Design and planning of a transdisciplinary investigation into farmland pollinators: rationale, co-design, and lessons learned

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To link to this article DOI: http://dx.doi.org/10.3390/su141710549

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Design and Planning of a Transdisciplinary Investigation into Farmland Pollinators: Rationale, Co-Design, and Lessons Learned

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Citation: Hodge, S.; Schweiger, O.; Klein, A.-M.; Potts, S.G.; Costa, C.; Albrecht, M.; de Miranda, J.R.; Mand, M.; De la Rúa, P.; Rundlöf, M.; et al. Design and Planning of a Transdisciplinary Investigation into Farmland Pollinators: Rationale, Co-Design, and Lessons Learned. *Sustainability* **2022**, *14*, 10549. https://doi.org/10.3390/su141710549

Academic Editor: Uta Schirpke

Received: 12 July 2022
Accepted: 18 August 2022
Published: 24 August 2022

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Abstract: To provide a complete portrayal of the multiple factors negatively impacting insects in agricultural landscapes it is necessary to assess the concurrent incidence, magnitude, and interactions among multiple stressors over substantial biogeographical scales. Trans-national ecological field investigations with wide-ranging stakeholders typically encounter numerous challenges during the design planning stages, not least that the scientific soundness of a spatially replicated study design must account for the substantial geographic and climatic variation among distant sites. 'PoshBee' (Pan-European assessment, monitoring, and mitigation of Stressors on the Health of Bees) is a multi-partner transdisciplinary agroecological project established to investigate the suite of stressors typically encountered by pollinating insects in European agricultural landscapes. To do this, PoshBee established a network of 128 study sites across eight European countries and collected over

50 measurements and samples relating to the nutritional, toxicological, pathogenic, and landscape components of the bees' environment. This paper describes the development process, rationale, and end-result of each aspect of the of the PoshBee field investigation. We describe the main issues and challenges encountered during the design stages and highlight a number of actions or processes that may benefit other multi-partner research consortia planning similar large-scale studies. It was soon identified that in a multi-component study design process, the development of interaction and communication networks involving all collaborators and stakeholders requires considerable time and resources. It was also necessary at each planning stage to be mindful of the needs and objectives of all stakeholders and partners, and further challenges inevitably arose when practical limitations, such as time restrictions and labour constraints, were superimposed upon prototype study designs. To promote clarity for all stakeholders, for each sub-component of the study, there should be a clear record of the rationale and reasoning that outlines how the final design transpired, what compromises were made, and how the requirements of different stakeholders were accomplished. Ultimately, multi-national agroecological field studies such as PoshBee benefit greatly from the involvement of diverse stakeholders and partners, ranging from field ecologists, project managers, policy legislators, mathematical modelers, and farmer organisations. While the execution of the study highlighted the advantages and benefits of large-scale transdisciplinary projects, the long planning period emphasized the need to formally describe a design framework that could facilitate the design process of future multi-partner collaborations.

Keywords: bees; bee pathogens; insect declines; landscape ecology; pan-European; pesticides; pollinators

1. Introduction

Animal pollinators support approximately 35% of global agricultural production, and an estimated three out of four of the main crops producing fruits or seeds for human consumption [1,2]. The volume of pollinator-dependent agricultural production has increased threefold in the last half century, with pollination services responsible for global crops now worth around USD 600 billion per annum [1]. In addition to providing essential ecological roles as pollinators of wild flowering plants, naturally occurring insect pollinators, such as Hymenoptera, Diptera, and Lepidoptera contribute significant crop pollination services [3–7]. Nevertheless, pollination deficits still exist for some crops, and commercial producers cannot depend entirely on populations of wild pollinators that fluctuate in abundance and species composition across growing seasons [8–10]. Managed bees, particularly *Apis mellifera* L. and *Bombus terrestris* L., are often used for pollination services in many large-scale agricultural production systems, and are thus mass produced and transported over considerable distances to meet these ends [11–13].

Over the past decade, several reports have indicated dramatic, long-term declines in wild insect diversity, abundance, or biomass (e.g., [14–17]). Although subsequent articles highlighted a need for caution and additional data before making generalisations concerning global insect declines (e.g., [18–21]) recent systematic reviews still suggest declines in some insect groups in some countries [22,23].

Towards the end of the last century, reports of massive honey bee colony losses became more frequent [24–26]. Many instances of honey bee mortality and colony collapse were attributed to poisoning by agricultural pesticides and/or increasing infestations of pests and pathogens, in particular the mite *Varroa destructor* [27,28]. Considerable honey bee colony losses still occur, and for the period between 1 April 2019 and 1 April 2020 beekeepers in the USA lost an estimated 44% of honey bee colonies, the second highest annual loss over the previous 10 years [29].

Several papers have described declines in abundance and diversity of wild insect pollinators, and, as with managed bees, these declines have been attributed to a range of abiotic and biotic stressors such as emerging pests and diseases, invasive species, intensification of agriculture, habitat loss, exposure to agrochemicals, nutritional deficiencies, and climate change [26,30–36] (Figure 1). Long-term trends in warming have led to some asymmetrical biogeographical and phenological shifts in flowering plants and their pollinators, potentially leading to their separation in space and/or time [37]. Over the short term, atypical seasonal weather such as early or warm springs and increased frequency of flooding and drought events has resulted in mismatches between flowering and emergence of early season pollinator species, loss of feeding and nesting habitats, and reduction in the quantity and quality of floral resources [38,39].

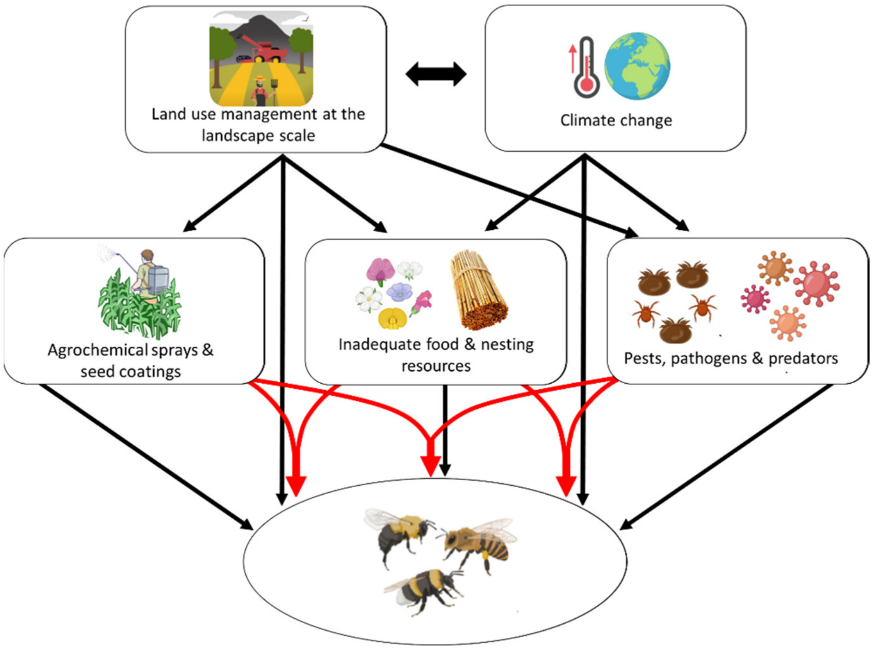

Figure 1. Illustration of the typical stressors with direct, indirect, and synergistic effects on wild and managed pollinating insects in agricultural landscapes. Synergistic interactive effects are illustrated in red while direct and indirect impacts are shown in black.

Crop treatment with insecticides can have obvious direct acute effects on the mortality and longevity of both wild and managed insect pollinators (e.g., [40–44]). Additionally, sublethal exposure to pesticides, including herbicides and fungicides, can cause physiological disruption in bees, induce disorders in developing larvae, and impact feeding behaviour and flower visitation rates [45–48]. Nutritional deficits have been postulated as another cause of pollinator decline, even though there is often ambiguity when relating the abundance and diversity of floral resources with the performance of managed bees and wild pollinator communities [30,36,49]. Although many studies report positive relationships between farmland pollinators and floral diversity (e.g., [50,51]), counter examples also exist, or situations where the relationship is variable over time or dependent upon the taxa considered [49,52]. The positive effects on pollinators—and pollination services—produced by artificially increasing floral resources, whether this is part of hedgerow management or sowing of floral strips, can also be dependent upon a number of secondary factors including the wider landscape context (e.g., [53,54]).

Negative relationships have been demonstrated between wider land-use intensity, often measured in terms of inputs of nitrogen fertilizer or agrochemical use, and wild bee diversity [55] or honey bee performance [56]. Similarly, pollination services provided by wild bees, often a function of bee abundance and/or diversity, can be reduced in simplified, monoculture, landscapes compared with more complex and diverse agricultural settings [57]. Many of these patterns are not universal, however, and contrary situations exist where, for example, organic management and enhanced landscape complexity have no demonstratable beneficial effects on insect pollinators [55,58,59].

Many of the biotic and abiotic stressors described above are co-dependent to varying degrees, and one negative factor can exacerbate the negative effects of another or lead to synergistic chains of accumulating impacts [34,60]. For example, agricultural intensification can lead to loss of semi-natural habitat, with subsequent decreases in local flora, which in turn requires bees to forage over extended distances resulting in weakened individuals with increased risk of pesticide exposure [61]. A restricted or monotonous diet can result in bees being susceptible to pesticide toxicity (e.g., [62]), while bees weakened by insecticide poisoning can, subsequently, be more susceptible to pests and diseases [63–66]. Low availability of floral resources can also escalate pathogen transmission among pollinators by increasing the likelihood that multiple individual insects, of the same or different species, visit the same individual blossoms [67]. Finally, concurrent exposure to multiple agrochemicals can lead to overall negative impacts that are greater than a simple sum of the individual effects [68]. In order to provide a more complete description of the nexus of synergistic stressors encountered by pollinating insects and how these stressors interact in their effects on the insects, it is therefore necessary to assess the incidence and magnitude of all abiotic and biotic stressors concurrently [32,69].

