WildPosh

D1.2 Field Site Monitoring Protocol

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Lead beneficiary: RHUL

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1 Preface

This document is a deliverable for the WildPosh project, funded under the European Union's Horizon Europe Research and Innovation Action under grant agreement No. 101135238.

The aim of this document is to present the WildPosh Field Site Monitoring Protocol to introduce the selected methodology and harmonise the monitoring across the site network.

2 Summary

This document outlines the field monitoring protocol for the WildPosh project. It consists of guidance on how to select field sites to establish the site network, data management, plant and pollinator surveys, sample collecting, and grower surveys.

The site selection guidance outlines criteria for ideal site selection with details on certain basic and detailed data to be collected for each site. The data management plan outlines practical guidelines for collecting and handling data and a sample labelling scheme to ensure consistency across the site network. For conducting plant and pollinator surveys, the protocol adapts familiar Safeguard methodology. The sample collecting guidelines include detailed instructions for collecting samples with the selected methods to analyse pesticide residues in various environmental matrices. These matrices are nectar, pollen, leaves, soil, water, and pollen collected from *Bombus* colonies. Included is guidance on how to handle, store and ship the samples. Finally, the grower surveys are designed to collect information on pesticide use in the crops adjacent to the field sites.

3 List of abbreviations

EU	European Union
GDPR	General Data Protection Regulation
WP#	Work Package #
GIS	Geographic Information System
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
PIWET	Państwowy Instytut Weterynaryjny
RHUL	Royal Holloway University of London
UFZ	Helmholtz Centre for Environmental Research GmbH - UFZ
DDP	Delivery Duty Paid

Further abbreviations given in Section 7.3 for sample naming.

4 Field site selection

In each country the site network consists of 10 field sites. A field site is a protected area, semi-natural habitat, calcareous grassland, or similar, with abundant flowering plants, and is situated next to conventionally farmed agricultural crops, five of which are wheat and five of which are oilseed rape crops.

Criteria for the field sites:

- Five sites adjacent to winter-sown wheat and five adjacent to winter-sown oilseed rape crops.
- Minimum of 2km separation (edge to edge) between sites.
- No barriers between the crop edge and the site. This means roads, ditches, hedges etc. Overall, there should be minimal obstructions from the crop edge to a distance of 20m from the crop edge.
    - The crop edge is measured from where the actual crop plants grow, not from the border of the farmland and the seminatural habitat.
- The site should be at minimum 40m deep from the crop edge to allow the maximum sampling distance of 20m from the crop edge without impacts from the other direction. There is no minimum width, as long as all sampling can be carried out.
    - A shorter depth of 25m can be allowed if no pesticide impacts can be expected from the other direction, for example if the site is bordering a forest on the other side.
- The sites should come from different farms, but a wheat and oilseed rape site from the same farmer is fine.
- The site should not be on a slope, to avoid biases to the run-off direction.
- You need to be able to identify the farmer of the crop, to allow surveying them for pesticide use.
- You need to have permission to place 3 bumble bee nests on the site.
- You need to have permission to sample soil, water, and plant material from within the site.
- There are no set criteria for the exact type of the seminatural habitat, but preferably it should be consistent within each country.
- If you can identify a prevailing wind direction, choose the side that is away from where the wind is coming from.

These criteria present the ideal site and may be relaxed if such sites are not available. If you cannot find sites that have no barriers in between, then a site with a light wire fence or a small and shallow ditch might be the next best choice. A less-than ideal site is still better than no site at all, and ultimately the aim is to monitor the situation as it exists. A goal of the sampling is to monitor spray drift from the crop, and for this any obstructions between the crop and site are important to consider, as is the distance between the site and the crop edge (most critical for sampling is the area from the crop edge to 10m from the edge). Although ultimately only 10 sites are required, it would be best to identify additional candidate sites (15-20 in total), to allow flexibility in site selection after detailed

landscape data has been generated (see Section 7), or in case anything surprising happens (e.g. a farmer withdraws).
Preference may further be given to sites that are:

- Close to home institution to reduce traveling times.
- Next to crops where contact with the farmer is already established.
- Known to form a gradient of pesticide use regimes.

There is no need to establish a gradient of features like size, across the sites. The only gradient we are looking for is the amount of pesticide use, however this is typically not possible to know beforehand and therefore is expected to emerge through variation in pest infestation, and consequent spray regime. This is why the sites should not be located on the same farm to avoid the same pesticide use regime by a farmer.

4.1 Definition of a field site

Going with the above criteria, a site can be more precisely defined from the centre point of the crop edge, forming a sampling area that is 20m deep (Fig. 1). There is no minimum or maximum width of the site, as long as it allows all samples to be taken and is at least 20m away from other crops.

Figure 1: Illustration of an example field site, defined by the centre point of the edge between the focal crop and the semi-natural habitat. The crop edge is marked by a red line, and the centre point is marked by a red point. The sampling area is a 20m deep strip along the crop edge, illustrated as a box with green shading. As there needs to be a 20m distance from any potential sampling point to any other crop, a 20m “buffer zone” can be imagined outside of the sampling zone, illustrated as another green box without shading.

The buffer zone can be relaxed to 5m if the site is surrounded by something other than crops. Figure and map data by Google (from Google Earth).

5 General site data

For each site, certain basic data are recorded:

- Site code (see section 7.3)
- Distance from crop where semi-natural habitat type begins.
- Latitude and longitude. Use the center point along the crop edge.
- Inclination (slope) of the site
- In case a barrier or additional distance exists between the crop and the field site, describe the barrier in as much detail as possible:
    - Composition (e.g. wire fence)
    - Width and height (or depth if it is a ditch)
    - Any other noteworthy details. Pictures are always helpful, so take as many as are needed to visually describe the barrier.
- Type of crop (wheat or oilseed rape).
- When *Bombus* hives were installed and removed
- Crop types of surrounding landscape (see section 6)

5.1 Kit needed to gather general site data

- Data collection template
- Tape measure
- Clinometer kit for measuring inclination.

6 Detailed landscape Data

For modeling purposes more detailed landscape data for each site will be generated. Site network members need to send the coordinates of each site to the coordinators at UFZ. Using a combination of GIS tools and high-resolution remote sensing data, UFZ will generate habitat maps within a 1 km radius around each site, following the EUNIS classification system. It is essential to gather some information in the surrounding landscape, such as crop types, as these cannot be accurately identified using satellite imagery alone. Thus, ground-truthing at each site will be required.

- As soon as you have the candidate sites, but by the beginning of February at the latest, send the coordinates to Christophe Dominik at christophe.dominik@ufz.de
    - Latitude/longitude; WGS 84/EPSG:4326; decimal degrees

- The coordinates in the right format look like: 51.42480, -0.56670

The team at UFZ will send requests for ground truthing the identities of the surrounding crops for 500m and 1km radius of the site to each partner by the end of February (prioritizing the Spanish partner, due to their earlier season).

7 Data management plan

An overall data management plan is provided for WildPosh by WP8, available at the WildPosh website internal repositories (deliverable D8.3 Data Management Plan).

The EU pollinator hub will be used as data repository for the final datasets. During the project when sharing raw data, the WildPosh website internal repository will be used.

The idea of “As open as possible, as closed as necessary” is used with the accessibility of the generated datasets, meaning that much of the data will be publicly open and accessible in the data repository for anyone interested.

7.1 Work Package 1 data management

Within the site network we will produce a large amount of biological data, and in addition meta-data relating to the samples, and landscape data. All data will be collated and stored in a standardized format, where possible also employing automatic data quality checks. The goal is to provide templates for data collection, that are not only easy to use, but also minimize the risk of collecting data incorrectly.

- GDPR issues need to be considered when any personal data are being handled. In particular this relates to farmer surveys. Data should be stored securely, for example on university network drives system. Do not store any critical data on USB sticks or other devices that may be lost, and do not share any data outside of the project.
- Collecting and handling data will by itself require some resources from site network members, for example to digitize collected data from paper copies.
- If possible, appoint a specific person to handle data management in your group.
- To ensure consistent data collection across the site network, standardized data collection templates are available and should be used by all personnel doing the fieldwork. These templates also include space for metadata relating to the sampling event, such as collection time and weather conditions, and space for extra notes.
- The data collection template should be as easy to use as possible with minimal interpretability required, but nevertheless provided with instructions regarding what measurements are taken, the units of measurement, and the form of data

that should be recorded (e.g. codes, percentages, presence/absence, continuous measurement, etc.).

7.2 Data collection in practice

- Separate data collection templates are provided for collecting data in the field.
    - A diagram of everything needing to be sampled will also be provided to aid fieldwork.
- In the field data can be recorded as printed hard copies of the provided templates (using a clip board) or in digital form to an Excel file with a tablet or a laptop.
- A separate Excel file is provided that will act as a master data file, and the data from the field templates should be collected into the master file as soon as possible. The master file has sheets with the same fields as the field templates.
- If using printed templates, it is a good idea to use a folder type clip board and a waterproof ink pen or pencil to record data.
- Photograph the sheet as soon as it is done as a back-up.
- If entering data digitally, use the digital version of the template and create a file for each sampling event in the same fashion as you would use the paper version.
- Similarly, a backup should be taken of the digital files and the data entered into the main Excel file as soon as possible.
- Enter the recorded data from the paper or digital templates into a master Excel file as soon as possible, preferably on return to the home institution. If errors are spotted, these may be still corrected when the sampling event is fresh in memory.
- Send a copy of this Excel master file after each sampling round to WP1 data coordinator (Matti Leponiemi, matti.leponiemi@rhul.ac.uk) **within one month of completing the sampling round**. Add to the file name the date when the data was entered (see section 7.4).
- Also send a digital copy of all raw field data to WP1 data coordinator (Matti Leponiemi, matti.leponiemi@rhul.ac.uk).
- The data for the site network will be collated at RHUL.
- Store all raw data and paper copies at the home institution in case they are needed later to resolve issues or for audits.
- The master Excel files will form the master data (“Single Source of Truth”) file that will be used for any future analyses or manipulations.
- The collated data will be sent to the WildPosh data repository for storage and will be available at the WildPosh web page for appropriate WildPosh partners.

