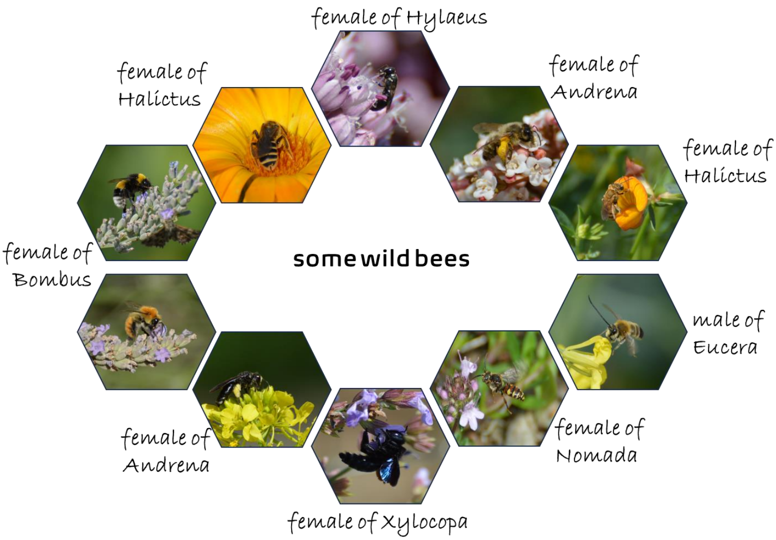
We started by establishing the boundaries of the farm, and marking the 4 transects (A,B,C,D,) for monthly monitoring.

boundaries of the farm

Position of the 4 transects

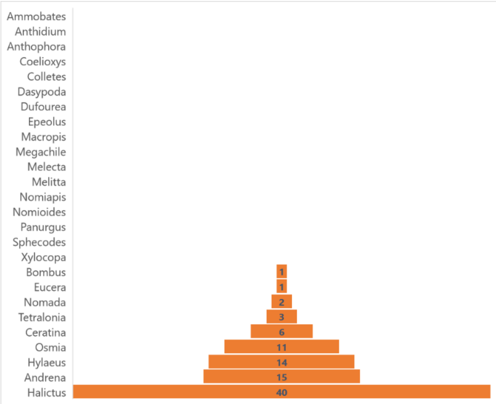
4.2 Farm Report: Which pollinating insects live on your farm?

Honey bees are not the only pollinators of plants! There are more than 1000 species of wild bees in Italy (for examples, see photographic card below), which together with wasps, flies, butterflies and beetles carry pollen from one flower to another, enabling cultivated plants to produce abundant fruit and wild ones to reproduce and create a rich and varied landscape. Bees are highly specialized for collecting pollen and nectar from flowers. Both the quantity and quality of fruit and vegetable crops depend on the state of wild pollinator populations.



This project was specifically concerned with the types of wild bees at [*Farm Name]*, and therefore also the flowers available to nourish them. The following graph shows the bees captured by group and percentage.

There were many bees of the "**Halictus**" group. The number recorded is indicated on the bar. Different genera of bees belong to the group (there are two examples in the photographic card above), including small- and medium-sized bees that visit many different plants.



There were many bees of the "**Andrena**" group: the Andrena genus alone contains 214 different species of bees present in Italy, many of which have well defined food preferences. Due to their great variety, many are still relatively little known: e.g. no details are available on where they nest or how they feed.

We also sampled some specimens of the "**Eucera**" and "**Tetralonia**" groups, known for the long antennae of males, and bees of the "**Hylaeus**" and "**Nomada**" groups, which at first sight look more like wasps than bees.

What groups are missing? These are bees that escaped the monitoring campaign because they were rare or absent. Some are probably present in areas surrounding the farm; longer and/or more extensive monitoring may have intercepted them. The "**Anthophora**" group, for example, contains bees that are very good flyers but are mostly active at the beginning of spring: the monitoring did not intercept them.

Rarity, on the other hand, may be linked to needs that are not met by current farm conditions, or to environmental needs that are not met by the farm environments monitored. In the first case, measures could be taken to favour such bees, Certainly more detailed study could provide more information about absent groups.

4.2 Farm Report: How are the bees in your company?

To estimate the variety of bees, the LIFE 4 Pollinators project uses an indicator called **BEE Indicator**, developed by the project research team. The indicator was related to other variables: the data on bee biodiversity (from sampling in May, June and July) was analysed on the basis of survey information on the agricultural management of the farm and its landscape context. In a hilly context with ditches, hedges, woods and uncultivated land, a fairly high value of the indicator is likely. On the plains, where intensive agriculture is more common and landscape naturality is reduced to a minimum, a lower value is more probable. The more favourable the agro-environment is for bees, the greater the number of species present in an agro-ecosystem, increasing the value of the indicator. The maximum rating is 5 stars, indicating that the farm is an excellent hotel for bees!!!

From an environmental point of view, 5 stars is equivalent to sustainable management, attentive to the environment, and a landscape rich in semi-natural habitats with abundant graduated blooms throughout the period of bee activity. A rating of 1 star indicates a serious situation for pollinators: resources are almost completely absent and the agro-ecosystem has too few natural components.

[*Farm Name]* achieved a **score of 3 out of 5 stars**. Although the spectrum of pollinating bees is quite good, there is **room for improvement** especially regarding the list of bee groups that were not found.

4.3 How to interpret the result?

Landscape, farming practice and suggestions

Your farm is in a highly urbanized or intensively cultivated landscape. Natural habitats are almost completely absent: almost all the land is cultivated or paved, so pollinating insects have no habitat for nesting or flowers to visit.

Your farming methods are limiting wild bee biodiversity. Pesticides are probably having negative effects on pollinating insects. Field and road edges and ditches may be excessively mowed or treated with herbicides, preventing the growth of flowering plants.

We recommend reducing and remodulating the use of pesticides in an attempt to encourage pollinating insects. For bees to return to the farm, some uncultivated areas must be left, where mowing is reduced to a minimum and where no chemical products are used.

Your farm is in a predominantly agricultural landscape. Natural habitats are reduced and fragmented: the biodiversity of pollinators is compromised by the absence of ecological corridors between habitats suitable for insects.

Your farming methods are probably depressing Apoidea biodiversity. Pesticides could be used in a more environmentally friendly manner. Ditches, field edges and roads are probably mowed often, preventing the growth of wildflowers essential for the life of bees..

We recommend increasing areas suitable for pollinators. Uncultivated patches are essential on a farm: the availability of areas with wildflowers and untilled soil is important for encouraging the return of wild bees.

Your farm is in a varied landscape:with natural elements and waterways, as well as cultivated and built-up areas. The agro-ecosystem plays a fundamental role in the conservation of wild bees. It should allow bees to find food resources such as wildflowers (absent in forests and paved areas).