The exposure and responses of pollinators to biotic and abiotic stressors can vary considerably over different physical scales and across geographic regions. Therefore, large-scale, multi-site field studies are increasingly being employed to obtain field-realistic descriptors of typical pollinator environments, which allow quantification of variation in stressors among locations, and can be generalized to a wide range of insect taxa occurring in agricultural ecosystems. In the European arena, this has led to a growing number of studies of insect pollinators involving multiple research partners and study sites distributed over several countries (e.g., [70–75]). As the geographic range and scientific scope of these multi-national field studies continues to grow, and the number of partner organisations increases, the study design process and the network of interactions among the different partners and stakeholder groups also becomes increasingly complex. As such, the effective and efficient design of such field studies that ensures maintenance of scientific rigour and the needs of diverse stakeholders, while accepting the constraints imposed by budget, logistics, and labour availability, presents a significant trans-disciplinary challenge (see [71,76,77]).

The 'PoshBee' project (Pan-European assessment, monitoring, and mitigation of Stressors on the Health of Bees) is a multi-institute project funded by the EU Horizon 2020 initiative that commenced in 2018 [78]. The overarching aim of PoshBee is to investigate the range of responses exhibited by insect pollinators when simultaneously exposed to multiple environmental stressors, and the project has adopted a wide-ranging, hierarchical investigative approach, involving highly controlled laboratory experiments, semi-field cage trials, and toxicological modelling. As a reference point for many of these laboratory and semi-field experiments, PoshBee also included a pan-European field investigation to gain insight into the field-realistic levels of chemical, nutritional, pathological, and habitat-based stressors encountered by farmland insects. By using standardized populations of managed bees as bioindicators or 'sentinels', the field study aimed to determine the potential impacts of these stressors on insect pollinators at physiological, individual and population levels.

The detailed planning of the PoshBee field investigation occurred over several months and involved multiple academic partners, agricultural organisations, beekeepers, and other stakeholder groups. The individual components of the field study evolved via an iterative co-creation process so as to meet their individual goals, and these separate protocols were then further modified so as to produce a manageable and practicable overall study design. With the many diverse partners in PoshBee it was not uncommon to encounter conflicting viewpoints between and across stakeholder groups or research disciplines concerning the aims of the field study or with respect to how the limited time and labour should be allocated. In this paper, to share our experiences and provide guidance for future multi-partner, multi-country field studies of similar magnitude and complexity, we describe the rationale behind each component of the field study and the lessons learned in designing and conducting the PoshBee field investigation.

2. Partner Organisations and Method Development

The design of the PoshBee field study involved input from 27 distinct organisations, including nine field teams, five farmer organisations, six beekeeper organisations, and an additional seven 'Tier II' organisations involved in analysis of the various samples and data obtained (Supplementary File S1). One institute (Trinity College Dublin; TCD) was assigned the responsibility for field study coordination, finalising methods, and data management. Further input and moderation were provided by members of the PoshBee management team to ensure that the field study met its original objectives, maintained its role in the overall project scheme, and provided the data and samples required for completion of other PoshBee work packages [78]. Additionally, because the project aimed to obtain information offering value from multiple perspectives (e.g., biological; ecological; agricultural; legislation) it was also required to recognize the needs of the different stakeholder groups (e.g., academics; farmer organisations; beekeepers; conservationists; policy makers) in terms of the quantity and form of data that were collected.

Given the physical scale of the field study, and the number of components that required completion in a limited time frame, the major issues that arose from the multi-partner co-design process were:

(i) the evolution and adoption of a co-design process acceptable to all study partners that allowed fair discussion and contributions from different sources to be assimilated into final design decisions;
(ii) selecting sub-sets of partners to assist with development of distinct aspects of the field study (e.g., site selection, sampling protocols, landscape analysis, laboratory analysis, social engagement);
(iii) the collaborative production of standardised protocols for each component of the study that met the objectives of the different stakeholders, remained integrated with overall project aims, and were acceptable to all partners;
(iv) capacity building of the partner institutes in terms of network creation, acquisition of new skills and techniques, and development of logistical awareness.

The method development process was initiated during the project 'kick off' meeting in August 2018, and continued during a subsequent Annual General Meeting of all partners in January 2019. In terms of general planning, these were the only two occasions when the majority of team members convened for face-to-face discussions. Further fine tuning of each study component, and overall logistics and decisions concerning the field sampling sequence, were determined by dialogue among study partners via telephone conversations, video conferencing, and, primarily, email group discussion chains.

Collecting input from all stakeholders at an early stage of project development helped to refine research questions and ensured that the scope of the field study encompassed the aims and objectives of all network members (Figure 2). These initial discussions were also used to identify aspects of the project where further clarification or debate were needed, and where stakeholder requests were flagged as being out of scope or impractical in terms of field study logistics [79,80].

The project management team identified and assigned study partners to assist with the development of aspects of the field study appropriate for their areas of expertise (Figure 2). In many cases, this involved creating working groups consisting of Tier II organisations involved with the analysis of biological samples (e.g., pesticide load; palynology; nutrition; pathogens) and also field teams that would be involved with the collection of the required samples.

As draft methodological protocols were produced, they would be subjected to rigorous scrutiny by other partners to ensure they would meet its aims and provide the quantity and form of data required (Figure 2). Objections were often raised by Tier II partners in terms of the form or quantity of samples collected, or by field teams about the practicality of the proposed method under field conditions and time limitations. In these situations, an iterative co-design cycle commenced whereby modified protocols were produced to address issues that had been raised, along with an explanation of why and how some changes or compromises were required (Figure 2). The modified designs were distributed to the group until they were deemed satisfactory by all partners. Nevertheless, modifications to one field protocol could have ramifications for the resources now available for others, in which case their design cycles were reinitiated with the new limitations in place.

Clarification and further refining of methodologies were reinforced via a practical workshop involving team leaders, field workers and practical demonstrators. The workshop provided an opportunity for specialist methods and techniques (e.g., extraction of bee stomach contents; collection of bee haemolymph; *Apis mellifera* colony assessment; identification of bee parasites; efficient collection of pollen samples) to be demonstrated and practiced by field teams prior to the initiation of field work. The protocol development process ultimately resulted in the production of a volume of documents providing step-by-step instructions for the methods and timing of each element of the field study (Supplementary Files S2 and S10; [78]).

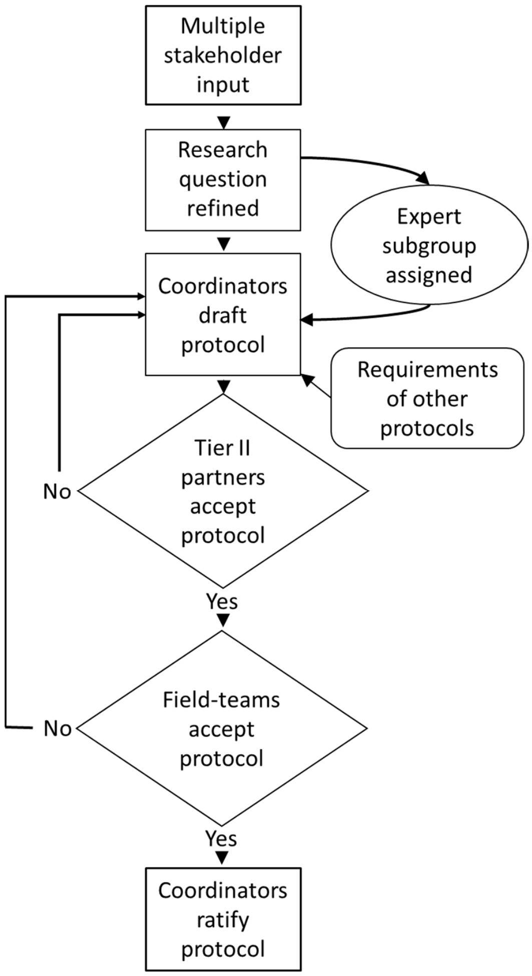

Figure 2. Illustration of the iterative co-design process for each aspect of the PoshBee field investigation. Each component of the field study was designed to provide information for various stakeholders (e.g., academics, beekeepers, farmers). A subgroup of partners was selected who, along with the project coordinators at Trinity College Dublin, produced an initial draft protocol. This draft then went through several iterations based on the views of the Tier II partners involved in analysis of samples, data, etc., and the field teams who judged whether what was being suggested was practical given the labour available, time constraints. When all partners were satisfied the final version of the protocol was signed off by the project coordinators.

By dividing the development process among partners, each component of the study could be signed off by project leads as they were completed, thus accelerating the development of the overall study design. Nevertheless, a period of nine months was still required to co-design, fine-tune, and finalise the series of standardized protocols required for the field study, and this time was in addition to two years already spent on the initial project proposal and establishment of the over-arching project framework.

3. Overall Aims and Rationale behind Study Components

The overall aim of the PoshBee field study was to quantify multiple biotic and abiotic stressors encountered by managed and wild bees in agroecosystems across the main European biogeographic zones. Additionally, it was aimed to assess the effects of these stressors on sentinel bee species at molecular, physiological, individual and colony levels. The groups of stressors to be investigated included agrochemical and heavy metal exposure, prevalence of pathogens and parasites, and quantity and quality of nutritional resources. This portfolio of direct stressors was embedded within landscape-scale measures of habitat quality and agricultural intensification. To evaluate the potential direct or indirect impacts of these stressors we also measured several proxies for sentinel bee 'health', such as colony growth, developmental integrity, gut microbiota, and molecular responses. Although the overall aims were primarily designed for the investigation of insect pollinators in agricultural settings, we envisaged that this general approach to stress-response relationships could be applied or adopted to non-agricultural situations such as (semi-)natural landscapes, managed conservation areas, or urban ecosystems.