7.3 Sample labelling scheme.

During WP1 all data and samples will be labelled using a consistent naming scheme, which includes project code with the work package number, a three-letter country code (Table 1), three letters (Table 2) and number for site code, a letter and two numbers for sample type and distance (Table 3), and the sampling date. As an example:

- WPosh1_GBR_OSR02_N01_26-05-2025

Table 1: Country codes used in WildPosh

Country	Code
Estonia	EST
Germany	GER
Spain	ESP
United Kingdom	GBR

Table 2: Crop codes used in WildPosh

Crop code	Code
Oilseed Rape	OSR
Wheat	WHE

- The site code consists of the crop code and a number from 01-05, as there are 5 sites of each type.

Table 3: Sample type codes used in WildPosh

Sample	Code
Nectar	N
Pollen	P
Leaves	L
Soil	S
Water	W
Bombus pollen	B
Transect bee/fly to ID	T

- The sample type code is accompanied by the sample number with two numbers, for example the first nectar sample is N01.

- For landscape data, only the first two parts (country_site) are needed.
- With all numbering, a leading zero must be used (e.g. 01).
- The date is written using the standard format DD-MM-YYYY. Do not use a different format.
- All samples collected from the field must labelled with these codes;
    - Country code, site code, sample code, and date

To illustrate, the example sample “WPosh1_GBR_OSR02_N01_26-05-2025” would be a sample collected from the United Kingdom, from a site adjacent to the second oilseed rape crop, and the sample would be a nectar sample at 1m distance from the crop, collected on 26th of May in 2025. The project code will remain the same throughout WP1 data collection but will help subsequent data processing when datasets are combined with other work packages, datasets from public databases, or other projects.

Most samples are collected at predefined distances from the crop edge, and this distance is part of the sample name. The ideal sampling scheme is explained in section 8, with ideal distances from the crop edge.

7.4 File labelling

Template Excel files with multiple sheets are provided for partners. These templates may be used instead of printed paper templates. The templates will be provided and follow this labelling:

- WPosh1_CountryCode_SiteCode_Protocol_Date

A separate master data file is provided where the data from the separate templates for each sampling round should be filled as soon as possible (see section 7.2). Give the file a date that represents the date when it was filled in and sent:

The master files should be named WPosh1_CountryCode_Date.xsls

8 Overall site monitoring scheme

At each site the selected matrices will be sampled at given distances from the crop edge (Fig. 2). The samples can be mostly taken anywhere along the imaginary line that is at the specific distance from the crop edge. For example, a plant material (leaf) sample is taken at distances 1m, 4m, 7m, 10m, 15m, and 20m from the crop edge. One sample can then be thought of as “a distance”, e.g. one nectar sample is taken at distance of 1m, another sample at a distance of 3m, and so on. The list of samples to be taken is given in Table 4, and the full sampling protocols for each sample type are presented in the following sections.

In addition to sampling these matrices, plant and pollinator surveys will be conducted following methodology adapted from the Safeguard protocols (sections 9 and 10), as well as some basic site data (section 5).

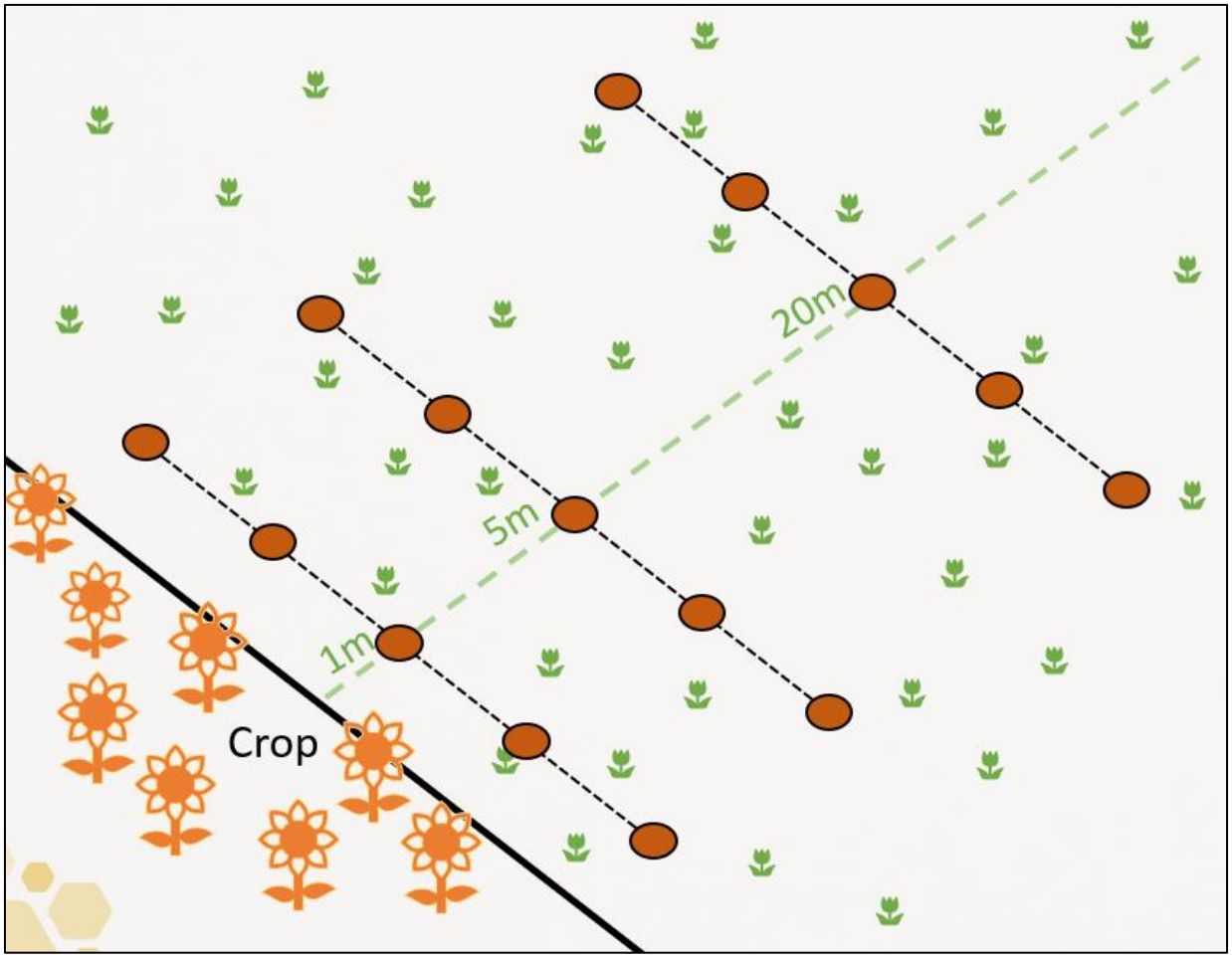


Figure 2: Illustration of the sampling scheme, with example soil samples being taken at 1m, 5m and 20m distance from the crop edge.

There are seven distances from the crop edge to take nectar samples, creating the densest matrix to be sampled. While other samples will be taken only once during the season, nectar will be sampled in two rounds, once in the middle of the season and again at the same time as other samples.

Other matrices will be sampled at three or six distances from the crop edge. The sample numbers are defined by the number of samples that can be analysed for pesticide residues in the project (by PIWET and ANSES).

Table 4: Number of sampling rounds for each matrix, the number of samples per sampling round, the volume/weight required per sample, and the distances from crop edge where the samples are to be taken.

Matrix	Rounds	Samples	Amount	Distances from the crop
Nectar	2	7	20 µl	1m - 3m - 5m - 7m - 10m - 15m - 20m
Pollen	1	6	50 mg	1m - 4m - 7m - 10m - 15m - 20m
Leaves	1	6	50 g	1m - 4m - 7m - 10m - 15m - 20m
Soil	1	3	50 g	1m - 5m - 20m
Water	1	3	500 ml	1m - 5m - 20m
Bombus pollen	1	3	600 mg	NA

- The nectar, pollen, leaf and soil samples should all be taken during the same day!
- Water samples can be taken separately

The sampling should be done with minimal variation from the ideal sampling distance. In case the presented sampling distance cannot be achieved, for example due to obstructions between the crop edge and site or because there are no flowers to be sampled at a certain distance, you may deviate from these distances. This can be done by increasing the “bucket” where samples are taken. For example, it is likely that you cannot always find flowers at a distance exactly 1m from the crop edge. In this situation you may increase the variation from 1m, sampling anywhere between 0.5m-1.5m. The width of this sampling line (=variation from the distance) must be recorded. Similarly, in case a sample cannot be taken at a certain distance, it should be taken at a distance as close to it as possible. It is still better to take a sample at a different distance than the ideal, rather than to take no sample at all. Again, all deviations from ideal sampling parameters must be recorded, as they will be needed for analysis of the data.