Your farming methods do not seem to have an excessively negative effect on the apoidea community, but certain things could be improved to favor biodiversity. The use of plant protection products prevents less tolerant bees from living on the farm. The frequency of mowing could depress plant biodiversity which needs to be sufficiently abundant and varied to constitute a valid food resource for the most demanding wild bees.

We recommend agricultural methods that increase the naturalness of your farm's agro-ecosystem. Wildflower strips between rows, planting new hedges and leaving vegetation along ditches helps create a network of micro-habitats useful for pollinators.

Your farm is in a landscape where there are natural elements and the agro-environment favors their connectivity. Trees, shrubs and pond edges are homes to many flowering plants and can support a good variety of wild bees.

Your farming methods do not seem to depress the availability of food resources and nesting sites for pollinating insects. Measures such as wildflower strips and selective mowing seem to be favouring the community of pollinating insects. The use of pesticides appears to be prudent and sustainable.

We recommend continuing sustainable and biodiversity-friendly methods. Consumers willingly sustain farms that protect the environment. We therefore recommend you participate and apply for Pollinator Friendly Farm certification!

Your farm is in a very favourable landscape for pollinators, with uncultivated meadows, bushland and waterways, or it is predominantly agricultural with agro-environmental characteristics such as to host a great biodiversity of wild bees.

The farming methods seem particularly attentive to the needs of pollinators. Crop treatments are reduced to a minimum or absent. Mowing seems reduced and flowering plants seem favored by methods such as wildflower strips.

We recommend continuing to use measures which favor the biodiversity of apoidea. Artificial shelters for bees, such as bee hotels, could be a further educational and communication tool to enhance your commitment to the protection of pollinating insects. A farm like yours can certainly obtain Pollinator Friendly Farm certification. The ecolabel tells consumers that they are preserving biodiversity when they purchase your products!

4.4 Replications: Concluding remarks

Here we report one of eight replication results with details on how the replication was conducted and how the results were shared with direct stakeholders. Farms receive a report in the language of their country, and in a few cases further networking enabled deeper discussion and understanding of the issues.

The results of replications were also discussed in scientific contexts:

EU CAP Network workshop Promoting pollinator-friendly farming, Ljubljana, Slovenia, 17-18 June 2024

XII European Congress of Entomology, Crete, Greece, 16-20 October 2023 (Book of Abstract, pp. 237). Title: “Promoting pollinators among farmers in the LIFE 4 Pollinators project”.

EURBEE9-9th European Congress of Apidology, Belgrade, Serbia, 20-22 September 2022 (Book of Abstracts, p. 314) . Title: “A first approach to design a new biodiversity indicator based on wild bees for rural development plan”

5. Identification of protection measures for integrated agriculture

5.1 Integrated agriculture and the quality system

Integrated agriculture or integrated production is an agricultural production system with low environmental impact. It seeks to minimize the use of synthetic chemical substances, water and energy in order to protect the environment and human health without reducing the quality of the product. Methods that impact the environment are only used when strictly necessary on the basis of cost-benefit analysis of environmental and economic needs.

In the previous CAP 2014-2022, the rules for integrated production (measure 10.1, Italian RDP list) became even more rigorous with introduction, since 2016, of the National Integrated Crop Management Quality System (SQNPI). This system identifies crop production obtained in compliance with *regional* integrated production regulations and aims to protect consumers through safety, traceability and cultivation processes that safeguard the environment and human health.

Compliance with SQNPI procedures is certified by accredited control bodies based on the ISO 17065 standard. It confers the right to apply a "sustainable quality" logo (ecolabel depicting a bee) to products. Compliance with the quality system was initially voluntary. Under the current CAP 2023-2027, integrated agriculture measures are SRA01/ACA01. A major innovation is mandatory compliance with SQNPI and enforcement by one of the certification bodies that approve use of the logo.

SISTEMA DI QUALITÀ NAZIONALE
PRODUZIONE INTEGRATA

5.2 Integrated agriculture in Emilia-Romagna region

In Emilia-Romagna region (ER region), plant production covers over 1 million hectares, which is more than 8% of national cultivated area. Total production exceeds 8 million tonnes, approximately 15% of overall national production. Integrated agriculture has a long tradition in the ER region; it was introduced and has been encouraged for over 30 years and the crops included have increased year by year. In 2023 and 2024, the area of integrated production covered approximately 95,000 ha, about 10% of the total cultivated area. Integrated production is partly funded by SRA01 (Integrated production), SRA19-3 (Reduced use of plant protection products) or with sectoral intervention (former CMO) on fruit, vegetables and potatoes, which must all be registered in the SQNPI.

The technical standards for integrated production are set out in "**Integrated farming system guidance**", which is divided into General Guidance, Technical Cultivation Standards and Post-Harvest and Other Processed Production Standards. In addition, the ER region periodically publishes the "Bulletin of Integrated and Organic Production", which contains information and technical advice to support farmers who decide to use integrated production. The Bulletins are constantly updated with indications that emerge in technical coordination meetings and they inform farmers of any further new constraints. The ER region also makes data available regarding the development of forecast models of adversities and aerobiological monitoring, which provide further operational indications for crop protection.

Thanks to these tools and to an efficient assistance and control network, integrated production in Emilia-Romagna is considered one of the most advanced in Italy.

5.3 Proposal for the implementation of the integrated production guidance of the Emilia-Romagna region

The rules of integrated agriculture already include measures that benefit pollinators through reduced use of chemical products compared to conventional agriculture. However, additional steps could be taken to implement the regulations, such as further indications to directly safeguard pollinators and product safety.

As part of Action B.4 of the LIFE 4 Pollinators project, two project outputs, the Farmers' Manual and the Code of Conduct envisage indications for the protection of pollinating insects. In line with these indications, we intend to propose measures for implementing the integrated production regulations in General Guidance and the Technical Cultivation Standards.

In **GENERAL GUIDANCE**, we propose including some indications in the Farmers' Manual for promoting pollinating insects and their diversity through correct management of the agricultural landscape (Figure 5), ecological infrastructure and habitats for the foraging and nesting of pollinators.

Other indications that could be added to General Guidance, besides those already envisaged for integrated agriculture and in the project's Code of Conduct, regard mitigation of exposure to plant protection products and possible collaboration with beekeepers for crop pollination by managed honeybees.

