POLLINATOR AND BOTANICAL TRANSECT PROTOCOL

WP1: ESTABLISHING AND TESTING CO-ADAPTATIVE MANAGEMENT IN A EUROPEAN POLLINATOR RESTORATION NETWORK
TASK 1.2: STANDARDISE SURVEY AND POLLINATION EXPERIMENTS AND INTEGRATION TO LONG-TERM MONITORING OF WILD POLLINATORS

Deliverable D1.2

02 December 2024

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RestPoll
Restoring Pollinator habitats across European agricultural landscapes based on multi-actor participatory approaches

Prepared under contract from the European Commission

Grant agreement No. 101082102
EU Horizon Europe Framework Programme

Project acronym:	**RestPoll**
Project full title:	**Restoring Pollinator habitats across European agricultural landscapes based on multi-actor participatory approaches**
Start of the project:	Oct 2023
Duration:	48 months
Project coordinator:	Professor Alexandra-Maria Klein University of Freiburg, Freiburg, Germany www.restpoll.eu
Deliverable title:	RestPoll case-study protocols
Deliverable n°:	D1.2
Nature of the deliverable:	Demonstrator
Dissemination level:	Public
WP responsible:	WP1
Lead beneficiary:	ALU
Citation:	Ouin, A., Fornoff, F., Acloque, A., & Andrieu, E. (2024). *Pollinator and botanical transect protocol*. Deliverable D1.2 EU Horizon RestPoll Project, Grant agreement No. 101082102.
Due date of deliverable:	January 2025
Actual submission date:	February 2024

Deliverable status:

Version	Status	Date	Author(s)
2.0	Draft	02 Dec. 2024	Annie Ouin [INPT], Felix Fornoff [ALU], Amandine Acloque [INPT] and Emilie Andrieu [INRAE]

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Funded by the European Union

This project receives funding from the European Union's Horizon Europe Framework Programme under project No. 101082102.

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Preface

The RestPoll project aims to provide society with tools to reverse wild pollinator declines, including the restoration of pollinator habitat. This protocol aims to assess the diversity and abundance of the most important pollinators: bees (Hymenoptera: Apiformes), hoverflies (Diptera: Syrphidae) and butterflies (Lepidoptera: Rhopalocera), the flowers available for pollinators and the vegetation at representative transects within study sites. This pollinator sampling protocol is based on the STEP project (Status and Trend of Pollinator in EU) protocol as used in Scheper et al. (2015) and Holzschuh et al. (2016).
We supplemented this protocol with "expert alternatives" (see gray **) focusing on the recording of pollinator activity to allow more detailed analysis including the construction of plant-pollinator interaction networks.

Related document(s):
- Case Study Area (CSA) set up protocol
- Pollinator_flower_vegetation_transects_RestPoll_WP1.xlsx

1. Pollinator transects

1.1. POLLINATORS

The following pollinator groups will be surveyed:
- All butterflies in the Papilionoidea superfamily (formerly Rhopalocera) will be identified directly in the field, or captured and photographed for identification and then released.
- All hoverflies (Diptera: Syrphidae) will be identified directly in the field (e.g., *Episyrphus balteatus*), or captured and stored for later identification.
- All wild bees (Apiforme) and *Apis mellifera* will be captured with the exception of obvious species (e.g., A. *mellifera*, female of *Halictus scabiosae*) depending on the entomofauna and the observers' skills.

European lists of species are made available on the online RestPoll file server (bees and hoverflies: Reverté et al., 2023; butterflies: Wiemers et al., 2018)

1.2. LOCATION

The pollinator transects will be carried out at one transect of 150m long per sampling site, dividing the transect into three 50m long sub-transects. When possible, the transect will be fixed for all surveys across flight seasons and years. The transect should be situated at the centre of the site and/or at least at 10m distance from the habitat border to avoid pronounced edge effect. The 150m long transect is walked once for 15min (5min for each 50m sub-transect) to record bees and hoverflies over a width of 2m (1m on each side), and a second time for 15min to record butterflies over a width of 5m (2,5m on each side) (Figure 1). There have to be a rest period of at least 15min between the two walks.

The ideal situation is a unique straight line of 150m. If the study site is very small, it might not be possible to position a straight line of 150m, then a W shape ("zigzag") is

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recommended (Figure 2). Avoid “pure” go and return, which would increase the risk of double counting.