It became apparent at an early stage in project development that it was infeasible to empirically test all combinations of stressors in an orthogonal, fully factorial experimental design. Instead, the field study design aimed to provide as broad an assessment of exposure to and impacts of stressors as possible within the logistical and financial constraints typical of field projects of this scale. Because the field study involved multiple components and involved participation of field workers and specialists from many organisations, the rationale behind each component of the study required separate consideration and debate as well as clear explanation to other members of the network. This discussion formed the first step in the development of the efficient cooperative design process described above, which integrated each stakeholder's knowledge, input, and requirements (Figure 2).

The reasoning, rationale, aims, and the issues encountered for each component of the study and how any problems were resolved are summarized in Table 1. The sections below expand on these issues and provide additional details and explanations regarding project development and how the final structure of the PoshBee field study was determined.

3.1. The Sentinel Bee Species

To improve consistency over the whole PoshBee site network it was necessary to use managed bees as sentinel species, which would enable the same species to be sampled in all countries, allow more controlled starting conditions, and foster uniformity in aspects of husbandry and sampling schemes. Additionally, the use of the same sentinel species in other work packages of the PoshBee project, such as laboratory and semi-field experiments, would allow more straightforward cross-referencing of results and a more mechanistic understanding of stress-responses relationships observed in the field.

There are many differences in the ecology, behaviour, and resource use among wild and managed bee species, and conservation efforts to support the health of honey bees may not always benefit other taxa (e.g., [81]). Nevertheless, many wild pollinators co-occur with their managed counterparts, and the sharing of floral resources, both mass-blooming crops and naturally occurring flowers, is commonplace [49,82,83]. Consequently, we made a general assumption that, at least on a local scale, our sentinel bees and naturally occurring pollinators would be exposed to similar levels of agrochemicals and, potentially, similar loads of pests and pathogens [84,85]. The results obtained from the sentinel bees in terms of the stress-response relationships could, therefore, with some justification be extrapolated to co-occurring wild pollinators in these sites [86].

Table 1. Summary of issues raised during the development and planning of the PoshBee managed bee field study design, how issues were resolved, and how these ideas translated to practice (PPPs—plant protection products).

Section	Issue to Be Considered	Resolution	Details
Section 3.1	Bee species vary in their response to stressors. Bee exposure to agrochemicals linked to foraging range. Bee species affected by different pests and diseases.	Use more than one species of sentinel bee species. Use bee species with different typical foraging distances. Use bee species with different social structures.	Three species of sentinel bees used: *Apis mellifera*, *Bombus terrestris*, *Osmia bicornis*
Section 3.2	Study requires same crops in all partner countries. Stress-pollinator responses vary on different crops. Agrochemical use differs on perennial and annual crops.	Adopt widely grown, economically important, European crops. Use crops that typically involve use of PPPs. Use one perennial and one annual crop.	Two study crops used: - Oilseed rape (annual) - Apple orchards (perennial)
Section 3.2	Geographical variation in intensity of bee stressors. Bee health and responses to stressors affected by climate. Bees exposed to multiple stressors simultaneously. Different stressors may/may not be correlated. Honey bee husbandry practices vary among countries.	Include multiple countries in study design. Use sites that offer a range of climatic conditions. Select sites that provide range of land use intensities. Accept variation due to local crops and honey bee management. Attempt to standardize other variables as much as possible.	Eight European countries involved in field study: CHE, ESP, EST, GBR, GER, IRL, ITA, SWE
Section 3.2	Field studies can have low statistical power. Proximal sites not considered statistically independent. Between-site variation required for meaningful results.	Use large number of independent replicate sites. Space sites so they can be considered statistically independent. Develop a gradient of land-use intensity.	A total of 128 study sites were used. Sites at least 3 km apart and selected to provide agricultural intensity gradient.
Sections 3.3 and 3.5	Bee health influenced by available nutritional resources. Bees respond to habitat diversity on a local scale. Bees respond to habitat diversity in wider landscape.	Assess abundance and diversity of local floral resources. Assess local habitat composition. Assess wider landscape context. Assess abundance and diversity of local pollinator communities	Habitat assessed/classified using: - floral and pollinator surveys. - field boundary habitats. - neighbouring-field habitats. - GIS landscape analysis 1 km.
Section 3.4	Pesticide exposure varies among bee species. Pesticide contamination differs between colony matrices. Pesticide concentration may bioaccumulate in nests. Pesticide exposure will vary depending on which products have been used and when they were applied.	Assess chemicals in foraging (female) bees. Compare chemicals in different components of the nests. Compare pesticides in floral resources with those in bees/nests Obtain information on PPPs use and application dates.	Chemicals assessed in: - foraging bees of three species. - pollen and nectar of crop flowers. - pollen stores of three bee species. - wax/royal jelly *A. mellifera* hives. - farmer information on PPPs.
Section 3.6	Diversity of bee pests and pathogens varies spatially. Different bee species may face different pathogens. Commercially produced bees can be infested.	Test for multiple pests and pathogens. Use multiple bee species for pathogen testing. Test bees for pathogens before exposure to field site conditions.	Evaluated bees for multiple pests. Tested all sentinel bees for same suite of multiple pathogens. Test bees prior and post field exposure
Section 3.8	Variation in flowering phenology among countries. Variation in typical methods used different partners. Variation in the range of experience and skill sets of field workers in different partner countries.	Standardize relative sampling times. Standardize all field sample collection and assessment methods. Standardize measurements and form of data collected. Provide training in all field methods.	Sampling based on crop flowering times. Workshop to train field workers. Produced: - detailed manual of method protocols. - videos illustrating sampling methods. - standardized data collection templates.

Discussion of candidate species to be used as the sentinel species highlighted that even with managed bees, different taxonomic groups have different ecological requirements, phenologies, life history traits, foraging behaviour, and sensitivity to stresses (Table 1). Additionally, interspecific variation in typical foraging ranges can result in individuals being more or less exposed to stressors such as agrochemicals, diseases, and parasites. It became apparent, therefore, that although saving costs and time, using only a single sentinel species would provide only a limited view of the pollinator environment. To address the issues outlined above and meet the overall aims of the project, it was decided that the sentinel bees should involve:

(i) a suite of bee species rather than a single species;
(ii) species that have previously been used as indicators of abiotic/biotic stress in pollinators;
(iii) species that exhibit variation in life histories, typical colony sizes, and nesting behaviours;
(iv) species that vary in their typical foraging distances;
(v) species that are commercially available in all countries taking part in the study.

Ultimately, it was decided to use three managed species of bees as sentinel species: *Apis mellifera*, *Bombus terrestris* and *Osmia bicornis* L. *Apis mellifera* is already used as an indicator of pesticide contamination [87] and measures of *A. mellifera* colony and individual performance have been related to agricultural land use. *Bombus terrestris* and *Osmia bicornis* are increasingly used to examine effects of field-realistic insecticide exposure [42] and have been recommended as model species for risk-assessment [88]. In terms of life histories, *A. mellifera* and *B. terrestris* are both social species whereas *O. bicornis* is a solitary stem-nesting species, although it can form aggregations within nesting sites. Additionally, whereas *A. mellifera* colonies generally consist of thousands of workers and drones, commercial *B. terrestris* colonies generally contain around a hundred individuals.

In terms of the scale of the surrounding landscape the sentinel bees would encounter, it was necessary to consider the differences in foraging ranges of the three species. Solitary bees, including *Osmia*, typically forage <1 km from the nest [89–91], whereas estimates for *A. mellifera* foraging distances range from <1 km up to 6 km [92–94]. Estimates of typical foraging distances for *B. terrestris* include 1.5 km [95], <2 km [96], 2.5 km [97], but can be as much as 10 km [98].

From a practical point of view, it was aimed to standardise the initial populations and maintenance of the sentinel bees in as many ways as was feasible, but because of differences in the typical methods used in the different partner countries some concessions were made. Each field site network partner was instructed to use *A. mellifera* equipment and practices representative of their local region, which resulted in good standardisation of practices within each country but naturally resulted in some inter-partner variation. For example, local sub-species of *A. mellifera* were used and hives were constructed of different materials (e.g., wood; polystyrene; Supplementary File S3). Similarly, standardized commercial *B. terrestris* colonies, supplied with a queen and approximately 80 workers, were sourced from local suppliers in each country, so that the Irish and UK field teams purchased colonies of their local subspecies, *B. terrestris audax*, and the remaining countries used *B. terrestris* (Supplementary File S4).

The UK and Ireland teams encountered difficulties in obtaining sufficient numbers of locally-sourced *O. bicornis* pupae. Also, *O. bicornis* is a recent arrival in Ireland and some partners expressed concern over releasing genetically dissimilar individuals that had been sourced from mainland Europe. Therefore, as a group, it was decided not to use *O. bicornis* as a sentinel species in the UK and Ireland, even though it was conceded this would result in gaps in the overall data set. In the remaining countries, all partners obtained *O. bicornis* as pupae from the same commercial source and followed the same standardized guidelines for release of foundation stock and recapture of nesting individuals [99].

3.2. Study Site Selection

Stress-response relationships affecting pollinators in agricultural landscapes vary at multiple spatial scales: for example, among countries, among areas of differing agricultural intensification, between crops, and between the centres and margins of fields [75]. It was recognized that the geography and scale of agricultural land differed significantly among the countries taking part in the field investigation, so to ensure some commonalities it was essential to formulate site-selection criteria to promote a coherent structure to the pan-European network (Table 1). From early discussions and debates it was agreed by study partners that to meet the aims and objectives of the field study the site network was required to:

(i) include sites in geographically separated European countries to encompass multiple biogeographic zones with differing climates and seasonality;
(ii) involve perennial and annual mass blooming crops that were found in all partner countries and were strongly associated with insect pollinators;
(iii) reflect gradients of agricultural intensification typical of each partner country;
(iv) have sufficient independent replication within and among countries to enable robust statistical analysis of the data obtained.