8.1 Monitoring timeline

The exact timing of events will vary depending on the country. The first nectar sampling should be completed during the **peak of oilseed rape flowering** (~95% flowering). In addition to the nectar sampling, plant and pollinator surveys will be carried out, and this is also when the bumblebee colonies will be placed onto the sites.

The second sampling event, when all matrices should be sampled, should happen **two weeks after the end of oilseed rape flowering** (end of flowering when ~95% of crop no longer flowering). The plant and pollinator surveys will also be done at this timepoint, and bumblebee colonies will be removed. You may ask the farmer, or check the sites yourself, or both, to ascertain flowering state. These timings are the same for oilseed rape and wheat sites.

To be done as soon as possible:

- Find field sites.
- Send site coordinates to UFZ (by the beginning of February)
- Order any kit and consumables required

Early in the season, before sampling:

- Ground truthing the field sites
- Order *Bombus* colonies
- Prepare *Bombus* colonies
- Prepare sample collection equipment
    - Print and attach required labels etc.

During peak oilseed rape flowering:

- Place the *Bombus* colonies on the sites
- Nectar sampling event
- Plant and pollinator surveys

Two weeks after oilseed rape flowering:

- All matrices are sampled
- Plant and pollinator surveys
- Remove *Bombus* colonies.

Later in the season:

- Water sampling if not done during main sampling
- Farmer surveys

How much time is required to complete a sampling round will depend on the human resources available. Each country should plan to have two sampling teams of 3-4 people, both going to one site per day, each to a different site. This requires 5 sampling days to be scheduled for both sampling rounds. However, additional visits may be required to sample water or to remove the *Bombus* colonies.

Right after the sampling round has been completed and all samples have been taken, the samples should be sent to the appropriate institutions for analysis. Instructions for shipping samples are at the end of the protocol for each matrix. The digital data templates should be sent to RHUL at most one month after sampling has finished. Completing the pollinator data may take longer, due to the need for species identification, and these data can be sent separately later (please inform RHUL of your expected timeline).

9 Survey of pollinators

The Safeguard method is adapted for the pollinator surveys. A 250 meter transect is used to survey butterflies first and then bees and hoverflies. Five 50m sub-transects are further used for floral surveys (Section 10). The survey is done at each sampling event. A modified Pollard walk method is used to survey the pollinators along the transect [1]. The walking speed should be practiced prior to doing the transects, so that you are reliably walking 250m in 15 minutes.

9.1 Overall survey conditions

Surveying should be done only under good weather conditions, meaning no rain, and the temperature should be above 15 °C on a sunny day, or 20 °C if it is cloudy, but under 33 °C. Avoid the hottest midday hours. Wind should be 5 or less on the Beaufort scale [2].

Depending on the region the ideal time for the transects is between 09:30-17:00. It is better to do the survey another day than doing it later than this, but it is also better to do a survey outside the ideal time rather than not at all. The surveys are expected to take approximately 1.5 – 2.5 hours per site.

- Transects are variable and recorders can decide the location of sub-transects. Document the transect placement with GPS.
- Record the time of the start and end of the transect, temperature (start and end), estimated cloud cover (average % across transect time), and wind speed using the Beaufort scale (see Annex 1).
- One person can do both transects, or two can do them independently. In any case the butterfly transects should be done first, as butterflies are more likely to be scared off.
- If a pollinator cannot be identified or caught, it should still be recorded with as much taxonomic information as possible.

9.2 Butterfly transects

- Transect length is 250m in 15 min, using 50m (3min) sub-transects.
- Transect width is 5m (2.5m to each side of the person)
- Record the transect using Geotracker GPS-app.
- The species are recorded in the field. Record every species, whether on a flower, flying around, or engaged in any other activity.
- Butterflies are identified with expertise on the wing, otherwise they are caught with a net and identified with a field guide or an app (e.g. Obsidentify)
- If the butterfly is on a plant, the plant species is recorded for pollinator-plant interactions.

- Handling time is not included in the 15min, so stop the clock while handling specimens or writing notes.

9.3 Bee and syrphid fly transects

- Transect length is 250 m in 15 min, using 50 m (3 min) sub-transects.
- Transect width is 2m (1m to each side of the person).
- Record the transect using Geotracker GPS-app.
- Unless the identification can very clearly be done in the field the species should be caught, stored individually in respectively labelled tubes, and identified in the lab.
    - Tube labeling should follow the sample naming scheme (section 7.3)
    - For example, WPosh1_GBR_OSR2_T01_26-05-2025
    - Bumble bee queens should not be collected but identified in the field.
    - Place the tube in the cold box as soon as possible and store in -20°C at the home institution. Process the tubes as quickly as possible.
- Record every species, whether on a flower, flying around, or engaged in any other activity.
- If the bee or fly is on a plant, the plant species should be recorded for pollinator-plant interactions.
- Handling time is not included in the 15min, so stop the clock while handling specimens or writing notes.

9.4 Kit needed for pollinator surveys

- Data collection template
- A device to record GPS coordinates (e.g. mobile phone)
- A stopwatch to record transect time
- A net to catch flying insects
- Collection tubes for bees or syrphids to be identified in the lab
- Pre-printed labels for pollinator collection tubes
- Guides for pollinator ID (including mobile apps).
- Thermometer (e.g. in the car)

10 Floral survey

The Safeguard methodology is adapted to perform floral surveys on field sites. The floral survey also utilizes the transects employed in the pollinator survey (Section 9). The survey is done at each sampling event, so during the first nectar sampling and the main sampling event.

- Place a 1x1m quadrat randomly (i.e. not targeting particularly flower-rich patches) at the midpoint of each sub-transect, 5 in total (see section 9.2 and 9.3).
    - A flag may be placed instead of a quadrat to mark later quadrat placement.
- Measure at each quadrat
    - The percentage floral cover of each flowering species (or highest taxonomic level feasible)
    - The percentage of vegetation cover,
    - The average highest sward height at the center of the quadrat.
- Also record the species name of the ten most common flowering plants within the whole transect area, and a percentage cover estimate of them.
    - If the flower cover is low, it might be easier to estimate the number of floral units and the mean size of a floral unit. The percentage cover respective to the transect area can then be calculated later.
    - The floral unit may be defined as convenient (e.g. single flower, or inflorescence), as long as this is kept constant for the count of that species.
    - Count individual floral units up to 100, but you may then shift to estimating intervals in steps of 50.
- To aid in plant identification, mobile apps like PlantNet, Obsidentify or Flora Incognita can be used.
- Pictures should be taken of the quadrats and the most common species to allow later confirmation.

10.1 Kit needed for floral surveys

- Data collection template
- A device to record GPS coordinates (e.g. mobile phone)
- Guides for plant ID (including mobile apps).
- 1x1m quadrats.
- Small flags to mark quadrat positions in each sub-transect.
- Camera to take pictures of the 1x1m quadrats and most common species.
- A tally counter (optional but may be useful).

11 Collecting pollen samples from flowers

Pollen will be collected from flowers using a pollen vacuum device, called the Electronic Pollen Sampler or E-PoSa [3]. It is powered by a USB-port, and a power bank or a laptop is required to power it. Not all power banks work, so check the compatibility of your devices in advance. A laptop battery will power the device for a day, but power bank capacity requirement was not tested.

- Pollen sampling is expected to be one of the more time-consuming tasks on the sites and should be prioritised. You might need one person for this task alone.
- The sampling should be planned on a day when all the samples from one site can be taken during the same day.
- If sampling cannot be achieved on the same day, you will need to retake all of the samples.
- Use lab gloves when sampling and change the gloves between each sample (that is, pollen from one distance from the crop, e.g. 1m).

The electronic pollen sampler is straightforward to use. It is a handheld electronic vacuum device that can be used to suck pollen directly from the flowers. When the tip of the vacuum is moved around the anthers, pollen is gathered into a collection tube (Fig. 3).

As collecting the pollen is time-consuming, we need to be careful in collecting enough pollen and avoiding any losses. Therefore, the collection tubes should be pre-weighed to allow precise measurement of yield in the field. In the lab the pollen is transferred from the collection tube into a tube that will be used directly for the pesticide residue analysis. The amount of pollen needs to be precisely weighed at this step, as it will not be weighed again at PIWET. This is to avoid any losses of sample due to moving it from one tube to another.

- One sample consists of 50mg of pollen pooled from any flowers found on the site at the specified distance.
- Record the species where pollen was collected from.
- Enough collection tubes should be prepared beforehand, one tube per sample.
- Be aware that some pollen might be lost when transferring the pollen from the collection tube, so slightly more than 50mg should be collected.
- After a sample has been gathered, seal the tube using parafilm and place in a cool box over ice packs.
- Transport the samples to the lab as quickly as possible, within the same day. If you cannot further process the samples the same day, store them in the freezer at -20°C.
- Transfer the sample from the collection tubes in the lab into a single analysis tube, measuring precisely the weight of the pollen.
- Mark tubes according to the sample naming scheme. Sample name slips should be printed beforehand using a laser printer, not hand-written.
    - See section 7.3 for sample naming.

- Fill sample summary sheet with details of the sampling event.

Figure 3: Extracting pollen using the electronic pollen sampler in the field. Gloves should be used when collecting actual pollen samples.

11.1.1 Preparing pollen collection tubes

Collection tubes are prepared from 5ml Eppendorf tubes, filter paper (Whatman 114), and 75 µm metal mesh filter (Fig. 4A). Some cutting tools and a drill will also be helpful.