The length of the transect can be extended beyond 150m by adding 50m segments (thus adding 5min of sampling per added segment). However, for the overall analysis, only 150m will be retained.

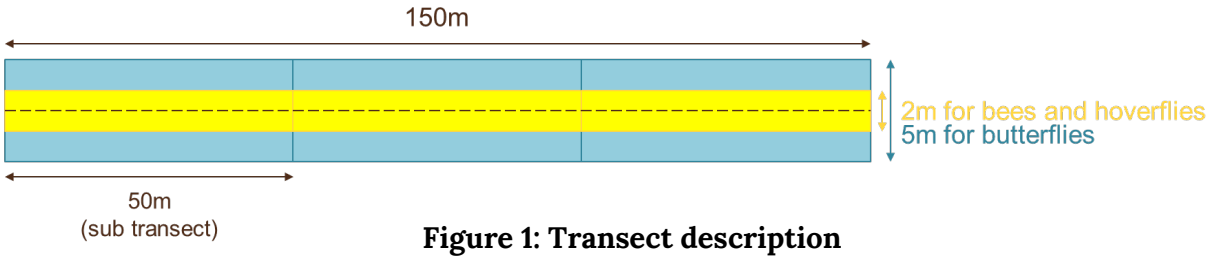

Figure 1: Transect description

Figure 2: Example of transects in real conditions

1.3. WEATHER CONDITIONS

Optimally, pollinator transects should be conducted when the vegetation is dry and wind speed is low. All surveys will be carried out on relatively calm wind conditions (Beaufort number < 3, see Table 1). Temperature should be above 17°C and measured in the sun, because the temperature in the shadow is often below 17°C in spring, and pollinators fly nevertheless. The weather considerations could be adapted to each study region (e.g., it must be at least 13°C in the shade if sunny, at least 17°C if cloudy for the butterfly/bee transect monitoring in GB) but it should be consistent and recorded for each cases study area.
Generally, it is better to collect data even if the weather conditions are not perfect (given that the same conditions prevail at all the sites within a case study area) than to have no

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data at all. Sunshine is more important than temperature. Weather conditions at the beginning and at the end of the transect should be noted (Appendix A.2 Field sheet: pollinator records).

Table 1: Beaufort scale for estimating wind speed

Beaufort number	Land conditions
0	Calm. Smoke rises vertically.
1	Smoke drift indicates wind direction. Leaves and wind vanes are stationary.
2	Wind felt on exposed skin. Leaves rustle. Wind vanes begin to move.
3	Leaves and small twigs constantly moving, light flags extended.
4	Dust and loose paper raised. Small branches begin to move.
5	Branches of a moderate size move. Small trees in leaf begin to sway.
6	Large branches in motion. Whistling heard in overhead wires. Umbrella use becomes difficult. Empty plastic bins tip over.
7	Whole trees in motion. Effort needed to walk against the wind.
8	Some twigs broken from trees. Cars veer on road. Progress on foot is seriously impeded.
9	Some branches break off trees, and some small trees blow over. Construction/temporary signs and barricades blow over.
10	Trees are broken off or uprooted, saplings bent and deformed. Poorly attached asphalt shingles and shingles in poor condition peel off roofs
11	Widespread damage to vegetation. Many roofing surfaces are damaged; asphalt tiles that have curled up and/or fractured due to age may break away completely.
12	Very widespread damage to vegetation. Some windows may break; mobile homes and poorly constructed sheds and barns are damaged. Debris and unsecured objects are hurled about.

1.4. CONDUCTION

The surveys will be carried out in spring and summer seasons, during at least two consecutive years (i.e., 2024-2025 or 2025-2026 or 2024-2025-2026). A minimum of three surveys per year will be conducted between March and September with an interval of about 5-8 weeks between each survey. Exact timings should be decided by the case study areas leads and should aim at surveying maximal diversity and abundance of pollinator species across the year and under the location specific climatic conditions and the current weather situation. The number of surveys per year can be extended beyond three, but only three will be retained for the overall analysis.