In order to encompass the major European biogeographic zones and EU regulatory areas, and to create approximate north–south and east–west gradients, eight European countries were selected to establish the study site network (Figure 3): United Kingdom (GBR) and Ireland (IRE) (Atlantic zone); Sweden (SWE) and Estonia (EST) (Boreal zone); Germany (GER) and Switzerland (CHE) (Continental zone); Spain (ESP) and Italy (ITA) (Mediterranean zone).

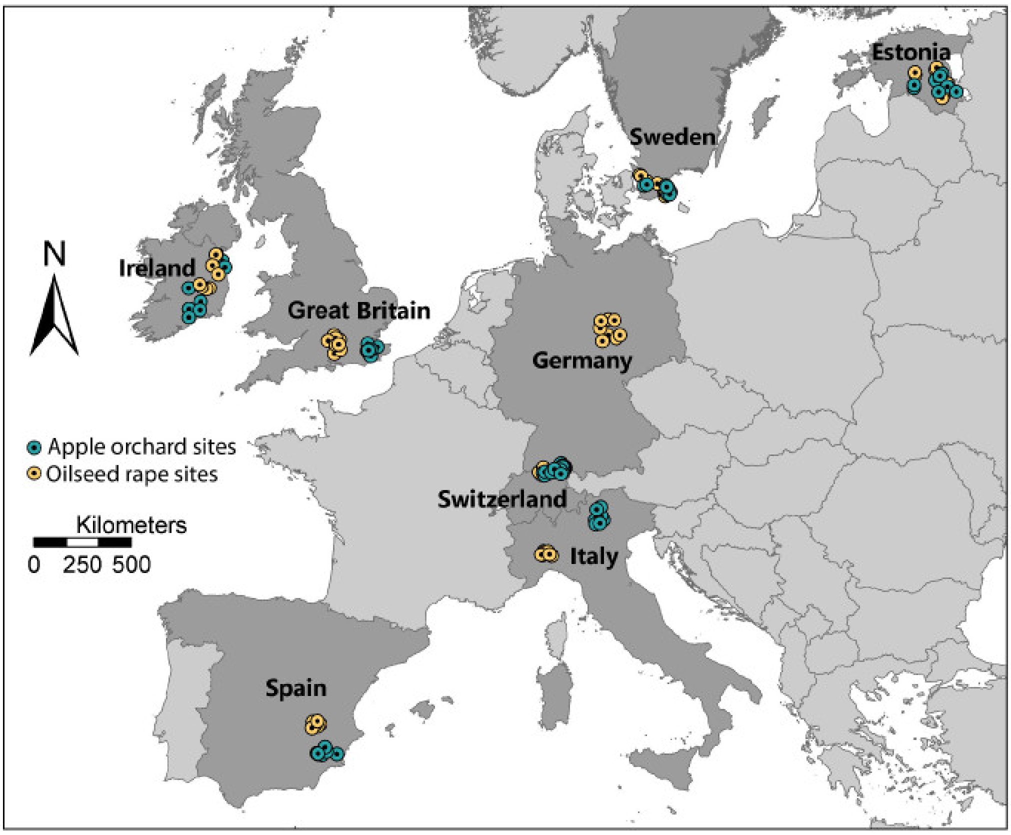

Figure 3. Distribution of apple and oilseed rape locations that formed the PoshBee site network, with eight sites of each crop in each of the eight participating countries. Note clustering of crops in some of the countries, and proximity of German apple sites to Swiss field sites.

Two study crops were selected: oilseed rape (OSR) representing a typical annual arable cropping system and apples representing a perennial horticultural system. These crops are grown throughout Europe, are insect-pollinated, and economically important [10,41,82,100]. Approximately 30 million tonnes of OSR are produced by EU member states on 11 million hectares (2020 data; agridata.ec.europa.eu; accessed on 1 March 2021), whereas approximately half a million hectares of apple orchards produce around 12 million tons of fruit each year (www.statista.com; accessed on 1 March 2021). In terms of field sampling practicality and logistics, both crops provide mass flowering events in late spring/early summer and are considered important sources of pollen and nectar for managed and wild bees within agricultural landscapes [5,101].

In order to achieve gradients in agricultural intensity, apple sites were selected based on variables such as orchard size, tree density, average tree height, or pest management strategy (e.g., organic; integrated pest management; conventional). For OSR, sites were selected based on the area of surrounding land that was also used for OSR, cereals, or improved grassland, which acted as a proxy for general agricultural intensification. The focal crops provided a valuable contrast in terms of the frequency and duration of agrochemical treatments. Conventional or integrated pest management of OSR generally results in short term, 'pulsed' application of a low number of pesticides, whereas conventional or integrated pest management of apples consists of a more or less 'continuous' spraying regime of various herbicides, fungicides, insecticides, and growth promoters from flowering through to fruit production.

Due to the high variation that can occur in entomological and botanical field data, and because field studies in this sphere of research often adopt a correlation approach, such investigations require sufficient sample sizes to achieve acceptable statistical power to detect weak effects [55,75]. To achieve acceptable but practicable sample sizes in each country, it was decided that eight sites of each crop in each country were required. This resulted in each crop being replicated 64 times overall, and a site network of 128 replicate sites in total.

For statistical independence it was necessary to distribute sites so they were not considered to 'overlap' in terms of the foraging ranges of the three sentinel bee species (see above). From a practical viewpoint, however, it was also necessary to consider that time spent on site could be extended if travel time between sites was minimized. After some debate, it was decided that selecting sites at least 3 km apart would promote statistical independence of sites while still allowing multiple sites to be visited in a single field day. Some leniency in site selection was allowed because of how the study crops were distributed in different countries. For example, in four countries, the UK, Germany, Spain and Italy, the OSR and apple study sites were in distinct regions, whereas in the remaining countries, Ireland, Estonia, Switzerland and Sweden, the OSR and apple sites were interspersed (Figure 3).

To account for inter-country variation, some properties could not be set across all study sites. For example, the cultivars/varieties of crops were those adopted by local growers to suit local environmental and market conditions, so were not standardized across countries or even for study sites within countries. Similarly, there was no requirement to select or reject sites on the basis of longitude, latitude, or altitude as these components would reflect the geography of the growing areas in each country. No minimum crop area was set as field size varied considerably across the different partner countries. For example, Switzerland had the smallest OSR fields with a mean area 2.3 ha, compared with the OSR fields in Germany that had a mean area of 51 ha (Supplementary File S5).

A number of practical considerations also proved critical when finalising study site selections. For example, to reduce travel time and associated costs, preference was given to sites located close to home institutions or clustered within particular growing regions. Additionally, preference was given to established contacts and/or farmers who were willing to provide information on farm management, specifically their use of agrochemicals.

Because the project was financed by EU funding, it was necessary to consider the rights of participating growers with respect to EU general data protection regulations (GDPR), and each of the field sampling partners were required to ensure their planned field work and data management followed their national GDPR guidelines. Additionally, informed consent forms and GDPR-related documentation were presented to growers indicating their rights, a summary of the field collecting plans, and how the project data would, and would not, be used (Supplementary File S6).

Although the relaxation of certain requirements led to some minor confounding of site properties among partner countries, the site selection process resulted in an overarching European framework that captured typical national variation in farm type, cropping styles, and landscape diversity. Across all eight partner countries, the study area spanned over 3000 km, from Spain (latitude 38°) to Estonia (latitude 59°; Figure 3), and a series of within-country agricultural intensity gradients nested within an overall gradient based on international differences.

3.3. Landscape Descriptors

A primary aim of the field study was to classify sites in terms of agricultural intensity, which, could be used as a general proxy for the stressors experienced by insect pollinators (Table 1). The spatial scale at which the landscape is considered can influence how the relationships between land use intensity and pollinator performance are perceived [74,102], so to describe the quality of the study sites at various spatial scales relevant to insect pollinators it was recognized that landscape descriptors were required to:

(i) quantify land use patterns in the wider surrounding landscape that would allow the production of agricultural intensity gradients;
(ii) depict local farm habitat quality in terms of features immediately adjacent and/or surrounding to the study site;
(iii) classify local habitat quality in terms of its immediate value to pollinators;
(iv) describe management and stewardship practices taking place at the study site, including agrochemical use and productivity.

Our solution to meet all of these requirements was to adopt a hierarchical suite of geographic information system (GIS) tools and field-based methods that would obtain meaningful landscape data at multiple scales, whilst acknowledging the limited time available for conducting habitat surveys or landscape classifications. The wider-scale landscape features were described using GIS analysis of high-resolution remote sensing data, so that for each site all landscape features within a 1-km radius were identified and manually digitized. Land cover features were then classified into final categories relevant to agricultural intensity and/or semi-natural habitat quality, such as crops/orchards, grasslands, woodlands, wetlands, waterbodies, roads, and urban areas. The land cover data allowed for the calculation of a 'landscape diversity' index for each site, and the classification of landscape profiles using multivariate analysis. At such spatial scales, clear gradients of agricultural intensity among sites (for both crops in each country) were readily identified.

Farmland pollinator assemblages are known to respond to the type and diversity of linear features in the landscape and the diversity of land use within their foraging ranges [103]. As a measure of habitat quality at a local farm scale we classified the types and diversity of linear features surrounding the focal field for each field site, and the types and diversity of land use in the fields immediately adjacent to the focal field using the European Nature Information System (EUNIS) habitat classification system (Supplementary File S7).