1. Drill or cut open the cap of the tube and cut the cap loose (Fig. 4B).
2. Cleanly cut away a few millimeters at the tip of the tube (Fig. 4C).
3. From the metal filter cut a piece that is large enough to cover the opening at the tip of the tube (Fig. 4D).
4. Heat up the metal filter enough that when you place the tip-end of the tube on the metal, it will be attached to the tube by slightly melting the plastic (Fig. 4E and 4F)
    a. A lab hotplate works well for this. Add a metal disc or a coin on the hot plate, place the filter on top of the disc, let it get warm, then quickly push the tube onto the filter. This way the hot plate will stay clean of plastic.
5. Cut away excess filter around the tip to make it cleaner (Fig. 4F).
6. Cut a piece of filter paper large enough to cover the tube cap (Fig. 4G) and place it between the tube and the cap (Fig. 4H).
7. Push the cap into the tube to close it, leaving the filter paper in between to act as a filter (Fig. 4I).
8. The filter tube may now be placed in the suction portion of the vacuum device, and act as a pollen collection tube (Fig. 4J).

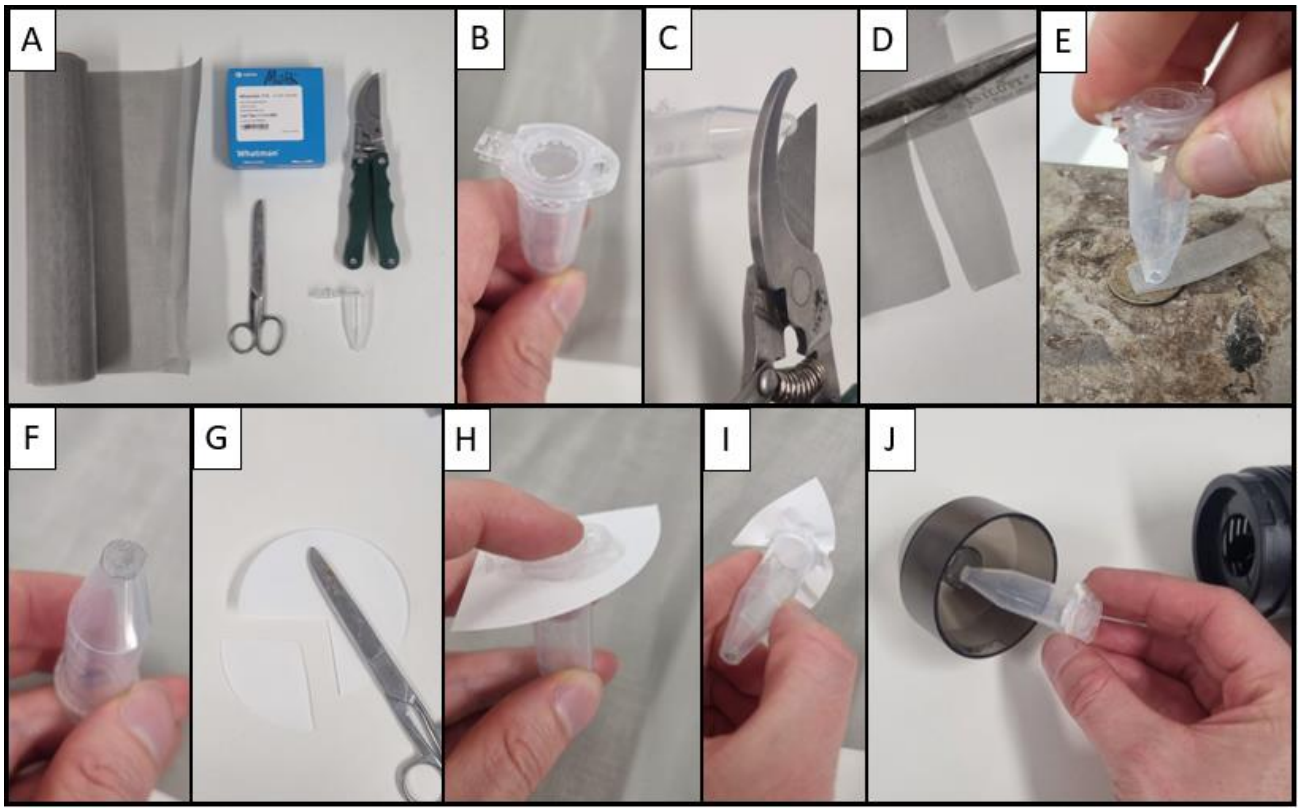


Figure 4: The pollen collection tube preparation process.

11.2 Kit needed for pollen sampling

- Electronic Pollen Sampler (https://amzn.eu/d/9hQ5c2R)
- Pollen collection tubes
  - 75 µm mesh metal filter (https://amzn.eu/d/9K9HT2c)
  - Whatman 114 filter paper
- Parafilm
- Data collection template.
- Cold box with ice packs to store samples.
- Tape measure
- Gloves
- A power bank or a laptop to power the electronic pollen sampler.
- A precision scale to measure the pollen amount

11.3 Shipping pollen samples

Pesticide residues from pollen samples are analysed at PIWET in Poland. To reduce costs, samples should be shipped once they have all been collected (stored at -20°C).

- Make sure samples are clearly labelled with the correct sample names (see section 7.3).
- Ship samples on dry ice, following appropriate shipping procedures (UN 1845).
- Use overnight courier for shipping.
    - Make sure there is enough dry ice to last an extra day or more. Even if using overnight shipping, delays in transport are to be expected.
- Always ship at the start of the week to avoid shipments getting stuck over the weekend in case of delays.
- All the expenses should be covered by the sending party! There can be no fees for the recipient.
    - Make sure to check the box for “Delivered Duty Paid (DDP)”
- The shipment should indicate no commercial value.
- **IMPORTANT NOTE:** Do not classify the contents as bee products!
- Fill, sign, and process the WildPosh material transfer form.

Send plant samples to the following address:

Tomasz Kiljanek
National Veterinary Research Institute (PIWET)
Department of Pharmacology and Toxicology
Partyzantow 57, 24-100 Puławy
POLAND

EORI number for the receiving institution is PL716001076100000.

12 Collecting nectar samples from flowers

Two methods can be used to collect nectar from flowers: microcapillary tubes (e.g. [4]) and a field centrifuge [5]. In sampling, priority should be given to plants that we know pollinators also prefer. However, in practice we may need to opportunistically sample what plants are available.

- Nectar sampling needs to happen early in the day, nectar amounts are typically highest earlier, and pollinators may also reduce the availability of nectar during the day.
- Nevertheless, do not bag flowers! Many field sites are publicly accessible.
- Do not sample on rainy or otherwise moist conditions. Rain or excess moisture will dilute the samples, especially when using centrifuge.
- The sampling should be planned on a day when all the samples from one site can be taken during the same day.
- If sampling cannot be achieved on the same day, you will need to retake all of the samples.
- Use lab gloves when sampling and change the gloves between each sample.

12.1 Microcapillary tubes

Use microcapillary tubes whenever the amount of nectar allows, and every time destructive methods cannot be used (e.g. protected sites). With microcapillary tubes nectar is sampled by placing the tube where nectar is located at the base of the flower (Fig. 5). Capillary action will draw nectar from the flower into the tube. The tube can be carefully moved around until no more nectar is drawn into the tube.

- One sample consists of 20 µl of pooled nectar from as many flowers as is required per distance (Table 4), the flowers all being at the same sampling distance from the crop edge. A deviation of 0.5m is allowed.
- For one sample collect nectar from the 5 most dominant flowering species (by relative abundance) at each distance. The nectar is pooled to form one sample.
- Record the relative contribution of each species to the sample in nectar volume.
- Enough capillary tubes should be ordered in all sizes. Sizes 0.5 µl, 1 µl and 2 µl are recommended.
- Use the largest tube that the floral morphology allows but be careful not to damage floral tissue to not retrieve tissue fluids.
- Also be careful not to contaminate the nectar with pollen.
- Collect at least 20 µl of nectar per sample, preferably slightly over to compensate for any losses that happen when nectar is extracted from the tubes.
- Record the species sampled and the collecting distance on to the summary sheet.
- Use the rubber bulb to empty microcapillary tubes into a pre-labelled centrifuge tube.

- Place the centrifuge tube in a cold box over ice packs.
- Transport the samples to the lab as quickly as possible, within the same day. If you cannot further process the samples the same day, store them in the freezer at -20°C.
- Transfer the nectar from the collection tubes into a 0.2ml centrifuge tube, creating a pooled sample of all species per distance class.
    - The pooled sample also consists of the nectar collected by centrifuge, if both methods were used to gather the sample.
- Mark tubes according to the sample naming scheme. Sample name slips should be printed beforehand using a laser printer, not hand-written.
    - See section 7.3 for sample naming.
    - If necessary, freeze the samples again at -20°C before shipping.
- Fill sample summary sheet with details of the sampling event.

Figure 5: Extracting nectar from a knapweed flower with a 2um microcapillary tube.

12.2 Field centrifuge

For small, low nectar volume flowers the field centrifuge can be used (Fig. 6). It is typically faster to use the centrifuge rather than capillary tubes. As the centrifuge is a destructive method, it should not be used in protected areas, with protected plants, or when picking flowers should be otherwise avoided. Sometimes, this may not be possible, and as the number of flowers required is often reasonable, however, it is unlikely to have a meaningful negative environmental impact.

The field centrifuge fits 1.5ml and 2ml tubes and as such only allows rather small flowers to be used. The kit comes with a carrying case and a 12V car plug, allowing use near a vehicle, but with an additional battery and cable the centrifuge can easily be used in remote field locations.