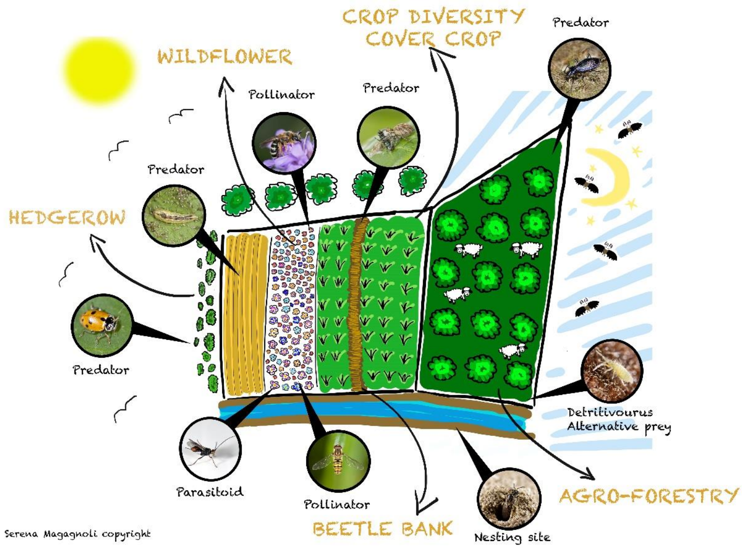


Figure 5 Figure taken from the Handbook for farmers of the Life 4 pollinators project, which illustrates the ecological infrastructures to be included in the farm to encourage insect pollinators and pest enemies.

In the Technical Cultivation Standards, especially the section on phytosanitary protection, our proposal is to complete the table of active ingredients allowed for the various crops with indications of their toxicity for bees (Figure 6). Active ingredients will be classified "slightly toxic", "moderately toxic" and "highly toxic" using smiley emojis of the same colours used in the publication. This will enable farmers to identify and choose the active substances least toxic to bees from those available for a specific crop or a specific pest.

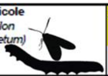
Figure 6 Emojis code proposed to be included in the Technical Cultivation Standards to indicate the toxicity of the active ingredients that allowed in integrated agriculture

Information on the toxicity of active ingredients can be obtained from a free publication "Toxicity for bees of active substances used in agriculture" (https://www.informamiele.it/tabelle-tossicita), updated every year by the "Technical board for appropriate agricultural methods and the protection of beekeeping in the seed, fruit and vegetable sectors" and published by the "Honey Market National Observatory".

Figure 7: Screenshot of the web page https://www.informamiele.it/tabelle-tossicita (12/08/2024)

We now give examples of the toxicity of active ingredients for two common crops, tomato and apple. The tables are in Italian, but Latin names of pests, name of chemicals and the proposed intuitive emojis code enable anyone to understand them.

AVVERSITA'	CRITERI DI INTERVENTO	S.A. E AUSILIARI
Afide Grigio *(Dysaphis plantaginea)*	**Soglia** **- in prefioritura:** **comparsa delle fondatrici** **- in post-fioritura:** **infestazioni in atto da caduta petali a frutto noce** **o in presenza di danni da melata**	Azadiractina Piretrine pure Sali potassici di acidi grassi Tao-Fluvalinate Sulfoxaflor Flupyradifurone Flonicamid Pirimicarb Spirotetramat
Afide lanigero *(Eriosoma lanigerum)*	**Interventi agronomici** - nelle potature limitare i grandi tagli - eliminare i rami colpiti **Soglia** **10 colonie vitali su 100 organi controllati con infestazioni in atto** Verificare la presenza di *Aphelinus mali* che può contenere efficacemente le infestazioni	*Beauveria bassiana* Sali potassici di acidi grassi Pirimicarb Sulfoxaflor Spirotetramat
Afide verde *(Aphis pomi)*	**Soglia** **presenza di danni da melata**	Azadiractina Piretrine pure Sali potassici acidi grassi Acetamiprid Sulfoxaflor Flupyradifurone Flonicamid Pirimicarb Spirotetramat
Carpocapsa *(Cydia pomonella)*	**Soglia** **- trattare al superamento della soglia di 2 adulti per trappola catturati in 1 o 2 settimane** - per il posizionamento dei trattamenti in I e II generazione si raccomanda di seguire le indicazioni dei Bollettini tecnici provinciali definiti sulla base del modello previsionale **- verificare su almeno 100 frutti/ha la presenza di fori iniziali di penetrazione e trattare al superamento della soglia dell'1%** **Tali soglie non sono vincolanti per le aziende che applicano i metodi della Confusione o della Distrazione sessuale** Installare la Confusione o la Distrazione sessuale all'inizio del volo Consigli - nelle aziende che negli ultimi anni hanno subito forti danni di carpocapsa si sconsiglia l'uso degli IGR (regolatori di crescita) - nei casi di perdita di efficacia di una o più s.a., si consiglia il prevalente impiego delle tecniche di confusione sessuale e del virus della granulosi - in I generazione si consiglia di utilizzare virus della granulosi - si consiglia di non utilizzare il virus in miscela con altri prodotti attivi nei confronti della carpocapsa - per problemi di incompatibilità si consiglia di non utilizzare il virus in miscela con prodotti rameici - al fine di prevenire l'insorgere di resistenze, si consiglia di evitare l'impiego ripetuto delle stesse s.a. sulle diverse generazioni del fitofago - al fine di limitare la consistenza delle popolazioni, impiegare i nematodi entomopatogeni che vanno applicati soprachioma sulla parte basale dei fusti, tra metà settembre e metà ottobre, in corrispondenza con precipitazioni o abbondanti irrigazioni; al momento dell'applicazione e per le ore successive occorre che la temperatura minima sia superiore ai 13 °C - in alternativa completa o parziale alla difesa chimica, si consiglia l'impiego di reti "AltCarpò"	Confusione e Distrazione sessuale Virus della granulosi Nematodi entomopatogeni (*) Triflumuron Tebufenozide Etofenprox Spinetoram Spinosad Acetamiprid Emamectina Clorantraniliprole Fosmet
Eulia *(Argyrotaenia pulchellana = Argyrotaenia ljungiana)*	**Soglia** **- I generazione: 5% di getti infestati** **- II e III generazione: 50 adulti per trappola o con il 5% dei germogli infestanti** Il momento preciso per l'intervento è indicato dai Bollettini tecnici provinciali sulla base delle indicazioni dei modelli previsionali	*Bacillus thuringiensis* Tebufenozide Indoxacarb Clorantraniliprole Emamectina Spinetoram Spinosad
Cimice asiatica *(Halyomorpha halys)*	**Monitoraggio** - a partire indicativamente da fine aprile ponendo attenzione, nelle fasi iniziali, ai punti di ingresso (vicinanza ad edifici, siepi, ecc.) - eseguire i controlli anche nel periodo degli sfalci e delle trebbiature delle colture erbacee ospiti (es. soia) e nel corso delle raccolte nei frutteti adiacenti, che possono provocare massicci spostamenti della cimice Monitoraggio visivo: - controllare la presenza di adulti, ovature e forme giovanili, su foglie e frutti con particolare attenzione alla parte alta delle piante - nelle prime ore del mattino la cimice risulta meno mobile Monitoraggio con trappole: - utilizzare trappole specifiche con feromoni di aggregazione da ispezionare periodicamente - installare le trappole sui bordi dell'appezzamento, a distanza di almeno 20-30 m tra loro - le trappole all'interno dei frutteti possono comportare l'incremento delle popolazioni e dei danni nel raggio di azione del feromone (circa 6/8 metri) - le trappole non forniscono una stima della popolazione ma facilitano il rilievo della presenza dell'insetto - non esiste al momento una soglia d'intervento **Mezzi fisici** - applicare reti antinsetto monofila o monoblocco con chiusura anticipando i primi spostamenti dell'insetto **Interventi chimici** - la situazione territoriale sulla presenza e diffusione della cimice è riportata nei Bollettini tecnici provinciali - gli interventi devono essere eseguiti sulla base dei riscontri aziendali - l'effetto abbattente dei trattamenti è legato soprattutto all'azione diretta per contatto quindi gli interventi vanno correlati alla presenza dell'insetto	Piretrine pure Acetamiprid Tau-fluvalinate Deltametrina Etofenprox Lamba-cialotrina Triflumuron