To obtain a measure of the potential quality of the study site to pollinators, we collected data on the abundance and diversity of field margin floral resources, and also surveyed wild pollinators as an indicator of general 'pollinator community health'. Many methods are used to assess the diversity and abundance of farmland pollinators and the floral resources available to them [104,105]. Because each site visit involved collection of multiple biological samples and measurements, time constrains meant it was necessary to employ relatively rapid floral and pollinator survey protocols that still provided ecologically meaningful measures of abundance and diversity. Consequently, a single simplified floral survey was performed at each site that provided an estimate of flowering species richness and a rapid abundance assessment by assigning floral units to an ordinal scoring system based on a 10-fold geometric sequence (i.e., no floral units ≡ 0; 1–10 floral units ≡ 1; 11–100 floral units ≡ 2; >100 floral units ≡ 3; [99]). This resulted in a single field worker being able to complete a floral survey in a single site visit.

As with the floral surveys, the pollinator surveys were required to provide meaningful data whilst enabling surveys to be completed by a single field worker within a limited time. The survey protocol also needed to be practicable in all participating countries, where major differences in insect abundance during the field work period could occur. Thus, the pollinator surveys consisted of standard transect counts involving a 50 m walk over a short (5-min) period and recording the flower-visiting insects 1 m either side of the observer [99]. To save time, only a coarse level of identification was used, and specimens were assigned to one of only five major pollinating groups (*Apis mellifera*, *Bombus* spp., solitary bees, Syrphidae, Lepidoptera). Although each individual pollinator survey was relatively rapid, there was still a need to gauge how the pollinator assemblages varied both spatially and temporally [49]. Therefore, pollinator surveys were performed at each site on three occasions, corresponding to early, middle, and late flowering of the focal crop (Figure 4), and on each occasion two 5-min surveys were performed in the centre of the crop and two were performed in the field margins.

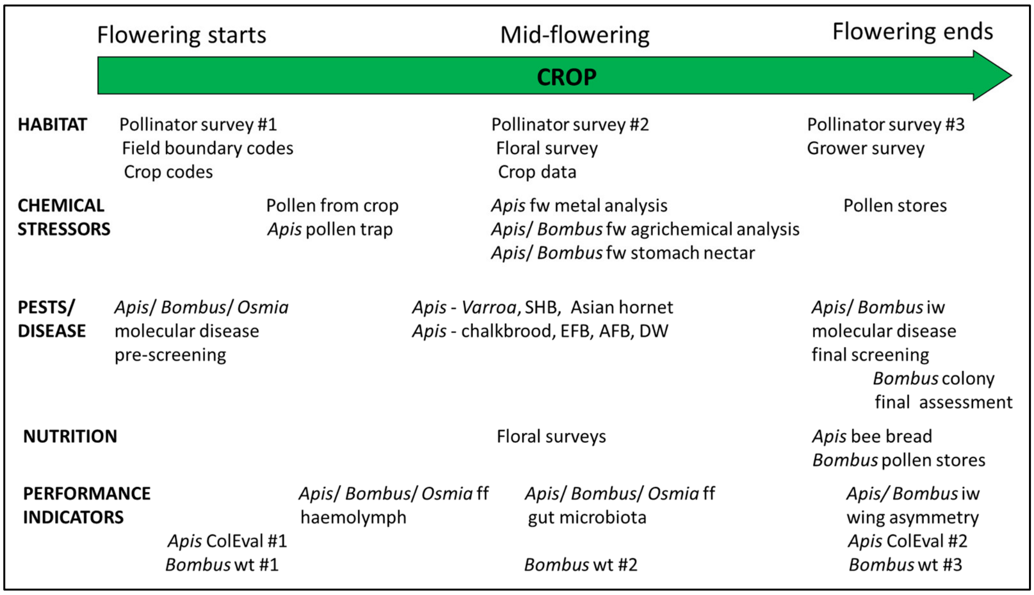

Figure 4. Relative timings of sample collection, measurements, and data recording for each site in the PoshBee field study. Timings were calibrated relative to the start of flowering rather than fixed to specific dates to account for the geographic separation among the different countries that were part of the site network. ff—foraging females; fw—foraging workers; iw—internal workers; wt—colony weight; ColEval—colony evaluations; SHB—small hive beetle; EFB—European foulbrood; AFB—American foulbrood; DW—deformed wing.

To gain further insight into the management of each study site and obtain additional details regarding the scale and output of each operation, each farmer was requested to complete a questionnaire at the end the growing season (Supplementary File S8). The survey, developed in collaboration with all stakeholders, was initially written in English and translated into local languages to ensure data were collected in a consistent manner. The resultant data pertaining to total and per hectare crop yields and inputs of agrochemicals could be used as additional scores to rank study sites in terms of agricultural intensification. Additionally, data provided by farmers allowed us to compare stated agrochemical use with the profiles of compounds identified by during chemical analysis by Tier II partners (Section 3.5).

3.4. Samples for Detection of Agrochemicals and Heavy Metal Contamination

Multiple pesticides have been found in honey samples worldwide, and different beehive matrices, such as wax and pollen stores, can contain different contaminant profiles [40,106–109]. In addition to insecticides, bees can also be exposed to fungicides and herbicides [45,48,110] and be exposed to several contaminants simultaneously, often with synergistic toxic effects [68,111,112]. Other toxic pollutants such as heavy metals can also be present in colonies at levels that alter bee behaviour and inhibit bee performance [113–115]. The use of copper- and sulphur-based compounds, in addition to mineral oils, for plant protection in organic farms creates an exposure pathway to these pollutants in what otherwise might be considered relatively benevolent agricultural settings [116]. Bees can encounter agrochemicals by contact, by ingesting nectar or water, or by collecting and storing contaminated pollen, and under field conditions bees could be exposed to toxins via several of these routes [117–120]. Therefore, investigation of the entire pathway of the potential contamination chain is required to give insight into the source, concentration, and likely impact of the chemicals on adult bees and developing larvae. After consideration of the above issues, the specific aims of the chemical analysis component of the PoshBee field study were to:

(i) elucidate the presence and concentrations of a wide range of chemical contaminants on/in adults of the three sentinel bee species;
(ii) assess different colony and nest matrices to establish the location and possible acquisition chain of contamination;
(iii) establish the most commonly occurring chemicals in each sample matrix, and the frequency of co-occurrence;
(iv) relate chemical contaminant profiles of bees and colony matrices with contaminants occurring in the pollen of the focal crops;
(v) relate chemicals observed in the various samples with products growers reported as being applied to the focal crop.

In order to meet the aims of this study component it was necessary to obtain multiple samples from each study site, all within the relatively narrow window of the main crop flowering period. To ascertain exposure of adult bees to chemical pollutants, female *Apis* and *Bombus* were collected as they returned to the colony after foraging events, whereas adult female *Osmia* were collected as they appeared near to the trap nests. By collecting adults of all three sentinel bee species during the same site visit, the agrochemical profiles obtained from the bodies of the three bee species could be meaningfully compared.

To establish potential contamination routes, pollen for analysis was collected from flowers of the main crop, from pollen traps attached to *A. mellifera* hives and, where possible, from the legs of returning *B. terrestris* foragers. Furthermore, to examine how contaminants could be passed on to developing larvae within the colonies, pollen stores were extracted from *A. mellifera* hives ('beebread'), *B. terrestris* colonies, and *O. bicornis* nesting tubes. To assess whether bees were exposed to chemicals from contaminated nectar, the nectar was extracted from the stomachs of female *A. mellifera* and *B. terrestris* as they returned from foraging visits.

To examine the presence, concentrations and co-occurrence of chemical contaminants, the different matrices were subjected to a suite of analytical processes (e.g., extraction, clean up and detection by chromatographic techniques). Three target compounds—sulfoxaflor, glyphosate, and azoxystrobin—were selected for quantification using LC-MS/MS based on their current use and their potential or known harmful effects on bees. Sulfoxaflor is a systemic sulfoximine insecticide used to control aphids, azoxystrobin is a QoI broad-spectrum fungicide, and glyphosate is an extremely widely used broad-spectrum systemic herbicide. An additional 70 potentially harmful agrochemicals, related analytes, and heavy metals were also assessed (Supplementary File S9).

By performing the above sample collection and chemical analyses, various sample-by-chemical data matrices could be produced, so that: the contaminant profiles of similar samples could be compared among sites; the contamination profiles of different samples could be compared within sites; and the level of co-occurrence of contaminants could be established so that the frequency of synergistic effects could be estimated. This chemical information would also be compared with the list of compounds that were applied to the crops obtained during the farmers' questionnaire.

3.5. Bee Nutrition and Pollen Analysis

Managed bees situated on mass flowering crops such as OSR and apples would, on first appearances, have convenient access to abundant supplies of pollen and nectar [35]. Examination of pollen traps and pollen stores, however, indicates bees must return to the colony with pollen obtained from non-crop flowers [121,122]. Even when super abundant, diets dominated by pollen from a single plant species do not promote optimal larval development and health of adult bees [62,123], and monotonous diets can also result in increased susceptibility to other stressors such as pesticide exposure and diseases [62,63,124].

As part of the PoshBee field investigation it was desirable to gain an insight into the diets of the sentinel bees in our study, both to gauge whether nutritional deprivation could cause direct negative impacts, and whether inadequate diets could exacerbate the effects of other stressors. As with other components of the field investigation, the final bee nutrition sampling program arose via discussions that considered the time and resources available to field workers and the quantities of material required by the Tier II analytical laboratories. Additionally, it was necessary to consider that samples of flower pollen, pollen from foraging bees, and pollen stores were also required for other components of the field study such as quantification of pesticides and other pollutants (Section 3.4).

The resulting hierarchical study scheme aimed to provide information along a nutrition pathway, from assessing the floral resources available to bees, what pollen was being stored, and the nutritional value of these pollen stores. To meet these goals, the objectives of this component of the field investigation where to:

(i) quantify the diversity and abundance of non-crop flowers available in the field boundaries;
(ii) determine the botanical origin, diversity, and nutritional quality of pollen in the food stores.