- One sample consists of 20 µl of pooled nectar from as many flowers as is required per distance class (Table 4), the flowers all being at the same sampling distance from the crop edge. A deviation of 0.5m is allowed.
- To extract nectar with a centrifuge, a modified centrifuge tube with a metal filter is placed over the nectar collecting tube (see section 12.2.1). You will need at least two of these tubes per sample, because the centrifuge needs to be balanced.
- Use new filter and collection tubes for each sample.
- Enough filter tubes for the centrifuge should be prepared beforehand.
- Place the flower on top of the filter-tube with the nectary facing downwards.
- Run the centrifuge for 30s to extract the nectar from the flower into the collecting tube. The tabletop centrifuge runs at 6000 rpm (2000 g) and cannot be adjusted.
- Record the species sampled and the collecting distance on to the summary sheet.
- In case multiple species are sampled for one sample, record the relative contribution of each species to the sample.
- The nectar yield can be observed visually, with a scale, or more accurately with capillary tubes or a micropipette.
- Collect slightly more nectar than needed to take losses into account, especially if estimating yield visually or with a scale.
- Be careful not to contaminate the sample with pollen or tissue fluids coming out of the flower.
- In moist or humid conditions, the centrifuge may extract additional moisture collected on the flower more easily than when using microcapillary tubes. Be particularly mindful about the conditions when using the centrifuge to extract nectar.
- Place sample tubes in a cold box over ice packs.
- Transport the samples to the lab as quickly as possible, within the same day. If you cannot further process the samples the same day, store them in the freezer at -20°C.
- Transfer the sample from the collection tubes (you should have at least two) in the lab into a single 0.2ml centrifuge tube, creating a pooled sample of all species per distance class.
    - Transfer also the nectar collected by microcapillary tubes into the 0.2ml tube if both methods were used to gather the sample.
- Mark tubes according to the sample naming scheme. Sample name slips should be printed beforehand using a laser printer, not hand-written.
    - See section 7.3 for sample naming.
- Fill sample summary sheet with details of the sampling event.

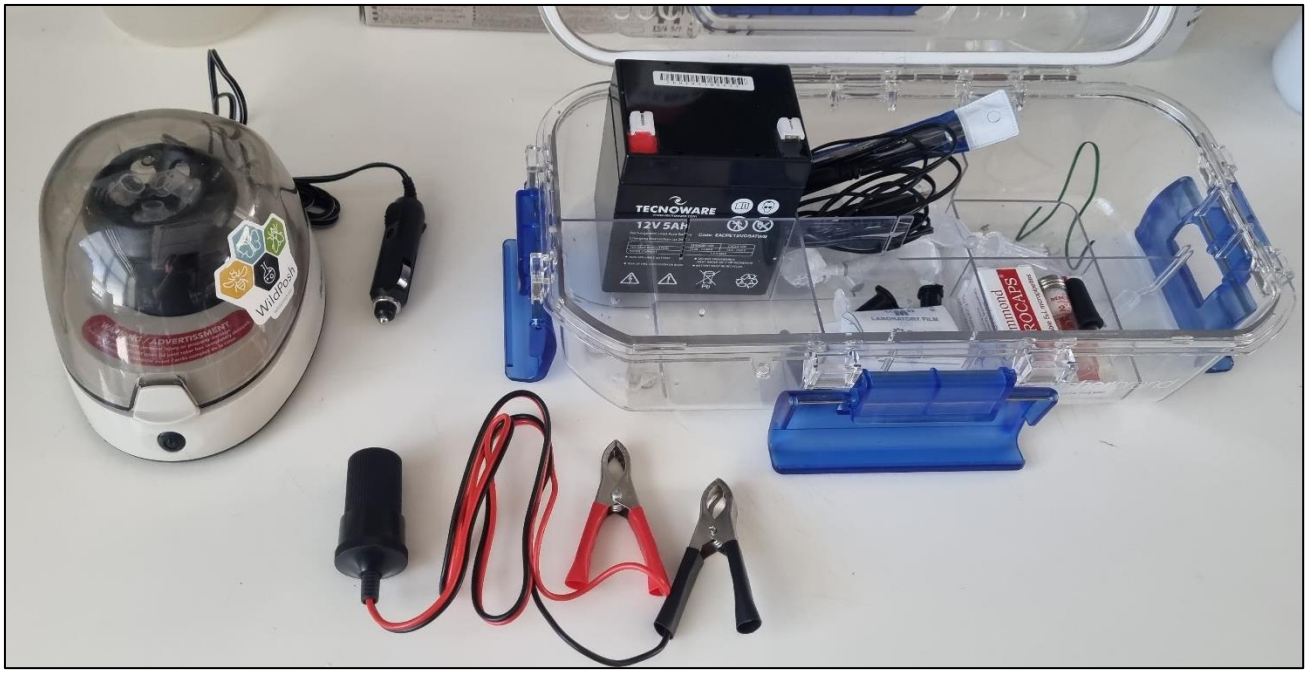


Figure 6: Field centrifuge kit. A tabletop centrifuge with a car plug, a cable with car socket that can be attached to a battery. A carrying case with a 12V battery and supplies.

12.2.1 Preparing the centrifuge filter tubes

Two 1.5ml centrifuge tubes, 75 µm mesh metal filter, and some cutting tools are needed to prepare the filter tubes (Fig. 7A). The process is similar to preparing pollen collection tubes in section 11.1.1.

9. Cut the tip of the tube at about the 1.5ml line (Fig. 7B)
10. Also cut the tube at about the 0.1ml line (Fig. 7C), leaving you with the middle section that is open from both ends (Fig. 7D).
11. From the metal filter cut a piece that is large enough to cover the opening at the tip of the tube (Fig. 7D).
12. Heat up the metal filter enough that when you place the tip-end of the tube on the metal, it will be attached to the tube by slightly melting the plastic (Fig. 7E and 7F)
    a. A lab hotplate works well for this. Add a metal disc or a coin on the hot plate, place the filter on top of the disc, let it get warm, then quickly push the tube onto the filter. This way the hot plate will stay clean of plastic.
13. Cut the extra filter mesh around the tip with scissors (Fig. 7G).
14. Insert the newly created filter tube into an intact 1.5ml centrifuge tube when ready to extract nectar from flowers (Fig. 7H).

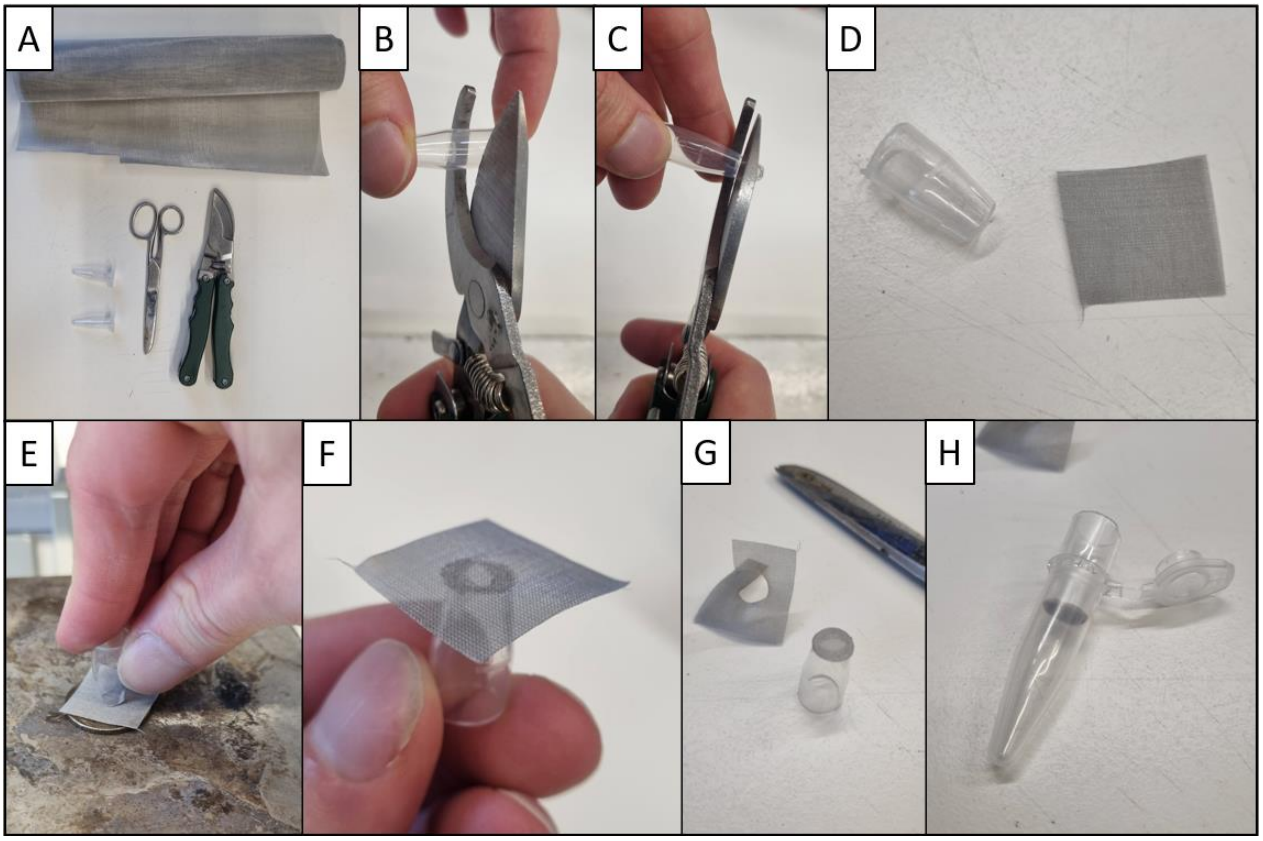


Figure 7: The nectar filter tube preparation process.