BATTERIOSI *(Pseudomonas syringae* pv. *tomato* *Xanthomonas campestris* pv. *vesicatoria* *Clavibacter michiganensis* subsp. *michiganensis* *Pseudomonas corrugata)*	**Interventi agronomici** - impiego di seme certificato, conservare la documentazione - ampie rotazioni colturali (almeno 4 anni) - concimazioni azotate e potassiche equilibrate - eliminazione della vegetazione infetta, che non va comunque interrata - è sconsigliato irrigare con acque provenienti da canali o bacini di raccolta i cui fondali non vengano periodicamente ripuliti da residui organici - trapiantare solo piante non infette dando preferenza a cvs tolleranti - sarchiature **Interventi chimici** Solo negli impianti ad alto rischio si può intervenire, prima della comparsa dei sintomi, al massimo 3 volte dopo la semina o il trapianto ad intervalli minimi di 6 -7 giorni	Prodotti rameici *Bacillus subtilis* Acibenzolar-S-metile
VIROSI (CMV, PVY, ToMV)	Per le virosi trasmesse da afidi in modo non persistente (virus del mosaico del cetriolo CMV, virus Y della patata PVY) valgono le stesse considerazioni generali di difesa Per il trapianto usare piantine ottenute in semenzali prodotti in vivai con sicura protezione dagli afidi Per le virosi trasmesse per contatto (virus del mosaico del pomodoro ToMV) è fondamentale l'impiego di seme esente dal virus o sottoposto a disinfezione mediante trattamenti fisici o chimici	
FITOFAGI **Elateridi** *(Agriotes spp.)* 	Evitare la coltura in successione ad erba medica per almeno 2 anni **Distribuzione localizzata dove sia stata accertata la presenza di larve secondo le modalità riportate nella Tabella 23 (Norme Generali) o in base a infestazioni rilevate nell'anno precedente** Con infestazioni in atto, effettuare lavorazioni superficiali nell'interfila per modificare le condizioni igrometriche e per favorire l'approfondimento delle larve nel terreno	*Beauveria bassiana* Cipermetrina Lambdacialotrina (*) Teflutrin (*)
Nematode galligeno *(Meloidogyne spp.)*	Sono presenti nei terreni prevalentemente sabbiosi **Interventi agronomici** impiego di varietà resistenti (Nemador, Trajan)	*Paecilomyces lilacinus* *Estratti di aglio* Geraniolo+timolo Azadiractina
FITOFAGI OCCASIONALI **Afidi** *(Myzus persicae* *Macrosiphum euphorbiae)* 	In generale, le infestazioni afidiche si esauriscono nell'arco di 10 giorni e sono ben controllate dagli ausiliari presenti in natura **Soglia** **attendere che almeno il 10% delle piante siano infestate da colonie in accrescimento presenti in 4 o 5 metri lineari cadauno, lungo la diagonale dell'appezzamento e, in ogni caso, verificare la presenza di insetti utili**	Piretrine pure Sali potassici di acidi grassi Olio minerale Azadiractina Maltodestrina Acetamiprid Sulfoxaflor Flonicamid Spirotetramat Flupyradifurone
Nottue terricole *(Agrotis ipsilon* *Agrotis segetum)* 	**Soglia** **1 larva/5 m lineari di fila in 4 punti di 5 m lineari ciascuno lungo la diagonale dell'appezzamento, su piante all'inizio dello sviluppo**	Deltametrina Cipermetrina Lambdacialotrina
Cimici *(Nezara viridula)* *(Halyomorpha halys)* 	**Limitare l'intervento alle sole coltivazioni ove è stata rilevata una presenza diffusa e significativa di cimici**	Lambdacialotrina Deltametrina Etofenprox Acetamiprid
Tripidi *(Thrips spp.)* 		Olio essenziale di aranci[o]
Dorifora *(Leptinotarsa decemline[ata])* 	**Soglia** **infestazione generalizzata**	
Ragnetto rosso *(Tetranychus urticae)*	**L'intervento è giustificato solo in presenza di focolai precoci di infestazione con evidenti aree decolorate delle foglie**	*Beauveria bassiana* Sali potassici di acidi gra[ssi] Olio minerale Maltodestrina Zolfo **Al mass[imo] 3 in[terventi] ha** Abamectina Bifenazate Acequinocyl Clofentezine Exitiazox Fenpiroximate Cyflumetofen
Nottua gialla del pomodoro *(Helicoverpa armigera)* 	**Soglia** **2 piante con presenza di uova o larve su 30 piante controllate per appezzamento** Si consiglia di controllare il volo con trappole a feromoni Si consiglia l'utilizzo di Spinosad e Indoxacarb sulle uova prima che schiudano	*Bacillus thuringiensis* Virus NPV nottua gialla Azadiractina Deltametrina Cipermetrina Etofenprox Lambdacialotrina Spinosad Spinetoram Indoxacarb Metaflumizone Emamectina Clorantraniliprole Metossifenozide

6. The Code of conduct and the "Pollinator friendly farm" certificate

Farmers can play a key role in the conservation of pollinators by implementing measures on their farm to support pollinators. The LIFE 4 Pollinators Code of Conduct proposes agricultural methods and measures that benefit pollinators. The measures fall in three main categories: reducing pesticide exposure, providing and enhancing foraging habitats, and providing nesting sites.

Suggestions for critical consumers are also offered.