Collecting data on floral diversity and floral abundance for each study site were already a planned component of the local landscape descriptors (see Section 3.3), and the amounts of nectar and pollen available to insect pollinators are generally correlated with floral density [125]. The diversity of nutritional resources accessible to the sentinel bees in our study would be dependent upon the species of flowers available within the foraging ranges of the different bee species (e.g., [126]).

To provide an indication of what pollen was being stored within the colonies, pollen stores were collected from all three sentinel species. This sampling was performed at the end of flowering of the main crop so it could be assumed the foraging bees had had access to the main crop when these stores were being laid down. This sampling point also considered that sub-samples of these stores were also required for pesticide analysis, where pollen obtained from the main crop, and the agrochemicals that had been applied to it, were a main point of interest (Section 3.4).

The pollen samples were homogenised and then separated into three sub-samples. One sub-sample was analysed for sterols, total amino acid concentrations, and amino acid profiles. A second sub-sample of the homogenized pollen stores was subjected to microscopic palynological analysis to obtain data on the species of plants present, and this was supported by a reference collection of named flower heads containing pollen grains obtained from each site during the field margin floral surveys (Section 3.3). The final pollen sub-sample was intended for pesticide analysis.

3.6. Bee Pests and Pathogens

Increasingly sophisticated screening and bioinformatic methods are detecting an increasing number and diversity of honey bee viruses [127]. New pathogens, such as novel strains of *Deformed wing virus*, and established diseases such as chronic bee paralysis continue to emerge and re-emerge, whereas others are replaced by newer, fitter variants [27,128,129]. Honey bees are also challenged by a range of pathogenic fungi and bacterial diseases, and their overall biotic stress load is expanded by invertebrate pests, predators, and parasites, such as Varroa mites, Asian hornets (*Vespa velutina*), and small hive beetles (*Aethina tumida*) [130–133].

Bumble bees and solitary bees are also susceptible to multiple pests and diseases [134–138] and there are increasing reports of disease associations between honey bees and bumble bees, and between honey bees and non-bee hymenopterans (e.g., [84,85,139]). Viruses and fungi that were considered primarily honey bee pathogens are now found in several species of bumble bees and mason bees, with interspecific transmission sometimes occurring via shared flowers [67,134,135].

In addition to applying a direct stress, high incidence of pests and pathogens can also indicate bees are experiencing other environmental stressors. There can be a correlation between general honey bee colony weakness and high pathogen infection rates [140–142] and bees become increasingly susceptible to pathogens when their diet is restricted and/or they have been exposed to agrochemicals or other toxins [62–65,124]. There is also increasing evidence that many bee pathogens, parasites, and pests act in a synergistic manner to cause multi-disease syndromes and conditions. For example, virus infection can lead to increasing rates of chalkbrood [143] and *Varroa destructor* is directly responsible for epidemic and lethal transmission of several viruses, such as *Deformed wing virus* [140,144]. Consequently, in order to gain a more detailed and holistic insight into the suite of biotic stressors the sentinel bees were encountering, we aimed to:

(i) record the prevalence of a suite of primary bee pathogens and pests across the site network;
(ii) calculate the frequency of co-occurrence of pests and pathogens to investigate potential ecological associations or synergistic effects;
(iii) compare the pest and disease profiles of three co-occurring sentinel bee species;
(iv) compare field assessment of major pathogens with infection rates obtained by laboratory analysis.

To meet these aims, adult bees were collected from each site and subjected to real-time quantitative PCR for detection and quantification of the following eleven pathogens: *Nosema apis*, *N. ceranae*, *N. bombi*, *Paenibacillus larvae* (American foulbrood; AFB), *Melissococcus plutonius* (European foulbrood; EFB), *Deformed wing virus* Type-A, *Deformed wing virus* Type-B, *Acute bee paralysis virus*, *Black queen cell virus*, *Sacbrood virus* and chronic bee paralysis virus. Although not exhaustive, this list of diseases was chosen to cover the main honey bee diseases occurring in Europe (including the notifiable diseases AFB and EFB), re-emerging diseases such as Chronic bee paralysis, and pathogens previously found to correlate with general colony health [128,142,145]. Samples of adult bees were collected prior to the bees being placed at study sites so that basal disease levels could be ascertained. Final samples of adults were collected after the bees had been exposed to field conditions for several weeks. By processing all three sentinel bee species through the same qPCR analysis, the incidence and pathogen load in each species and co-occurrence of pathogens in bees of different species at the same site could be estimated.

Each honey bee hive was also subjected to visual examination for the presence of diseases and pests under field conditions. Each hive was scored for cells and larvae showing symptoms of the notifiable diseases EFB and AFB, the prevalence of chalkbrood disease caused by the fungus *Ascosphaera apis*, and the presence of adult bees exhibiting deformed wing symptoms [78]. In addition to providing rapid results, especially important for notifiable diseases such as EFB and AFB, the data from these field examinations would be compared with results obtained from the molecular analysis to estimate levels of concordance.

Due to time constraints on each site visit, field assessments of invertebrate pests in the honey bee hives were mainly limited to rapid binary presence/absence scores. For example, the presence/absence of Asian hornet (*Vespa velutina* Lepeletier) was recorded at each site on at least three visits by visual inspection of the entrances to the hives. Small hive beetle (*Aethina tumida* Murray) infestations were assessed using basic vegetable oil-filled traps. Varroa mite (*Varroa destructor* Anderson and Trueman) is, currently, a much more common pest than Asian hornet and small hive beetle. Following input from the various beekeeper groups, to give a more quantitative measure of mite infestation rates it was decided to spend more time and effort on the assessments of this pest and use sticky traps in the bottom of hives to assess the daily mite drop [99].

Whereas honey bee hives could be opened and inspected during site visits, obtaining data on invertebrate pest loads of the other sentinel bee species was more complicated. Discussions among the field teams and research groups specializing in *Bombus* and solitary bees concluded it would be valuable to compare pest loads among species both within and across the site network. *Bombus* nests could only be opened and examined for pests at the end of the study, after the remaining bee population had been euthanized for final colony assessments (see Section 3.7 below). Each *Bombus* colony was dissected and the presence of pests and commensals such as mites, Diptera, hymenopterous parasitoids, vespulid wasps, cuckoo bees, ants, and wax moths was recorded. For *Osmia*, the assessment of invertebrate pests and natural enemies was a more involved process, where the presence of mites, hymenopterous parasitoids, and Diptera were assessed by maintaining occupied *Osmia* nest tubes until the following year, so allowing the pest species to complete their development and emerge as adults.

3.7. Indicators of Sentinel Bee 'Health' and Performance

In order to evaluate the relationships between environmental stressors and their impacts on bee health, it was essential to obtain quantitative measures of sentinel bee performance at each study site. The presence of pests and pathogens, already measured as in indication of biotic stress intensity, would naturally be used as a measure of bee health. However, as with the other components of the study, requirements of the different project stakeholders resulted in a hierarchical suite of performance measures being obtained for each of the sentinel bees. The overall aims of this component of the study were to:

(i) gain an insight into honey bee and bumble bee performance at the colony level;
(ii) estimate stressors encountered by developing larvae by examining body morphology of adult bees;
(iii) gain insight into the physiological condition of adult bees;
(iv) examine responses to stressors at the molecular level.

There is often a correlation between general colony weakness and high loads of pests and diseases [140]. Nevertheless, many of the beekeeper organisations involved with project planning and research groups concerned with general bee health suggested that some other general measure of honey bee colony performance was also required. The approach adopted was one based on the ColEval method where the number of bees, area of capped comb, and cells containing pollen stores were estimated on every frame present in the hive [146,147]. The assessments were performed when the hives were placed on site and then again prior to retrieval at the end of the study so that relative growth and development of the colony could be estimated. The growth of *B. terrestris* colonies was evaluated by weighing at regular intervals during the study period. To give an indication of colony size in terms of numbers of individuals, the number of adult *B. terrestris* (gynes, females, males) and the number of intact queen and male/worker cocoons were counted at the end of the study. For *Osmia*, the size of the aggregation of bees at each site was estimated by counting the number of occupied nest tubes [99].

At the individual level, we examined for nutritional or developmental stresses in developing bee larvae by obtaining samples of recently emerged adult bees from inside *A. mellifera* and *B. terrestris* colonies. General health was estimated by gauging the development of fat bodies and by using wing length and/or intertegular distance as indications of body size. Developmental stresses in these bees were further examined by calculating the degree of fluctuating asymmetry in their wings, which has previously been demonstrated to respond to pesticide exposure and other anthropogenic stressors [148–151].

In order to investigate physiological and molecular responses to environmental stressors, and potentially identify new stress-response relationships, proteomic and microbiological approaches were used. Haemolymph samples were collected from foraging bees and subjected to proteomic analysis to assess variation among proteomes and identify molecular 'spikes' associated with the presence of pathogens or chemical pollutants [152–154]. The gut microbiome profile of bees also responds to developmental perturbations and agrochemical contaminants [46,155] and, accordingly, we examined the gut microbiome of bees collected at the end of main crop flowering as these bees would have emerged from eggs and larvae produced during the field exposure period.

Overall, this diverse array of performance measures produced a comprehensive portrait of how the bees responded to environmental stressors occurring at each site, how these response variables were inter-related, and whether the same type or scale of responses were shown across the sentinel bee species.

3.8. Logistics, Timing, and Sample Moderation

Due to the number of measurements and samples required, devising a field sampling regime acceptable to all stakeholders was especially challenging. The relatively narrow crop flowering periods, together with the number of field sites, meant that field workers would be restricted to only a few visits to each site when the sentinel bees were exposed to flowers of the focal crops. Inevitably, losing field days due to inclement weather would further exacerbate these time limitations. Given these restrictions, the devising of a schedule for site visits and sample collection aimed to:

(i) create a sampling program that met the objectives of the wider field investigation and provided meaningful samples for end users;
(ii) create a timetable that provided each field team with clear guidelines concerning when each sample or measurement should be taken;
(iii) provide guidance on how the time spent and the labour available on each site visit could be optimized.