The centrifuge tube with the additional filter piece should fit into the centrifuge without a problem. As the centrifuge needs to be balanced, at least two nectar collecting tubes and filter tubes are needed per sample, and enough tubes should be prepared beforehand.

12.3 Kit needed for nectar sampling

- Data collection template.
- Cold box with ice packs to store samples.
- Tape measure
- Gloves
- 0.5 µl Microcapillary tubes
- 1 µl Microcapillary tubes
- 2 µl Microcapillary tubes
- 1.5ml centrifuge tubes.
- Field centrifuge kit (comes with additional carrying case and car socket adapter)
    - https://www.fishersci.co.uk/shop/products/myspin6-portable-kit-complete/17234243
- Filter tubes (see section 15.3)
    - 75 µm mesh filter (https://amzn.eu/d/9K9HT2c)

Highly recommended also:

- A 12V battery (e.g https://amzn.eu/d/00g7xKG5)
- Adapter for battery to car socket
- Battery charger
- Field scale to estimate nectar amount

12.4 Shipping nectar samples

Pesticide residues from nectar samples are analysed at ANSES in France. To reduce costs, samples should be shipped once they have all been collected (stored at -20°C).

- Make sure samples are clearly labelled with the correct sample names (see section 7.3).
- Ship samples on dry ice, following appropriate shipping procedures (UN 1845).
- Use overnight courier for shipping.
    - Make sure there is enough dry ice to last an extra day or more. Even if using overnight shipping delays in transport are to be expected.
- Always ship at the start of the week to avoid shipments getting stuck over the weekend in case of delays.
- All the expenses should be covered by the sending party! There can be no fees for the recipient.
    - Make sure to check the box for “Delivered Duty Paid (DDP)”
- The shipment should indicate no commercial value.
- **IMPORTANT NOTE:** Do not classify the contents as bee products!
- Fill, sign, and process the WildPosh material transfer form.

Send nectar samples to the following address:

Anne-Claire MARTEL
ANSES Sophia Antipolis
Unit of Honey Bee Pathology
105, route des Chappes
F-06410 Biot
France

EORI number for the receiving institution is FR13001202400043.

13 Collecting plant material (leaf) samples

- One sample consists of 50g of leaves (wet weight) collected at the specified distance from the crop edge (Table 4).
- Collect young leaves from 5 most dominant (by relative abundance) plants that are preferred nesting sites or larval food for pollinators.
    - This is based on the butterfly species identified in the first sampling round or based on knowledge of which plants are used by many butterfly species, if such plants can be identified.
- Wear lab gloves when collecting samples and change them when taking a new sample (at a new distance).
- If using scissors to gather the samples, clean them between samples with ethanol.
- Store the leaves in a 1l resealable polyethylene bag. 1l of packed leaves should be around 100g, but it is better to measure the weight with a scale.
- Record the species sampled and the collecting distance on to the summary sheet.
- Mark sample bags according to the sample naming scheme. Sample name slips should be printed beforehand using a laser printer, not hand-written.
    - See section 7.3 for sample naming.
- Tape the sample name slip on the bag with clear tape.
- Place sample bags in a cold box over ice packs.
- Transport the samples to the lab as quickly as possible, within the same day. Samples are stored in the freezer at -20°C.
- Fill sample summary sheet with details of the sampling event.

13.1 Kit needed for plant material sampling

- Data collection template
- Tape measure.
- Scissors
- 1l resealable polyethylene bags
- Gloves
- Scale to measure sample amount.
- Cold box with ice packs for sample storage.

13.2 Shipping plant material samples

Pesticide residues from plant material are analysed at PIWET in Poland. To reduce costs, samples should be shipped once they have all been collected (stored at -20°C).

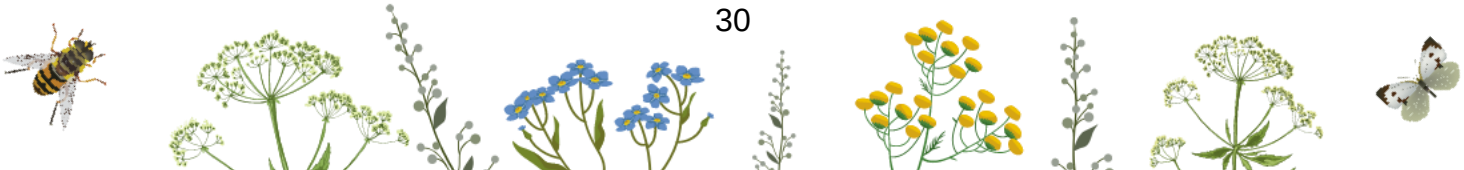
- Make sure samples are clearly labelled with the correct sample names (see section 7.3).

- Ship samples over dry ice, following appropriate shipping procedures (UN 1845).
- Use overnight courier for shipping.
    - Make sure there is enough dry ice to last an extra day or more. Even if using overnight shipping delays in transport are to be expected.
- Always ship at the start of the week to avoid shipments getting stuck over the weekend in case of delays.
- All the expenses should be covered by the sending party! There can be no fees for the recipient.
    - Make sure to check the box for “Delivered Duty Paid (DDP)”
- The shipment should indicate no commercial value.
- **IMPORTANT NOTE:** Do not classify the contents as bee products!
- Fill, sign, and process the WildPosh material transfer form.

Send plant samples to the following address:

Tomasz Kiljanek
National Veterinary Research Institute (PIWET)
Department of Pharmacology and Toxicology
Partyzantow 57, 24-100 Puławy
POLAND

EORI number for the receiving institution is PL716001076100000.



14 Collecting soil samples

To harmonize soil sample-taking, a GeoSampler (Buerkle) is used to take soil core samples (Fig. 8). The pipe has an inner diameter is 11mm, and a chamber length of 30cm. This covers the top layer that may be used by mason bees, but also the most typical nesting depths of ground-nesting bees and butterflies overwintering in soil [6].

Figure 8: Soil corer tool.

- One sample consists of five pooled sub-samples, each taken at the same distance from the crop edge (Table 4). The total sample weight needs to be 50g.
- The central sampling point is at the middle point of the crop edge, at the specified sampling distance from the edge.
- One sub-sample is taken at the central sampling point. Two further sub-samples are taken 2m from the central sampling point, and further two at 4m from the central sampling point, leaving 2m in between each 5 samples (Fig. 9).
- Wearing lab gloves, use the soil corer to extract a 30cm soil sample at each of the subsampling points. A sturdy rod or similar may be helpful in extracting the soil from the tool.
- If soil depth is insufficient to allow sampling as described above, take as many cores within the 8m long soil sampling transect as are required to generate a sufficient soil sample, and record deviations from the standard methods.
- After the 5 subsamples have been taken and stored, change the gloves and clean the corer with ethanol before taking a sample at another distance.
- Record the collecting distance and sampling depth on to the summary sheet.
- The soil corer retrieves about 50g of soil depending on the soil properties. In combination the five subsamples should result in well over the required 50g.
- Store samples in a resealable polyethylene bag.
- Mark sample bags according to the sample naming scheme. Sample name slips should be printed beforehand using a laser printer, not hand-written.
    - See section 7.3 for sample naming.
- Place sample bags in a cold box over ice packs.
- Transport the samples to the lab as quickly as possible, within the same day. Samples are stored in the freezer at -20°C.

- In the lab, mix the soil sample thoroughly and move slightly over 50g of the sample into a new pre-labeled bag. This bag will be shipped for analysis.
- Store the rest of the sample in a freezer as backup.
- Fill sample summary sheet with details of the sampling event.

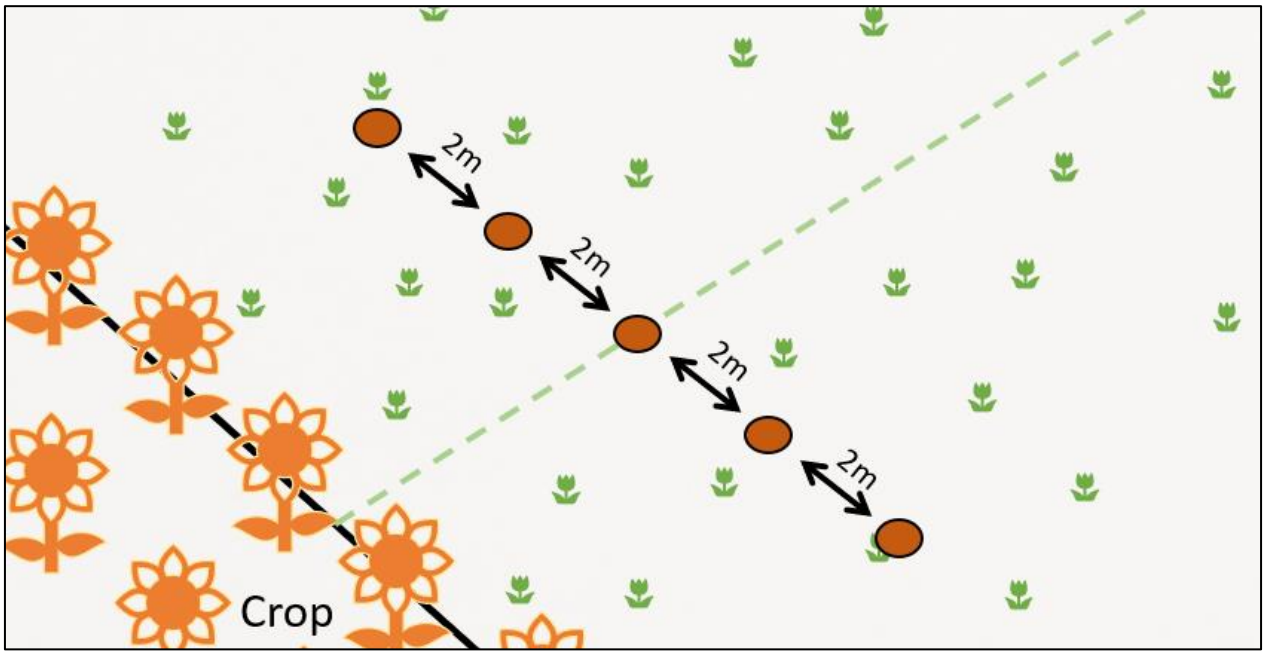


Figure 9: Schematic view of the soil sampling scheme. A central subsample is taken at the middle, and each further subsample 2m from the previous one.