Pre-remark on randomization: Always conduct surveys in pairs/triplets or quadruplets (hereafter groups) including each of your implementation types once, keep these groups

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across all surveys. Moreover, pollinator occurrence changes across the season and the time of the day. Therefore randomize/alternate the sampling order of groups per survey (start with a different group each survey) and of sites within a day (not always controls in the morning).
A transect consists of a very slow walk along a virtual line during which the operator only observes/counts/identifies/captures the individuals in front of him/her or to the side, but never turns around to look back. Pollinators will be observed for 15min per transect of 150m (5min per 50m sub-transect, i.e., a walking speed of 0,17 $m.s^{-1}$, i.e., 1m long covered in 6 seconds) and per survey. On the same 150m long transect, bees and hoverflies are recorded on a width of 2m (1m on each side) and butterflies on a width of 5m (2,5m on each side, 5m in front and 5m above). For each sub-transect, the total number of bees/hoverflies or butterflies that were nesting (or ovipositing), or flower visiting is noted. By default, the other individuals are considered to be flying**. 15min for bees and hoverflies + 15min for butterflies is the pure time spent for searching and catching the pollinators with the net. Taking into account the rest period between the two walks (15min), the minimum time spent on one transect for pollinator recording is 45min. In case of catching, the observer must instantly stop the stopwatch while the pollinator is transferred to a vial, labelled and notes are taken. Otherwise, the number of pollinators will be underestimated in sites with high abundances. Each pollinator individual will only be recorded once, for example a butterfly that crosses the transect multiple times, will not be recorded multiple times. The sex of the pollinator will be noted, in the field if possible (in the comments section), or in the lab when the individual was captured.

** *Expert alternative : The behaviour of each individual will be recorded: Flying, Resting, Nesting (or ovispositing), Flower visiting. For "Flower visiting", please note the flower species or at least the flower colour instead of "Flower visiting". Use the Field sheet in Appendix A.2bis instead of Appendix A.2*

In the field, each captured individual will be put into a uniquely labeled killing vial/container containing ethyl acetate vapor. Individuals of the same sub-transect can be pooled together in the same killing container. These individuals receive an individual label in the lab, where they are directly pinned and/or identified (or possibly barcoded), stored either frozen at -18°C or in a vial filled with clean alcohol before pinning is possible. Pollinators which were neither successfully caught nor identified to the species level in the field (although observed on the transect close enough for theoretically being caught) should nevertheless be noted together with as much taxonomic information as possible (morphotype or at least "hoverfly" or "bumble bee" for example) and the comment "not caught". These data can be used for abundance analyses at least.

For each survey, date, observer(s), time, wind speed (Beaufort scale, Table 1), temperature and cloud cover (visual estimation, Appendix A.1) will also be recorded (see Appendix A.2 Field sheet: pollinator records and template on the data repository). This information is gathered with a unique identifier for each sub-transect, consisting of the site id (i.e., the site type "CSA" and the identification number of the group of site types), the transect number, the sub-transect number and the survey number. The information will be entered in the template and composed by a formula.

1.5. SAMPLE LABELLING

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Each institution should use their own labelling system, but we suggest using a label with meaningful information such as:

RP24_S1_G1_L_1_12

with “RP24” for RestPoll project and the sampling year, the survey number (“S1”), the group number (“G1”), the site type (“L”: living lab implementation site, “I”: normal implementation site, “C”: in-control site, “O”: out-control site, “P”: positive control site), the sub-transect number and the individual number (“12” in the example).
A shorter labelling system, only consisting in the CSA name and a unique running number, such as ALU00001, may also be considered. In this case, make sure no label is ever duplicated, as the label only links to the sample, without giving any other information.

2. Flowering “insect-pollinated” plant plots

For each survey, all species flowering during that time (dicotyledons, including shrubs, trees, even very small ones, at species level and all crop species) will be recorded. The flower survey will take place in each sub-transect where bees and hoverflies data will be collected (i.e., in the 50*2m² sub-transects) or directly in the entire transect surface (i.e., 300m²). The cover percentage (Appendix 1) of each recorded flowering species will be recorded, with the exception of grasses and sedges (see Appendix A.3 Field sheet: flowering “insect-pollinated” plants record). When the cover percentage is below 1%, including in the presence of a single flower, the class “< 1%” will be indicated. At the same time, and for each sub-transect, mean vegetation height will be recorded, and total flower cover and bare ground percentage will be visually estimated (Appendix A.1).

The flowering “insect-pollinated” plant record should be conducted at least 20min before the pollinator transect to avoid disturbing the entomofauna, or just after the pollinator transects.