Because of the geographic spread of study sites, and differences in latitude and altitude, crop flowering seasons were staggered across different countries. During early project planning meetings, it became obvious that calendar dates could not be used to synchronise study site setups and sample collection. Instead, it was necessary to standardize setup times and sampling regimes to crop flowering patterns in each country. Each field team aimed to have sentinel bees on-site just prior to main crop flowering and then obtain the various samples and measurements in a predetermined sequence (Figure 4).

Outlines for the timetable were initially devised by the field study coordinators and presented to other stakeholders via email and during face-to-face planning meetings. Many components of the investigation had an obvious or necessary position in the study timetable. For example, samples of bees for pathogen testing and the initial honey bee hive assessments were required at the start of the study, whereas other samples, such as extracting pollen stores, were naturally taken at the end of the study period so we could be more certain they had been collected from the focal crop. Other measurements, such as weighing the *Bombus* nests and performing wild pollinator surveys, were performed three times during the flowering period so were naturally positioned in early, middle, and later site visits.

Because only a few sites could be visited within a single day, high levels of organization, time management, and contingency planning were required to optimize contributions of field staff available on each visit. The process of developing the timetable also resulted in some guidance on how each site visit might be made more efficient. For example, if foraging honey bees were required for heavy metal, agrochemical, and gut microbiome analyses, these samples could be collected together in the field and sub divided back at the laboratory. Advice was also provided in terms of which measurements could be performed during periods of inclement weather (e.g., floral surveys, landscape descriptors, crop measurements) and which samples could only be performed during fine weather (e.g., use of *Apis* pollen traps, collecting foraging bees, wild pollinator counts). To further aid with site visit planning, members of the field teams suggested that methodological protocols provide an indication of how long each task should take and how many persons were required to complete it [78].

Another outcome of the timetable development process was a realisation that the time available to meet all the aims of the field study was severely limiting, both in terms of the length of the crop flowering periods and the number and duration of site visits that could be completed in a single day. To reduce time wastage, some field teams subsequently revised their study site selection to shorten travel time between sites (Section 3.2). Timetabling issues also arose because field workers did not have sufficient time to collect the quantities of biological samples originally requested by the Tier II analytical partners. It sometimes became necessary to reinitiate discussions among project partners so that compromises could be made in terms of agreeing minimum viable sample sizes that could be practical obtained but still fit for purpose (Figure 2).

The production of the field work timetable was a key task in the overall study design process. Developing the timetable involved the consideration and amalgamation of the many individual methodological protocols, a recognition of the requirements of the Tier II partners processing the biological samples, and a review of the personnel required to complete these tasks in the field. It was also during this process that a greater awareness developed across project partners regarding the disparity between the designing of a multi-partner field study and the practicalities of executing that design once resource limitations were imposed.

3.9. Data Collection and Management

The field study, by its very nature, produced considerable amounts of biological and field data along with substantial amounts of meta-data relating to biological samples collected for subsequent chemical and pathological analysis. It was, therefore, necessary to consider all aspects of data management prior to the commencement of practical work to optimize data collection, storage, and security. With a view to future data sharing, there was also a need to produce high-quality data files that were easily comprehensible and would foster subsequent analysis and cross collaboration [156–158]. Additionally, the data collection, collation, security, and risk mitigation strategies for the PoshBee field investigation were required to comply with the overall PoshBee project data management and with wider GDPR issues. The project aimed to create an online centralised database maintained by project partners that gathered data from each component of the field study and laboratory analyses. Therefore, the overall objectives of the data collection and management process as part of the field investigation were to:

(i) collect high value biological data fit for purpose with respect to all project stakeholders;
(ii) ensure efficient and consistent data collection and formatting across all the field study partners;
(iii) produce digital files containing easily comprehensible raw data and meta-data that was amenable to future exploration by both internal and external users;
(iv) consider the practical issues related to data collection and management in terms of overall project logistics and availability of human resources.

The data collection process for each component of the field investigation was, as for the other components of the study, decided via a co-development process involving the different contributing partners. In terms of standardized collection of data, all written methodological protocols included templates stipulating what data were required for that study component (see Section 2; [99]). To improve ease of understanding for field workers, the data collection templates were directly linked to detailed instructions regarding the required measurements, units of measurement, and the form of data to be recorded (e.g., presence/absence; counts; character codes; continuous measurements). For audit purposes, and as an aid to solving potential future problems, meta-data (dates, time of day, field workers present) associated with each set of measurements and samples were also recorded. Explicit guidance was also provided to field workers in terms of data security (e.g., data collection checklists; regular digital data backups; photographing completed data templates in the field).

When producing the methodological protocols, it was necessary to ensure the data collected were fit for purpose while being mindful of the time and labour available to perform each task (Section 2). For example, although it might be desirable to simplify and accelerate data collection procedures in the field, it was also necessary to consider whether recording only low precision or coarse data would reduce their value in terms of future statistical analysis and validity of conclusions. The various stakeholders also needed to ratify whether the form of data collected could be converted into easily comprehensible and valuable information for different audiences (e.g., scientists; farmers; policy makers), and into concepts meaningful to the wider public.

Much consideration was given to establishing a series of codes that could be applied to the different data sets collected. These codes would be used to directly match labels and meta-data associated with biological samples (e.g., bee specimens; pollen; food reserves) that were collected by field teams for use by project partners in subsequent work packages. For example, standard three-character codes were applied to the data/samples obtained from the eight different partner countries (CHE, ESP, EST, GBR, GER, IRL, ITA, SWE) and for the different focal crops (OSR; APP). Similarly, a standard date formatting was agreed (dd-mm-yyyy) and established EUNIS codes adopted for landscape data (Supplementary File S7).

It became apparent during the field investigation that for each team and the project overall, data organization and management required a significant, but often overlooked, allocation of human resources. Where possible, efficiency was increased if a designated person in each field team took on responsibility for within-team data management issues. The data collection and management for the whole site network was then performed by project coordinators, who collected raw data files from each team and identified gaps or potential errors in the data at an early stage. All data from each component of the field study were collated into single files covering the whole site network, and final versions of the data and meta-data made available to all PoshBee partners via the project web page (poshbee.eu).

4. Discussion

4.1. The Trans-Disciplinary Design Process

The PoshBee field study represented a complex, trans-disciplinary project involving partners from over 20 academic, research and agricultural institutions, and collaborations with 100 s of local beekeepers and participating farmers. By the conclusion of the field work, over 750 *A. mellifera* colony evaluations had been performed, together with 350 inspections for Asian hornets, small hive beetles and Varroa mites. Floral surveys were performed at all 128 sites along with 1300 wild pollinator assessments. In total, approximately 60,000 *A. mellifera*, 10,000 *B. terrestris* and 1000 *O. bicornis* specimens were collected for subsequent testing for chemical contamination, diet quality, developmental anomalies, gut microbiota, and the presence of pathogens. In addition, over 1.5 kg of pollen from foraging bees, 2 kg of pollen stores from *A. mellifera* hives and almost 3000 haemolymph samples for proteomic analysis, were obtained.

There is increasing momentum towards publicly funded scientific research that has direct societal benefits, and transdisciplinary syndicates involving academics and practitioners are becoming an increasingly common mode of addressing complex ecological and environmental issues [159,160]. However, in terms of the process of designing and executing transdisciplinary research in agroecology and sustainable food production, there remains a lack of clear guidelines, a general research framework, and a common vocabulary [77,80,159,161]. Establishing effective collaborative research networks requires considerable effort and faces numerous challenges, not least the requirement that each network member is fully integrated into the co-design process, whilst concurrently appreciating the needs and skills offered by other members [77,80,159,161,162].

When involving diverse stakeholders, and even scientists from different disciplines, there can often be lack of shared framing for the same problem, with unique perspectives on how the problem should be approached and what a meaningful solution involves [159]. For a field study of the scale and complexity of PoshBee, it was desirable, and necessary, to engage and include input from a diverse range of practicing stakeholders and scientific partners with complementary expertise [161]. We aimed to directly connect scientists and practitioners by exchange of ideas and perspectives at an early stage of project planning, which we envisaged would lead to better and more straightforward integration of the academic and non-academic knowledge ultimately obtained [159]. Thus, the PoshBee field study can be viewed as an interdisciplinary science endeavour nested in a wider transdisciplinary approach (Figure 5). Initial face-to-face discussions among the various academic and practicing stakeholder groups were essential for reaffirming the original themes of join interest that had initiated the project, and, therefore, refining research questions to be answered by the field research (Figure 5). It was then necessary to design a hierarchical research scheme that would collect data acceptable to all partners and of a form that would transition easily into improved policy and practice (Figure 5) [162]. Overall, the co-design process synthesised multiple and diverse inputs to form a scheme of work that was comprehensible to all network members and elevated the whole process from being simply additive to a synergistic multi-partner research exercise. Both academic and non-academic partners benefited from mutual learning, networking, and forming links that could lead to future research collaborations. In addition to the scientific knowledge acquired, wider capacity building benefits for those involved in the planning process included development of personal and communication skills, project management, organisational competence, and managerial aptitude [80,160,162].