14.1 Kit needed for soil sampling

- Data collection template.
- Cold box with ice packs for sample storage.
- Tape measure to measure sampling distances and subsample locations
- Soil core sampling tool (Buerkle GeoSampler)
  - https://www.buerkle.de/en/soil-sampler-geosampler_p5350-5003
- Pre-labeled resealable polyethylene bags.
- A tool to push compacted soil out of the corer tool (e.g. wooden rod)
- Gloves

14.2 Shipping soil samples

Pesticide residues from soil samples will be analysed at PIWET in Poland. To reduce costs, samples should be shipped once they have all been collected. Until then, they should be stored at -20°C.

- Make sure samples are clearly labelled with the correct sample names (see section 7.3).
- Ship samples over dry ice, following appropriate shipping procedures for dry ice (UN 1845).
- Use overnight courier for shipping.
    - Make sure there is enough dry ice to last an extra day or more. Even if using overnight shipping delays in transport are to be expected.
- Always ship at the start of the week to avoid shipments getting stuck over the weekend in case of delays.
- All the expenses should be covered by the sending party! There can be no fees for the recipient.
    - Make sure to check the box for “Delivered Duty Paid (DDP)”
- The shipment should indicate no commercial value.
- Fill, sign, and process the material transfer form.

Send soil samples to the following address:

Tomasz Kiljanek
National Veterinary Research Institute (PIWET)
Department of Pharmacology and Toxicology
Partyzantow 57, 24-100 Puławy
POLAND

EORI number for the receiving institution is PL716001076100000.

15 Collecting water samples

As most other sampling and surveying of field sites needs to happen during good weather, taking water samples may not be possible on the same sampling day. Be prepared to have an extra sampling day only for water sample collecting after rainy days.

- One water sample consists of 500ml of water from puddles or other small water bodies, collected at a specific distance from the crop edge.
- In practice, water samples may have to be taken where they are found.
- A 500ml plastic bottle should be used for sample collecting, one bottle per sample.
- The bottle should be opaque to protect the sample from light. If necessary, cover the bottle with foil.
- Collect water samples with a 50ml or 100ml syringe wearing gloves, using a new syringe and changing gloves between each sample.
- Three syringes are needed per site.
- Always record the collecting distance.
- Mark sample containers according to the sample naming scheme. Sample name slips should be printed beforehand using a laser printer, not hand-written.
    - See section 7.3 for sample naming.
    - Tape the sample name slip on the bag.
- Place sample containers in a cold box over ice packs.
- Transport the samples to the lab as quickly as possible, within the same day. Samples are stored in the freezer at -20°C.
- Fill sample summary sheet with details of the sampling event.

15.1 Kit needed for collecting water samples

- Data collection template
- Tape measure
- 500ml plastic sample bottles (pre-labeled)
- 50ml or 100ml syringes
- Gloves
- Cold box with ice packs for sample storage.

15.2 Shipping water samples

Pesticide residues from water samples are analysed at PIWET in Poland. To reduce costs, samples should be shipped once they all have been collected (stored at -20°C).

- Make sure samples are clearly labelled with the correct sample names (see section 7.3).

- Ship samples over dry ice, following appropriate shipping procedures for dry ice (UN 1845).
- Use overnight courier for shipping.
    - Make sure there is enough dry ice to last an extra day or more. Even if using overnight shipping delays in transport are to be expected.
- Always ship at the start of the week to avoid shipments getting stuck over the weekend in case of delays.
- All the expenses should be covered by the sending party! There can be no fees for the recipient.
    - Make sure to check the box for “Delivered Duty Paid (DDP)”
- The shipment should indicate no commercial value.
- Fill, sign, and process the material transfer form.

Send soil samples to the following address:

Tomasz Kiljanek
National Veterinary Research Institute (PIWET)
Department of Pharmacology and Toxicology
Partyzantow 57, 24-100 Puławy
POLAND

EORI number for the receiving institution is PL716001076100000.

16 Setting up bumble bee colonies

Three bumble bee colonies will be placed on each site, 30 colonies per country. The main purpose of these colonies is to gather pollen from a wider area around the site.

It is advisable to order a couple of extra colonies in case anything happens during transport or setup.

16.1 Obtaining colonies

- Each site network member should have budget for three colonies per site and should find a local supplier of Biobest *Bombus* colonies in their area.
    - If buying from another country, make sure there are no legislative hurdles associated with importing colonies.
- The supplier should be contacted a month beforehand to make sure they have capacity to supply the number of colonies needed.
- The standard colony size is about 80 workers, but it should be communicated to the supplier that the colonies should be of equal size (also in terms of brood), as they are used for research purposes.
- When ordering the colonies, ask that the insulating cotton layer not be added to the colonies, but instead arrive separately. Not having the layer in place is helpful when checking for the natal queen and estimating the number of workers in the colony prior to placement in the field.

16.2 Colony setup

- Give each colony an ID using the same syntax by which samples are named (see section 7.3). The colonies are named corresponding to the site, and with a running “sample” number from B01 to B03.
    - As an example, a colony in the UK might be WPosh1_GBR_WHE5_B01
    - The colony name then is basically the same as the sample name, when pollen is collected from that hive.
- Before the colonies are placed in the field, make sure you can locate a queen, and record the number of workers on the data collecting template.
- Take a photo of the colony from above. Make sure the colony ID is shown in the photo.
- Weigh the colony, excluding the outer cardboard box and the Biogluc bottle.
    - Use a dark room under red light to perform these measurements. A desk light with a red bulb, or a red headlight also work.
    - Avoid wearing perfumes or using strongly scented deodorants/shampoo when working with the colonies in the lab.

16.3 Installing colonies

- Colonies are installed in the field during the first sampling round.
- Record the set-up date of the colonies. This will be part of the basic site data (section 5).
- The exact positioning of the colonies will vary by site, but a spot nearby the site sampling zone, that is sheltered by vegetation is preferable.
- Keep the Biogluc bottle open.
- A protective structure is necessary for the colonies to prevent attacks from ants, badgers, raccoons, or other animals, as well as rain.
    - These may be plastic or Styrofoam boxes, that are attached to the ground. Each country may decide the best type of structure, as long as it is consistent within the country.

16.4 Colony termination

The colonies are removed from the field sites after the second sampling round. The removal should happen at night, after foragers have returned to the colony.

- Close the colony entrance before collecting.
- Take a photo and weigh the colonies again, making sure the colony ID is visible.
- Place the colony in a plastic bag and seal the bag with tape. Label the bag with the colony ID and collection date.
- Fill the termination date and time on the site data template.
- Back at the home institution, freeze the colonies and store them in the plastic bags in -20°C before dissection.

16.5 Dissection of the colonies

- Take the colony out of the freezer and place on the workbench.
- Fill the colony ID and date on the data template.
- Record the numbers of
    - Adult and emerged workers
    - Males (drones)
    - Natal queen
    - New queens (gynes)
- Remove any wax cover carefully.
- Take a picture of the colony, making sure the colony ID is visible.
- Sort through the nest structure and record the number of intact
    - Worker/male cocoons
    - Queen cocoons
- Record the number of
    - Pollen storage cells

    - Nectar storage wax cups
- Clean the workspace and wash all equipment used, then proceed to the next colony.

17 Collecting pollen samples from *Bombus*

One sample of pollen is collected from the returning pollen foragers of each of the three colonies on the site, in total three samples per site. A sample consist of 600mg of pollen. Take care to avoid being stung!

17.1 Harvesting pollen from returning foragers

- Stand or sit in front of the colony and catch individual foragers into a 50ml Falcon tube, either directly or with a help of a hand-held insect net. Catch and store one bee at a time, for a total of 15-20 bees with full pollen baskets. Either all bees can be collected and kept on ice (see below) prior to collecting pollen from them, or this can be done one by one (depending upon the rate of pollen forager returns to the nest).
- Close the tube and place on ice for a few minutes to calm down the bee.
- Open the tube, take the pollen with tweezers, and let the bee fly away once it regains its senses.
- Wear gloves while collecting pollen and change them between each sample. Use different tweezers for each sample, or wash with ethanol between samples.
- Collect pollen into pre-labeled 1.5ml Eppendorf tubes.
    - See section 7.3 for sample naming.
- Use the field scale to determine that sufficient sample amount has been gathered.
- Place sample tubes in a cold box over ice packs.
- Transport the samples to the lab as quickly as possible, within the same day. Samples are stored in the freezer at -20°C.
- Fill sample summary sheet with details of the sampling event.