6.1 Categories of measures to be adopted

The category "Reduction of pesticide exposure" includes non-agrochemical pest management (agricultural, physical and mechanical methods and other low-impact control) or when chemical control cannot be avoided (e.g. when the economic threshold is exceeded or in the case of mandatory control), mitigation measures are suggested to reduce the impact on pollinating insects (e.g. use of products less toxic to bees, practical precautions based on weather conditions, time of day, anti-drift devices and other operational measures).

The category "Providing and increasing foraging habitats for pollinators" promotes the sowing of wildflower strips or the maintenance of wild vegetation at field margins and in non-productive areas of the farm; it also suggests selective mowing of nectariferous and polleniferous flora and diversifying crop species to prolong the duration of flowering periods.

The third category "Providing appropriate nesting sites and increasing ecological corridors" involves maintenance of ecological infrastructures at farm and landscape level (hedgerows, woody vegetation, roadsides, dykes, field boundaries, windbreaks and ditches), creation of artificial nesting-sites for wild cavity-nesting bees (bee hotels) and preserving bare ground and reducing tillage to support ground-nesting bees.

To obtain "Pollinator-Friendly Farm" certification, the farmer must comply with 3 measures of non-agrochemical pest management (1 mandatory and 2 optional), 6 mitigation measures to reduce the impact on pollinating insects (1 mandatory and 5 optional), 2 measures aimed at providing and increasing foraging habitats for pollinators (1 mandatory and 1 optional) and 1 optional measure to provide nesting sites and increase ecological corridors. The Code of Conduct also proposes non-mandatory measures to enhance sustainability through consumer choices, e.g. choosing organic, local and seasonal products.

Farms wishing to comply with the Code of Conduct should fill in the form at the end of this document, indicating the measures they intend to implement, and send the signed scanned form by email to amicidegliimppollinatori@gmail.com.

6.2 Verification

Verification that the measures are effectively carried out is done by documentation checks, requests for photos and/or visits to the site by LIFE 4 Pollinators personnel. Farms then obtain "Pollinator-Friendly Farm" certification for the current year. The audits aim to ensure that farmers are implementing the actions correctly and therefore effectively supporting wild pollinators.

Figure 8 Certificate given to farms that fulfill the commitments for the protection of pollinators contained in the Code of conduct.

Certified farms are listed on the project social media and website and shown on a map. The "Pollinator-Friendly Farm" ecolabel has no legal value as yet but acknowledges compliance with measures to protect pollinators. Thus the farm can testify pollinator-friendly production and obtain economic advantages. The possibility of certification mark status is being investigated.

7. Best practices for the protection of pollination networks

7.1 Plant-Pollinator Networks

A plant-pollinator network is defined as "*a group of local plant and pollinator species and the links between them that establish who interacts with whom*" (i.e. qualitative network; Figure 8). A network can also include a measure of the strength of the various interactions (i.e. quantitative network) as explained in the IPBES document (https://www.ipbes.net/glossary).

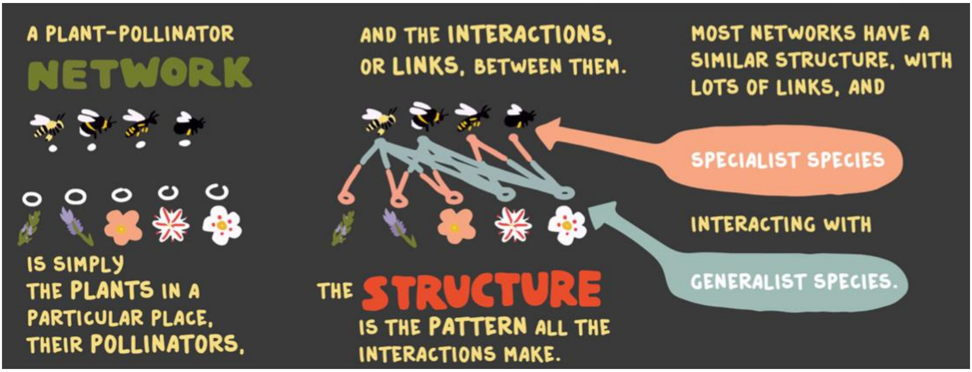


Figure 9: What is a plant-pollinator network? Extract from Fig. 1 An explanation of why it's important to protect the structure of plant-pollinator interaction networks in Khoury et al. (2019) https://rdcu.be/dMCV6 .

Plant-pollinator networks help us understand ecosystem complexity by summarising mutualistic interactions between pollinators and plants, their patterns and the ecological role of individual species in the community. Network analysis can therefore be used for conservation purposes, to make predictions to help management, and to assess the effects of concrete actions on pollinator and plant communities. Preliminary surveys of plant-pollinator interactions are the basis for any future monitoring. To guarantee ecosystem stability, ecological interactions and entire communities should be targeted with conservation measures, in natural, urban and rural environments (including agricultural systems).

Pollination networks are usually mapped through field surveys by specialists, but also expert-assisted citizen science activities, such as mini-bioblitzes or educational projects; photographic records uploaded by citizens on web platforms may provide reliable data that can be used to obtain descriptive networks of plant-pollinator interactions.

7.2 How and at what level can plant-pollinator networks be informative?

Here we report some case studies from LIFE 4 Pollinators. Network analysis of plant-pollinator interactions offers valuable insights into the biodiversity and ecological dynamics of a study area, regardless of data collection methods.

1) Scientific monitoring for data on plant-pollinator networks provides precise data, which may concern the complexity of interactions between plants and pollinators, and the specificity of these relationships. The network allows us to identify which pollinators (or pollinator functional groups) are generalists and which have more specialized roles. Taxonomic level is closely related to the monitoring protocol and to the experience of the sampler/observer and is defined in relation to the aim of the study. For example, Figures 9 and 10 show examples of the network at a single site at different times. The increase in complexity of interactions between main pollinator functional groups and wild plant species can be appreciated after implementation of pollinator-friendly measures in the area.