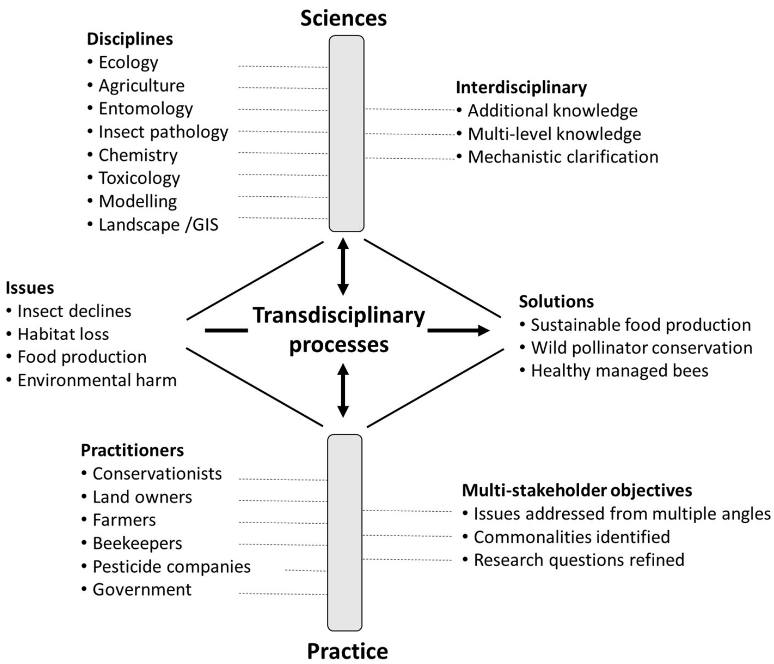

Figure 5. Illustration of the transdisciplinary processes involved when designing an investigation into the stressors affecting insect pollinators across a pan-European study network. Issues raised by diverse stakeholders including scientists from multiple backgrounds and non-academic practitioners result in interdisciplinary scientific projects and the identification of commonalities in the requirements of the different groups (adapted with permission from [161]).

4.2. Lessons Learned

Undoubtedly, the overall success of the field study was highly dependent upon the detailed planning, problem solving, and conflict resolution performed prior to the commencement of the field season. Nevertheless, the journey from planning to execution provided many valuable insights into the challenges and potential shortfalls in the design process of studies of this complexity and scale. In terms of recommendations and guidance for researchers developing ecological field projects of similar magnitude, we summarize the main lessons learned during planning and execution below:

1. The design process, interactions with collaborators, and communications with on-site stakeholders all require considerable time and resources: initiate these processes as early as possible.
2. Produce detailed written protocols and data templates for each element of the study and use wider stakeholder input to ensure these protocols will lead to the collection of data that are fit-for-purpose in terms of overall project aims.
3. Accelerate overall study design by delegating method development to sub-groups with appropriate expertise.
4. Assign one group as project managers that hold responsibility for method development, overall project planning, and form a hub for communications with all other team members.
5. Enhance capacity building and reinforce technical skills of field teams with training workshops and video demonstrations.
6. Early in the study design process, amalgamate protocols and logistics into a field timetable. Verify that what has been planned is achievable under the limitations imposed by budget, time, human resources, and typical field conditions. Identify which components of the study are not practicable, and therefore, where compromise or redesign is required.
7. During practical work, minimise omissions or errors by regular centralized monitoring of the progress of each field team in terms of sample collection and data recording.
8. Problems encountered in the field are very often related to shortages of time and/or personnel: consider how time on site can be maximised and made more efficient.
9. No matter how tempting, avoid adding excessive secondary elements to field work outside the scope of the main project, as these can detract from achieving primary aims.

Although time consuming, the production of a series of detailed protocols for each element of the study was considered highly valuable. For the field workers, these short documents clarified what was to be done, how it was to be performed, when the task should be carried out in terms of the crop flowering time, and, roughly how long the task should take. The practical workshop was highly valuable for field staff to gain supervised experience of new techniques, and also provided an environment where the accuracy of written protocols could be evaluated and estimates of timings compared with actual task performance times. The protocol documents provided instructions and templates for data collection, which in themselves were highly effective in optimizing and simplifying overall data management. The data management process was also aided by appointing one person in each field team to organize and submit interim data files to the central data organizers, who could identify gaps or possible errors when collating data from the whole site network.

The overall study design process took considerable time and was finalized just prior to the commencement of the actual field set up. Future studies would benefit from commencing this process as early as possible in advance of the planned field work start and accelerate the process by promptly identifying sub-groups of partners to work on components associated with their specific expertise. It was still essential, however, to designate one group as overseers of the overall study design process, who were responsible for integrating individual protocols into a workable and practical work scheme and communicating decisions to the whole partner network. The field study organizers engaged in conversations with different sub-groups regarding individual protocol development, but with a more complete knowledge of the wider context and resources available to perform each task. Often, there were several of these iterative discussions between the investigation team leaders and sub-groups of partners occurring concurrently, and this process formed the central means by which the overall study design was fine-tuned and finalized.

Overall, the problems encountered during the execution of the field work were very often associated with limitations imposed by shortness of time or the availability of field personnel. The short duration of the crop flowering periods was compounded by days lost to inclement weather, which could soon result in the build-up of a substantial backlog of required samples and measurements for some sites. Additionally, the considerable distances between sites in some countries meant that even during fine weather only a few sites could be visited in a single day. In hindsight, many of these practical failings were identified during early group discussions but the significance of their impact was not given enough consideration, especially as the study design and logistics timetable were finalized. Indeed, the situation was exacerbated somewhat by the addition of further measurements and samples to the study as a means of gaining additional benefits from the substantial study site network. It became apparent as the field work was underway that we had not fully considered some processes that could increase time on site (e.g., reducing travel time), boost efficiency when on site (e.g., using specialist teams for specific tasks), and contingency plans for what tasks could be performed in inclement weather. Future studies of this type would likely benefit from more detailed, and more realistic, evaluations of the work that can actually be achieved by field teams given the time available and the personnel allowed by the project budget.

5. Conclusions

In light of the physical scale and number of personnel involved, the PoshBee field study was extremely successful in terms of the quantity, diversity and quality of data obtained. Given that the overall achievements of the study far exceeded the sum of the individual parts, the investigation emphasised the many advantages of large-scale collaborative projects. Challenges encountered during the planning period highlighted the need for the development of a common, reproducible, rubric for the design of transdisciplinary projects in field ecology or agroecology, which should include a template for the co-design process, methods for people management, and initiate the use of a common vocabulary. By formally describing the co-design process in terms of mutual stages and sub-elements, and designating partners in terms of their roles, functions and inputs, commonalities between transdisciplinary projects will be more easily recognised and ultimately lead to a transparent design and planning format more readily adopted by future research syndicates.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su141710549/s1, Hodge Supplementary Files S1–S10.

Author Contributions: Conceptualization, S.H. (Simon Hodge), O.S., A.-M.K., S.G.P., C.C., M.A., J.R.d.M., M.M., P.D.l.R., M.R., E.A., R.D., P.B., D.M., R.J.P., T.K., T.D.B., D.S., S.H. (Steffen Hagenbucher), A.K., K.I., C.H., P.H., M.J.F.B. and J.C.S.; Data curation, A.B., N.C., M.L., A.-C.M., L.P., M.-P.R., E.D. and C.D.; Funding acquisition, A.-M.K., S.G.P., C.C., M.A., J.R.d.M., M.M., P.D.l.R., P.B., D.M., R.J.P., M.-P.C., M.J.F.B. and J.C.S.; Investigation, I.B., G.T., M.H.P.-P., D.W., E.C., G.D.P., P.M., J.M.S., R.R. and V.M.-L.; Methodology, A.-M.K., C.C., M.A., J.R.d.M., M.R., E.A., R.D., P.B., D.M., A.B., N.C., M.L., A.-C.M., L.P., M.-P.R., E.D., M.-P.C., K.A., D.A., S.N.V., T.K., I.B., C.D., G.T., D.W., T.D.B., E.C., D.S., G.D.P., P.M., S.H. (Steffen Hagenbucher), A.K., J.M.S., R.R., V.M.-L., K.I., C.H., P.H., M.J.F.B. and J.C.S.; Project administration, M.J.F.B. and J.C.S.; Validation, D.S.; Writing—original draft, S.H. (Simon Hodge); Writing—review & editing, S.H. (Simon Hodge), O.S., A.-M.K., S.G.P., J.R.d.M., P.D.l.R., E.A., P.B., R.J.P., A.B., M.L., A.-C.M., L.P., M.-P.R., E.D., M.-P.C., K.A., D.A., S.N.V., T.K., I.B., C.D., G.T., M.H.P.-P., D.W., T.D.B., E.C., A.K., J.M.S., R.R., M.J.F.B. and J.C.S. All authors have read and agreed to the published version of the manuscript.

Funding: This project received funding from the European Horizon 2020 research and innovation program under grant agreement no. 773921.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of The University of Reading's School of Agriculture, Policy and Development (protocol code 1046C and 29/05/19).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: The authors wish to thank all the farmers and apple growers who welcomed us and allowed us access to their properties to carry out this research, and all the beekeepers who assisted us. Two anonymous Reviewers provided many insightful and helpful comments on an earlier draft of this paper. Additionally we thank the people below for assistance with this manuscript, their much-valued discussions, and helping to put all the theory into practice: Håkan Albrektsson, Matt Allan, Sachin Anand, Adam Bates, Cristina Botias, Tomas Carling, Manuel Dietenberger, Daniel Dzul, Michael Gaffney, Andrés García, Ross George, Tiit Hallikma, Karin Hill, Madis Järvis, Laura Junk, Margret Jürison, R Karise, Vera Kaunath, Sarina Kivelitz, Jessica Knapp, Ronalds Krams, Egle Liiskmann, Irene Muñoz, S Naudi, Martin Nave, Peter Neumann, Norbertas Noreika, Lina Norrsell, Andrew O'Neill, Piero Onorati, Jenny Panziera, Kaarel Pent, Kjell Ritzén, Oleksandr Ronsevych, Aile Rummel, Micaela Sánchez, Christine Sandberg, Bettina Schaer, José Serrano, Glenn Svensson, Martin Svensson, Estefania Tobajas-Talavan, Veronika Wenz.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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