3. Full botanical record

This botanical record will be carried out without seeking total exhaustiveness (but rather a list of the predominantly present and dominant species). The observer can thus stay on his/her feets and does not have to be on his/her knees.
In each sub-transect, a full botanical record should be conducted at the best season for botanical record when most of the plants are visible and identifiable (before mowing for example) in a plot of 2m x 2m. One plot will be placed at the centre (diagonals crossing point) of each sub-transect (Figure 3), resulting in a total of three vegetation plots per transect. The cover percentage (Appendix 1) of each species will be recorded as if the species were alone in the sub-transect, i.e., the total percentage cover of the sub-transect may exceed 100% (see Appendix A.4 Field sheet: full botanical record). When the cover percentage is below 1%, including in the presence of a single flower, the class “< 1%” will be indicated. In the case of monocotyledons, which are more difficult to identify, identification can be simplified to "sedges" or "grasses". **

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** *Expert alternative : All species are identified, including monocotyledons.*

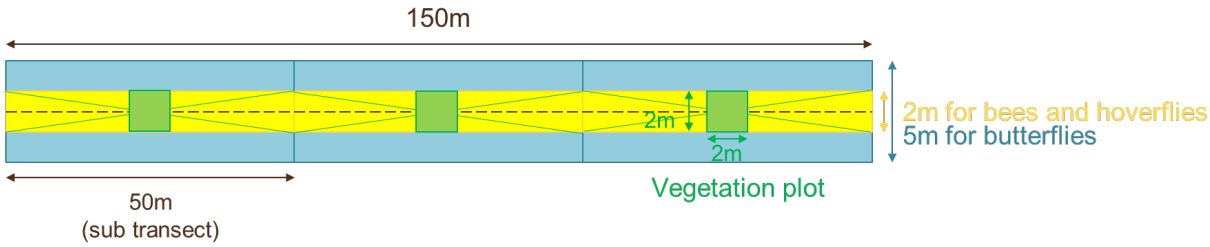

Figure 3: Location of the three vegetation plots in the transect

Date, UTM coordinates at the transect centre, land use (crop or grassland type), vegetation height, borders types and height of herbaceous boundary vegetation are also recorded (see Appendix A.4 Field sheet: full botanical record).

4. Data entry

All data shall be entered in the online file (Pollinator_flower_vegetation_transects_RestPoll_WP1.xlsx) and completed with species level identifications latest by the end of February of the subsequent year of the survey.
It is good practice to store a copy of the raw data sheets in a secure place. Therefore, all field sheets will be scanned and saved as one pdf per survey and survey type (pollinators, flowers, vegetation) at the online RestPoll file server.

5. Recommended equipment list

- 4 insect nets for catching butterflies (soft fabric, frame diameter ≤ 40cm, mesh ≤ 1mm)
- >50 vials (pre-filled with a small piece of tissue and 2 drops of ethyl acetate OR with cork chippings soaked with ethyl acetate)
- Pre-printed labels
- Thermometer
- Ziplock bags
- Stopwatch
- Measuring tape (50m)
- Field sheets, pencil, note pad
- 2m folding meter-stick (for vegetation height)

6. Acknowledgements

The authors wish to thank the contributors to this protocol, Alexandra-Maria Klein, Lynn Dicks, Michael P. D. Garratt, Claus Rasmussen, Jordi Bosch and Jeroen Scheper.

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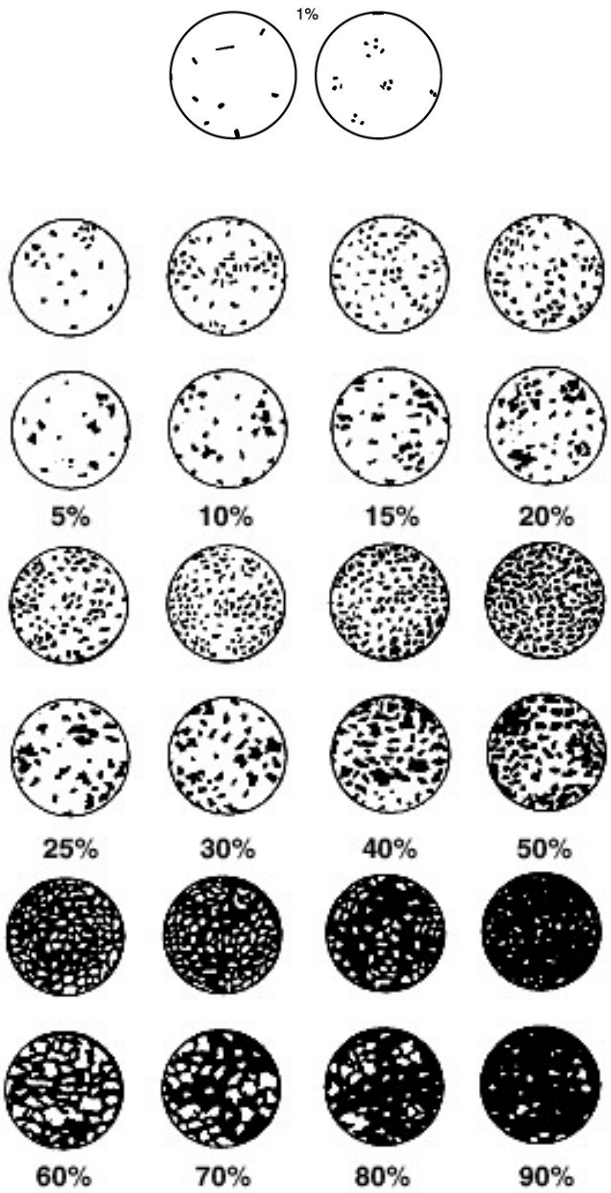
Appendix