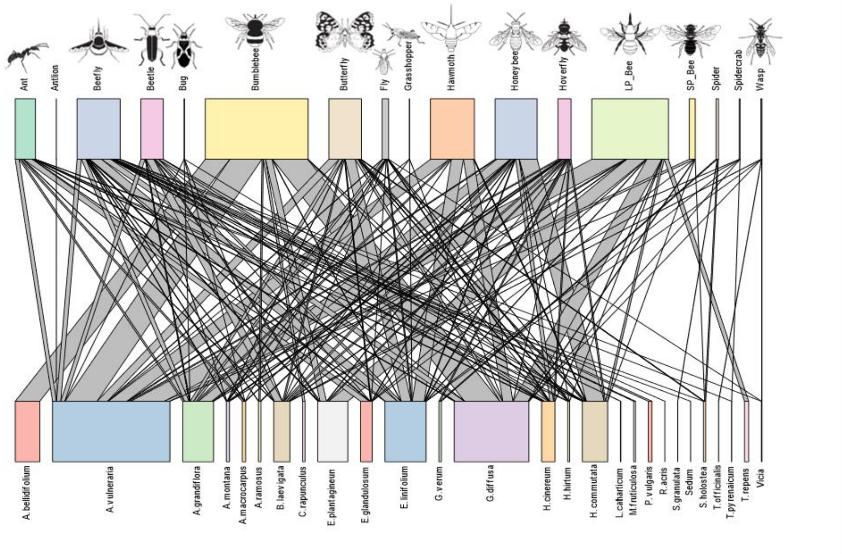


Figure 10: Example of a pollination interaction network conducted using the data from two consecutives bioblitzes carried out in the Sierra de Courel (Ancares - Courel ; ES1120001) during May 2022 and May 2023. Pollinator species were identified at the functional group level, and plants at the species level. The width of each bar represents the partial abundance of each organism or interaction.

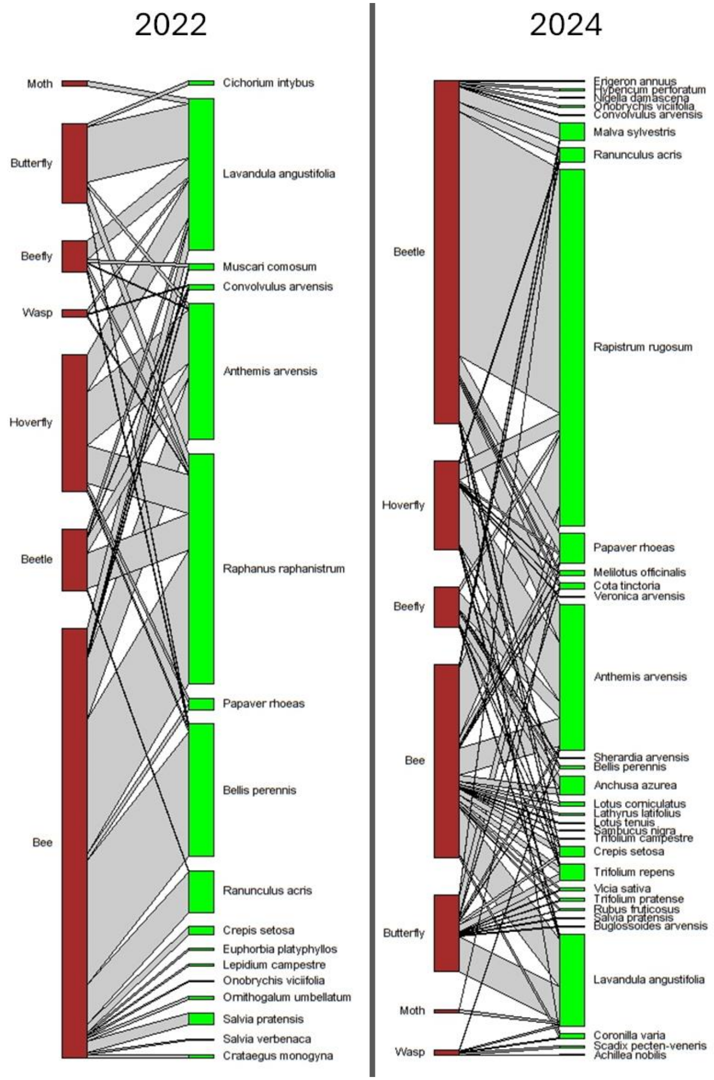


Figure 11 ; Plant-pollinator network at project study site in agroecosystem within the protected area "Parco della Chiusa" (SAC IT4050029) in 2022 (left) and 2024 (right), respectively before and after the implementation of pollinator-friendly measures.

2) Citizen science initiatives can contribute significantly to biodiversity data.

2a) By following standardized protocols accessible to non-experts, citizen scientists can collect quantitative data that may help assess the effectiveness of pollinator protection measures (Figure 11). This 'democratization' of data collection not only increases the dataset but also fosters community engagement and awareness.

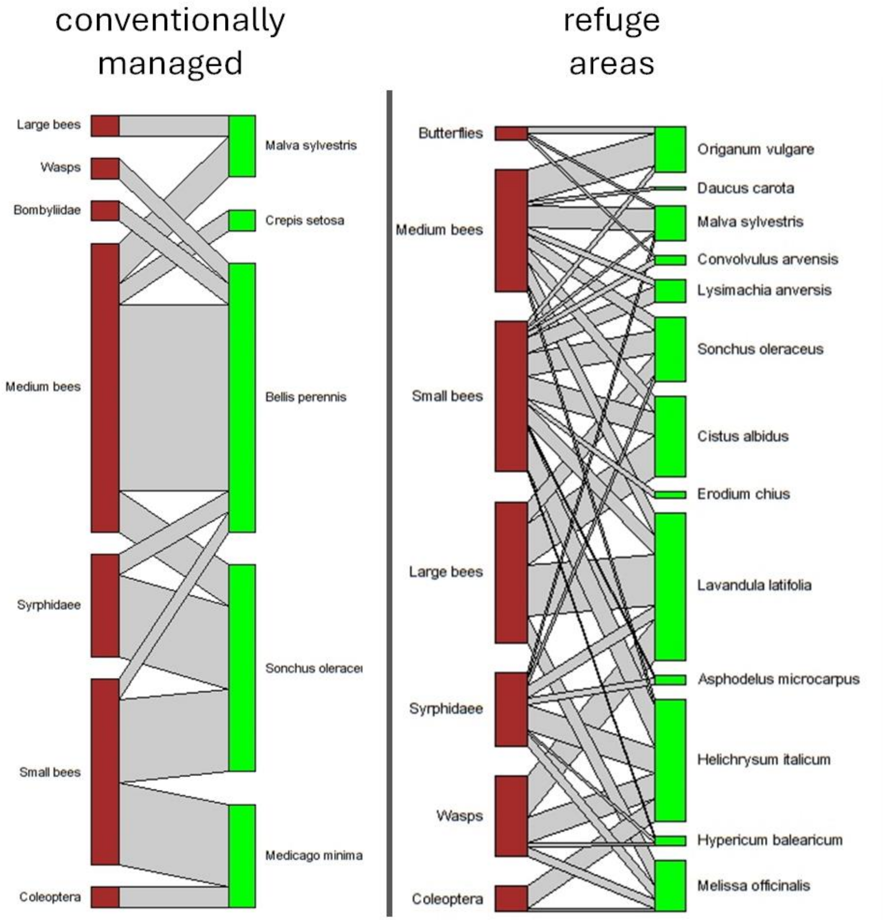


Figure 12: Plant-pollinator network resulting by combining all interactions recorded in three urban parks in Mallorca (Balearic Islands, Spain). The network on the left was obtained by sampling conventionally managed green spaces, while the one on the right comes from refuge areas for pollinators created in the same parks

2b) Qualitative data collected during bioblitzes, albeit without standardized protocols, can be useful to acquire a baseline picture for developing targeted conservation actions, such as the conservation of threatened and the management of invasive species (Figure 12).