17.2 Kit needed for sampling pollen from *Bombus* foragers

- Data collection template.
- 50ml centrifuge tubes (Falcon tubes).
- Insect net
- Pre-labeled 1.5ml Eppendorf tubes.

- Tweezers.
- Cold box with ice packs for sample storage.

17.3 Shipping *Bombus* pollen samples

Pesticide residues from *Bombus* pollen are analysed at PIWET in Poland. To reduce costs, samples should be shipped once they all have been collected (stored at -20°C).

- Make sure samples are clearly labelled with the correct sample names (see section 7.3).
- Ship samples over dry ice, following appropriate shipping procedures (UN 1845).
- Use overnight courier for shipping.
    - Make sure there is enough dry ice to last an extra day or more. Even if using overnight shipping delays in transport are to be expected.
- Always ship at the start of the week to avoid shipments getting stuck over the weekend in case of delays.
- All the expenses should be covered by the sending party! There can be no fees for the recipient.
    - Make sure to check the box for “Delivered Duty Paid (DDP)”
- The shipment should indicate no commercial value.
- Fill, sign and process the WildPosh material transfer form.
- **IMPORTANT NOTE:** Do not classify the contents as bee products! Pollen from bumble bee legs is not a bee product.

Send *Bombus* pollen samples to the following address:

Tomasz Kiljanek
National Veterinary Research Institute (PIWET)
Department of Pharmacology and Toxicology
Partyzantow 57, 24-100 Puławy
POLAND

EORI number for the receiving institution is PL716001076100000.

18 Grower survey

The grower survey is used to collect information on what pesticides have been used in the crops next to the WildPosh field site.

As early as possible after finding your potential field sites you should contact the farmers of the crops adjacent to your field sites and make sure they are willing to participate and be asked questions regarding the pest control measures they use. The survey will take place after the pesticide use regime has mostly ended, to make sure all information on the pesticides used is available.

Site network leaders of each country should translate, or appoint a person to translate, the survey into their local language well before conducting the survey. Two weeks before the survey, send the translated survey to the farmers, making sure they have received it.

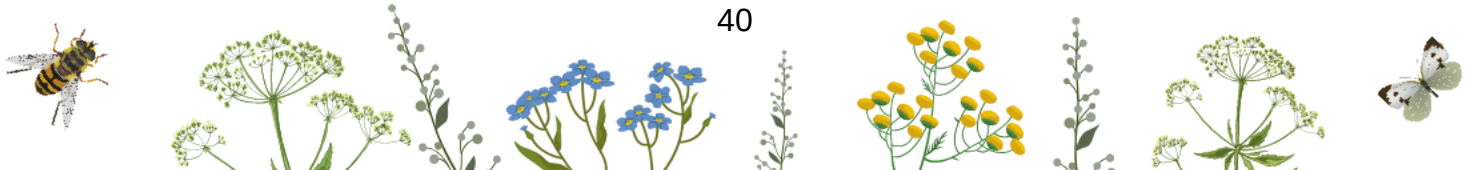
No personal information is asked for in the survey, but due to the open answers included in the survey that could be used to identify the person, the survey is not considered anonymous and should not be claimed to be such. Furthermore, the data cannot be shared with anyone outside WildPosh due to GDPR rules. Within WildPosh only partners directly using the data can have access to it.

18.1 Conducting the survey

Arrange a time to meet with the farmer to conduct the survey and encourage them to look over the survey beforehand, so they are familiar with the questions. Agree when and how the survey will take place (face to face, over the phone, or online). Face to face meetings are more likely to get responses and should be preferred. Online meetings should be avoided. When conducting the survey, you need to inform the farmer of the ethical statement. Emphasize that the data will be available only to selected individuals of the WildPosh project. Only summary data will be otherwise available.

It is recommended for you to read the questions to the farmer, and then enter the answer in the online form on a laptop. The answers should be entered in English regardless of the language used, to ensure standardised data. Similarly, if using paper forms, enter the answers immediately online to make sure interpreting handwriting does not become an issue. You may give example answers on request of the farmer or to prompt a response.

When entering data about chemical pest control applications, use the product name rather than the active ingredient. There are three slots available for application of a product. Include the date and rate of application for each of them, because the rate may not be the same in every application. If the three slots are not enough, add the information in the Notes-field. When entering data about the pesticide applications, be as detailed as possible.



18.2 Translations

A copy of the English survey is available in Annex 2 and can be sent upon request. When translating the survey, place the translated text below the English version in the document. Do not change the order of questions and translate as close as possible to the original wording. The important detail is that we are talking about the WildPosh field site, and the focal oilseed rape or wheat crop next to it.

When the translation has been made to your language, send the translated copy to Matti Leponiemi (matti.leponiemi@rhul.ac.uk). He will prepare the online survey based on the translation and send you the details to access the survey and answers.

Once you receive access to the online version of the survey, go through the questions and make sure everything is correct.

19 Overall kit checklists

- A folder to hold data collection templates
- Cold box with ice packs to store samples
- Gloves to wear during sampling
- Ethanol and paper wipes to clean equipment
- Tape measure to measure sampling distances
- Clinometer kit for measuring inclination.
- A device to record GPS coordinates (e.g. mobile phone)
- A stopwatch to record transect time
- A net to catch flying insects
- Collection tubes for bees or syrphids to be identified in the lab
- Guides for pollinator and plant ID (including mobile phone).
- Thermometer (e.g. in the car)
- 1x1m quadrats for plant transects
- Small flags to mark quadrat positions
- Camera to take photos of plants, quadrats, etc
- A tally counter (optional but may be useful).
- Electronic Pollen Sampler vacuum device
- Pollen collection tubes
- Parafilm to seal pollen collection tubes
- A power bank or a laptop to power the electronic pollen sampler
- A precision scale to measure the pollen (and other sample) amounts
- Microcapillary tubes (0.5µl - 2µl)
- 1.5ml centrifuge tubes for nectar samples in the field
- 0.2ml centrifuge tubes for nectar samples in the lab
- Field centrifuge kit
- Filter tubes for centrifuge
- A 12V battery for centrifuge
- Adapter for battery to car socket for centrifuge
- Battery charger for centrifuge
- Scissors to retrieve plant samples
- 1l resealable polyethylene bags (plant and soil samples)
- Soil core sampling tool (Buerkle Geosampler)
- A tool to push compacted soil out of the corer tool
- 500ml plastic sample bottles for water samples
- 50ml or 100ml syringes for water samples
- 50ml centrifuge tubes for catching bumble bees
- Tweezers to pick pollen from bumble bees

20 Acknowledgements

We thank WildPosh members, particularly members of work package one, for participating in the discussion developing the protocol.

21 References

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22 Annex 1 – Beaufort wind scale

From the Royal Meteorological Society (see https://www.rmets.org/metmatters/beaufort-wind-scale)

Wind Force	Wind Speed		Description	Specifications on land
	km/h	mph		
0	<1	<1	Calm	Smoke rises vertically
1	1-5	1-3	Light Air	Direction shown by smoke drift but not by wind vanes.
2	6-11	4-7	Light Breeze	Wind felt on face; leaves rustle; wind vane moved by wind.
3	12-19	8-12	Gentle Breeze	Leaves and small twigs in constant motion; light flags extended.
4	20-28	13-18	Moderate Breeze	Raises dust and loose paper; small branches moved.
5	29-38	19-24	Fresh Breeze	Small trees in leaf begin to sway; crested wavelets form on inland waters.
Do not survey above this level of wind!				
6	38-49	25-31	Strong Breeze	Large branches in motion; whistling heard in telegraph wires; umbrellas used with difficulty.
7	50-61	32-38	Near Gale	Whole trees in motion; inconvenience felt when walking against the wind.
8	62-74	39-46	Gale	Twigs break off trees, generally impedes progress.
9	75-88	47-54	Strong Gale	Slight structural damage (chimney pots and slates removed).
10	89-102	55-63	Storm	Seldom experienced inland; trees uprooted; considerable structural damage.
11	103-117	64-72	Violent Storm	Very rarely experienced, accompanied by widespread damage.
12	118+	73+	Hurricane	Devastation.

23 Annex 2 – Farmer survey

Site ID: _____

Q1 How big is the field next to the WildPosh site?

Q2 Which chemical growth regulators (auxins etc.), if any, did you apply to the field next to the WildPosh site?

Q3 Since your last harvest, which plant protection products (including herbicides, insecticides, fungicides, soap, copper etc.) did you apply to the field next to the WildPosh site?

For each product, please indicate when you apply the product and at what rate it is applied.

Q4 Which, if any, of these plant protection products did you apply to the field next to the WildPosh site using a tank mix? Please tick all that apply.

	Product name	Date of application	Application rate (l/ha)	Date of application	Application rate (l/ha)	Date of application	Application rate (l/ha)
Product 1							
[…]							
Product 15							

Q5 Which variety(s) of (oilseed rape/wheat) did you grow in the field next to the WildPosh site?

Q6 In the field next to the WildPosh site, which crops did you grow in the previous years? Please answer as many of these as possible.

- 2020
- 2021
- 2022
- 2023
- 2024

Q7 This year, which of the following describes the seeding strategy you used in the field next to the WildPosh site?

- Sow seeds treated with plant protection products
- Sow untreated seeds

Q7b Which (if any) plant protection products were these seeds treated with?

QX Additional notes