A.1 Estimation of percentage cover (for flower and clouds) (From: Terry, R.D. and Chilingar, G.V. (1955), and Borthwick, R. (2015)).



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A.2 Field sheet: pollinator records *(basic version)*

Date: Site id: Survey number: Transect number:		Site type: □ IMS (Implementation site) □ ICS (Internal control site) □ OCS (Out control site) □ LLS (Living Lab site) □ PCS (Positive control site) □	Observer(s):
Bees/hoverflies Time (start): Time (end):	Butterflies Time (start): Time (end):	Weather (start/end) - Bees - Beaufort number: / - Temp (°C): / - Cloud cover (%): /	Weather (start/end) - Butterflies - Beaufort number: / - Temp (°C): / - Cloud cover (%): /

Sub-transect	**Species (or morphotype)**	**Abund.**	**Label***	**Comment(s)**

BEES **

	F	**R**	**N**	**FV**
ST1				
ST2				
ST3				

BUTTERFLIES **

	F	**R**	**N**	**FV**
ST1				
ST2				
ST3				

HOVERFLIES **

	F	**R**	**N**	**FV**
ST1				
ST2				
ST3				

*: Only for individuals collected, it corresponds to the label assigned in the field (same label on the vial)

**: Flying (F), Resting (R), Nesting (N), FV (Flower visting)

A.2bis Field sheet: pollinator records *(expert version)*

Date: Site id: Survey number: Transect number:		Site type: □ IMS (Implementation site) □ ICS (internal control site) □ OCS (out control site) □ LLS (Living Lab site) □ PCS (Positive control site) □	Observer(s):
Bees/hoverflies Time (start): Time (end):	Butterflies Time (start): Time (end):	Weather bees (start/end) - Beaufort number: / - Temp (°C): / - Cloud cover (%): /	Weather butterflies (start/end) - Beaufort number: / - Temp (°C): / - Cloud cover (%): /

Sub tr.	**Species (or morphotype)**	**Activity** / Plant sp**	**Abund.**	**Label***	**Comment(s)**

**: Flying (F), Resting (R), Nesting (N), FV (Flower visting) with the name or at least the colour of the flower

*: Only for individuals collected, it corresponds to the label assigned in the field (same label on the vial)

A.3 Field sheet: flowering plants record

Date: Site id: Survey number: Transect number: Vegetation height (cm): / / Total flower cover (%): / / Bare ground (%): / /	Site type: □ IMS (Implementation site) □ ICS (Internal control site) □ OCS (Out control site) □ LLS (Living Lab site) □ PCS (Positive control site) □	Observer(s): Other/Comment(s):

Species	**Flower cover Sub-transect 1**	**Flower cover Sub-transect 2**	**Flower cover Sub-transect 3**

A.4 Field sheet: full botanical record

Date: Site id: Transect number: UTM Coordinates:	Site type: □ IMS (Implementation site) □ ICS (Internal control site) □ OCS (Out control site) □ LLS (Living Lab site) □ PCS (Positive control site) □	Observer(s):
Land use: Vegetation height (cm):	Border(s) type(s):	Other/Comment(s):

Species	**Coverage* Plot 1**	**Coverage* Plot 2**	**Coverage* Plot 3**

*: The species coverage is estimated as if the species was alone in the sub-transect, i.e., the total vegetation percentage cover of the sub-transect may exceed 100%