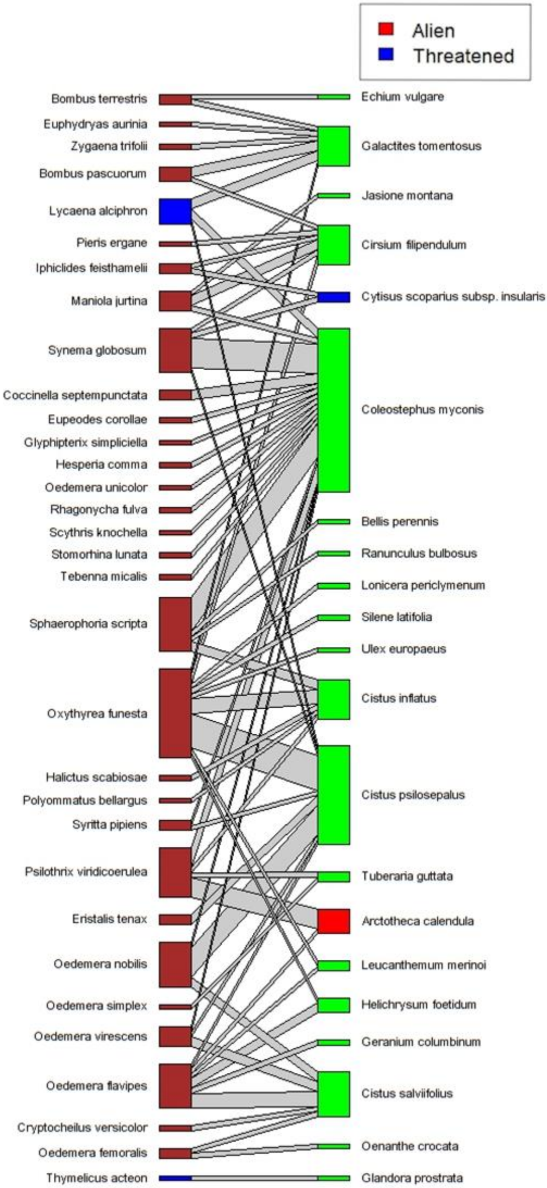


Figure 13: Plant-pollinator network obtained using data from a bioblitz carried out in May 2023 on the island of Ons of the Atlantic Islands of Galicia national park (ES0000254), Spain. In just a few days, citizen scientists were able to spot alien and endangered species.

7.3 Sampling methods to record plant-pollinator interactions

Transect / random sampling with hand net: walk for a defined distance (i.e. the length of the linear transect or random walk) and time (i.e. scheduled time of the day and duration), capturing insects that are visiting flowers (i.e. potential pollinators) and recording the species of flowering plants. The advantage of this technique lies in the possibility of recording plant-pollinator interactions and releasing the insects after identification in the field.

Plot sampling: observations conducted in one or more fixed squares (plots). Insects visiting flowers in selected plot(s) are recorded and/or captured, and the species of flowering plant are identified.

Floral resources: for a better understanding of relationships between plants and pollinators, it is advisable also to assess food resources. This can be done by random sampling in random plots of a given size, counting the number of floral functional units (individual flowers or inflorescences, according to species, e.g. number of capitulae for Asteraceae) in each plot.

Figure 14: Monitoring activity during the LIFE 4 Pollinators project.

The LIFE 4 Pollinators project proposes different sampling protocols, the methodologies of which are available in six languages from the project website (see download materials). As already indicated, several variants of transect sampling have been applied in the monitoring (Figure 13). The variants helped us address the aims of the monitoring activity: the protocols used to monitor the pollination networks (following pages) are an example of that already described in implementation of the BEE indicator (this document, pages 16-18). The main difference is the level of taxonomic identification. Two simplified protocols are described for citizen science in the Citizen Science Handbook, and the respective field recording sheets are also available as part of educational activity "Students4Pollinators" (Figure 14).

The choice of which monitoring protocol to adopt clearly depends on the objectives. Nevertheless, for conservation purposes, a standardized scientific methodology is urgently needed, and is currently being discussed among European experts. The proposal for a pollinator-monitoring scheme in Europe (Download PDF) provides detailed perspectives on methods to record pollinators, plant-pollinator interactions, and the corresponding flowering-plant communities, outlining different approaches in relation to research objectives.

FIELD PROTOCOLS AND STATISTICAL ANALYSES APPLIED TO POLLINATION NETWORKS

These protocols have been employed to obtain reliable scientific data on the diversity of the main pollinator groups (wild bees, wasps, butterflies, beetles, hoverflies and beeflies) and the ecosystem function to which they are related. To do this, data needed to be comparable across time (the duration of the project) to evaluate the ecological impact of the project (e.g. the pollinator-friendly measures proposed and adopted in the course of the project) using pollinators as bio-indicators. **Two protocols** were used, depending on the taxonomic knowledge and expertise of the people involved in the field surveys.

DETAILS ON SURVEY

Survey frequency and location: repeat the survey 3 times per site throughout the main flowering period; time and site should be selected according to the availability of flowers (e.g. April/early May, late May/June, July/early August).

Flower cover: The general aim is to assess environmental suitability for sustaining healthy populations of insect pollinators. Because flowers are essential for bees and other pollinators, a pollinator-friendly environment should provide abundant and diverse floral resources throughout the season. It is therefore essential to estimate flower cover in order to evaluate environmental quality for insect pollinators. Flower cover can be assessed before or after pollinator survey in 12 plots (1m x 1m) per site: six scattered throughout the field and six specifically in the transect. In all cases, the flower cover survey should be done in parallel with the insect survey, for example by stopping the insect survey every 15 min to measure one or two flower plots. Plots are preferably selected by randomized sampling, e.g. Locus-Map. In each quadrat, flowering plants are identified to genus or species level, and floral pollination units are counted (single flower, flower head or inflorescence, depending on the species).

Pollinator survey: we used two protocols as explained below.

Protocol version 1 (for expert surveyors) Pollinators are recorded (see the taxonomic categories below) along a transect possibly marked out along field margins or where there are wildflowers among crops and possibly entomophilous crops. Locus-Map, a multifunctional navigation application that records coordinates, time, length of transect, inserts photos and pauses recording, can be used. Individual insects are sampled by hand-netting when they visit a flower. They are kept in a Falcon tube (50 ml) in a cool dark container for the duration of the sampling event. They are released during breaks, keeping only 1-2 individuals per taxon (genus, species or morpho-taxonomic group) as reference samples. Only insects visiting flowers are collected (whether they are collecting pollen, nectar or essential oils, or are resting or mating), and the plant taxonomy is recorded (at genus or species level).

The overall duration of a pollinator survey is 90 minutes/day/site.

Protocol version 2 (simplified, for non-experts)

Pollinator surveys are performed in a plot. For 5 minutes, individual insects (identified in broad groups as mentioned above) are counted each time they enter the plot. Flower-visiting insects are captured only if they cannot be identified.

The overall duration of the pollinator survey is 120 minutes (60 minutes in the morning, 60 minutes in the afternoon).

INSECT GROUPING AND IDENTIFICATION

For protocol version 1: (genus/species level or morpho-taxonomic group)

- Bee genus, Apoidea, order Hymenoptera
- Wasps, Vespoidea, order Hymenoptera (morpho-groups)
- Beeflies, Bombyliidae, order Diptera (morpho-groups)
- Hoverflies, Syrphidae, order Diptera (morpho-groups)
- Other flies, Calliphoridae, order Diptera (morpho-groups)
- Butterflies, order Lepidoptera (morpho-groups)
- Moths, order Lepidoptera (morpho-groups)
- Beetles, order Coleoptera (morpho-groups)

For protocol version 2 (Identification in broad groups)

- Honeybees, Apoidea, order Hymenoptera
- Bumble bees, Apoidea, order Hymenoptera
- Large bees (larger than honeybees), Apoidea, order Hymenoptera
- Medium bees (honeybee size), Apoidea, order Hymenoptera
- Small bees (clearly smaller than honeybees), Apoidea, order Hymenoptera
- Wasps, Vespoidea, order Hymenoptera
- Beeflies, Bombyliidae, order Diptera
- Hoverflies, Syrphidae, order Diptera
- Other flies, order Diptera
- Butterflies, order Lepidoptera
- Moths, order Lepidoptera
- Beetles, order Coleoptera

NETWORK ANALYSES

STATISTICAL ANALYSIS (WITH R)

Different types of software can be used for network analysis. R, a free software environment for statistical computing (R Core Team, 2021) is very useful and widely used by academics. R has various packages specific for ecological and network analysis. For example:

- Vegan: a package that can be used to calculate major biodiversity indices (such as the Shannon index, Pielou evenness index and Simpson dominance index) and to manage ecological data (Oksanen et al., 2022).
- Bipartite: a specific package for ecological network analysis. It provides tools to analyse and visualise interactions between distinct groups, defined as nodes (Figure 2), and the properties of the network itself, such as connectivity, nestedness, modularity and robustness (Dormann et al., 2009);
- Igraph: this package can be used for network analysis, including node-ownership analysis. It can be employed to analyse the role of species within the network, considering aspects such as centrality, betweenness, and other interaction dynamics (Csardi & Nepusz, 2006).

To thoroughly explore the dynamics of an ecological network, it is essential to analyse specific properties that provide crucial details about its structure and resilience.

Connectivity, for example, is the percentage of interactions recorded out of all interactions theoretically possible, or an indicator of connection density in the network. The greater the connectivity, the greater the complexity of the network, hence its resilience to disturbance. Nestedness is an intriguing concept that reflects the link between specialist and generalist species. It highlights how some specialised species, with few interactions, are linked to generalist species and vice versa. In other words, it quantifies the network's flexibility in interacting with a wide range of species. Modularity is a fundamental proxy that reveals the presence of modules or highly interconnected species that form groups within a network. This property may unveil subgroups of ecological interactions that could have significant implications for network stability. Robustness, on the other hand, is crucial for evaluating a network's ability to maintain its structure in response to perturbations, such as the loss of a species. A robust ecological network can adapt to and resist such events, ensuring the persistence of interactions.

Network analysis is not limited to general measures; it also reveals the specific role of each species. Some strong species with many connections take key roles and can be fundamental for network structure. On the contrary, other species play a bridging role, connecting different species and thus contributing to network cohesion.

BIODIVERSITY INDICES

Plant and animal communities are often analysed using biodiversity indices to measure their diversity and structure (Magurran, 2004). Common, widely used indices include:

- Shannon Index (H): This index evaluates species richness in relation to relative abundance (Shannon, 1948). It considers both species richness and the proportion of individuals of each species, providing an overall measure of community diversity (Minachilis et al., 2023);
- Pielou Index (J): Also known as Pielou's equity index, this metric expresses how uniformly relative abundances are distributed among the species (Pielou, 1966). A value of J close to 1 indicates a uniform distribution, while lower values indicate dominance of certain species;
- Simpson's Dominance Index (D): This metric expresses the probability that two individuals, randomly selected from a set, belong to the same species (Simpson, 1949). Its value ranges from 0 to 1, where values close to 1 indicate dominance of a species in the community.

Combined use of these indices provides comprehensive insight into the biodiversity and structure of a community (Magurran, 2004). Examining interactions in food webs, such as those between predator and prey, host and parasite, or in our context plant and pollinator, offers a further precious approach for understanding community structure and formulating conservation strategies (Olesen et al., 2007). The analysis is based on an analogy between interactions of species and human social dynamics, regarding species as "nodes" in an ecological network (Dunne et al., 2002). Network topology offers an indication of ecosystem structure and stability as well as highlighting its resilience in the face of environmental changes and threats. A more complex network, characterized by a number of interactions equally distributed between different species or nodes, proves more robust to extinction events and environmental perturbations (Montoya et al., 2006). Network analysis also reveals "key species", those essential for ecosystem cohesion, and the nature of the interactions, distinguishing specialized species, involved in few interactions, from generalist species, involved in more interactions (Montoya et al., 2006). From a conservation perspective, these key species provide valuable guidance for conservation projects. They can act as key elements in the ecosystem or as particularly crucial specialists, loss of which could trigger significant disruption (Olesen et al., 2007). A tangible example of this principle is the possible extinction of a specialist plant species following loss of its pollinators, negatively impacting seed production and overall plant fitness. In conclusion, ecological network analysis emerges as a fundamental tool for understanding the dynamics of biological interactions and guiding targeted conservation strategies (Dunne et al., 2002; Montoya et al., 2006; Olesen et al., 2007; Petanidou et al., 2008; Lázaro et al., 2021).

BOX 4: REFERENCES

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